TESIS DE LA UNIVERSIDAD DE ZARAGOZA

2024

429

María Victoria Marco Benedí

Fenotipo clínico de la Hipercolesterolemia Familiar en la era del tratamiento prolongado hipolipemiante del Siglo XXI

Director/es

Civeira Domingo, Fernando

Laclaustra Gimeno, Martín

ISSN 2254-7606

Prensas de la Universidad Universidad Zaragoza

Universidad de Zaragoza
Servicio de Publicaciones

ISSN 2254-7606

Universidad Zaragoza
1542

Tesis Doctoral

FENOTIPO CLÍNICO DE LA HIPERCOLESTEROLEMIA FAMILIAR EN LA ERA DEL TRATAMIENTO PROLONGADO HIPOLIPEMIANTE DEL SIGLO XXI

Autor

María Victoria Marco Benedí

Director/es

Civeira Domingo, Fernando
Laclaustra Gimeno, Martín

UNIVERSIDAD DE ZARAGOZA
Escuela de Doctorado

Programa de Doctorado en Medicina

2022

TESIS DE LA UNIVERSIDAD DE ZARAGOZA

VICTORIA MARCO BENEDÍ

2021

Fenotipo clínico de la Hipercolesterolemia Familiar en la era del tratamiento prolongado hipolipemiante del Siglo XXI

Departamento de Medicina

Directores

Fernando Civeira Murillo

Martín Laclaustra Gimeno

Fenotipo clínico de la Hipercolesterolemia Familiar en la era del tratamiento prolongado hipolipemiante del Siglo XXI

Autora:
Victoria Marco Benedí

Directores:
Fernando Civeira Murillo
Martín Laclaustra Gimeno

Universidad de Zaragoza
Facultad de Medicina
Doctorado en Medicina

2021

Memoria presentada por: VICTORIA MARCO BENEDÍ

-

para optar el Grado de Doctor por la Universidad de Zaragoza
Dirigida por
Dr. FERNANDO CIVEIRA MURILLO
Dr MARTÍN LACLAUSTRA GIMENO

Diciembre 2021

D. FERNANDO CIVEIRA MURILLO, Doctor en Medicina, Catedrático del Departamento de Medicina, Psiquiatría y Dermatología de la Universidad de Zaragoza.

D. MARTÍN LACLAUSTRA GIMENO, Doctor en Medicina, Profesor del Departamento de Medicina, Psiquiatría y Dermatología de la Universidad de Zaragoza.

Codirectores de esta Tesis Doctoral, CERTIFICAN Que la Memoria de Tesis Doctoral titulada: "Fenotipo clínico de la Hipercolesterolemia Familiar en la era del tratamiento prolongado hipolipemiante del Siglo XXI", presentada por DÑA. MARÍA VICTORIA MARCO BENEDÍ, ha sido realizada en la Unidad Clínica y de Investigación de Lípidos y Arteriosclerosis del Hospital Universitario Miguel Servet bajo su dirección, de acuerdo a los objetivos presentados en Proyecto de Tesis aprobado por el Departamento de Medicina, reúne los requisitos para ser presentada por su autora para optar al Grado de Doctor por la Universidad de Zaragoza y autorizan su presentación en la modalidad de compendio de publicaciones.

Zaragoza, 13 diciembre de 2021.

A mis padres,

A mis hermanos y sobrinos,

A mi familia, que siempre está cerca,

A todas las personas que hicieron posible este trabajo,

A mi abuela Margarita, que con 91 años me dio la mejor lección de vida:
"La vida se vive con ilusión y esperanza"

Agradecimientos

Cuando se llega al final de una tesis doctoral, uno se da cuenta de que es el "comienzo de un principio", y que, gracias a la ayuda de muchas personas, la tesis está finalmente concluida. El destino me abarcó en el mundo de la Hipercolesterolemia Familiar, cuando todavía planteaba mis inicios de investigadora en el ámbito de la nutrición. Tropiezos del camino o como si de una *serendipia* se tratara, se me brindó la oportunidad de dedicarme a una enfermedad genética muy frecuente y sin embargo gran desconocida para la mayoría de las personas que la padecen.

Es por ello, mi más sincero agradecimiento y de manera especial, a mi director de tesis, el Catedrático Fernando Civeira, por aceptarme a realizar esta tesis doctoral. Gracias a su apoyo, su confianza depositada en mi trabajo. A su humildad y generosidad para estar siempre cerca en este camino arduo de la investigación. Su manera desinteresada para aportar y guiar ideas, que muchas veces necesitaban las mías propias. Su inmensa capacidad de trabajo y conocimientos que me han servido de motivación para no decaer en los momentos más complicados. Le agradezco también, el haberme facilitado los medios necesarios para el desarrollo de la tesis y abrirme las puertas de su gran y ejemplar carrera investigadora. Su sabiduría, que me ha orientado a ser una persona cada vez más resiliente, a pesar de los "obstáculos del camino". Gracias por formar parte de mi "familia laboral".

Le agradezco, a mi director de tesis, el profesor Martín Laclaustra, ejemplo de trabajo bien hecho, perfeccionista e intenso investigador. Por su capacidad creativa y plena disposición de los amplios conocimientos que precisa. Gracias por la serenidad que me has transmitido en momentos de intensidad científica. A mi compañera y "coach" de mi tesis doctoral, la Dra Ana Cenarro. Por ser ejemplo de madurez, precisión y meticulosidad en cualquier trabajo de investigación. Por sus acertados consejos, que han calmado mis "dudas científicas existenciales". Gracias por tu apoyo y tu amistad. A Ana Bea, por su profesionalidad y compañerismo justo a tiempo. Su buen carácter que facilita el desarrollo de cualquier actividad pese a las circunstancias. Gracias por estar cerca. A la Dra Estíbaliz Jarauta, por su amplia experiencia en la clínica, su dedicación y cariño por sus pacientes. Gracias.

Por otro lado, un agradecimiento especial para todos y cada uno de los pacientes que desinteresadamente se han ofrecido a lo largo de estos años, tanto en mi trabajo en ensayos clínicos, como en mi tesis doctoral. Gracias a ellos, hoy el conocimiento de la Hipercolesterolemia Familiar es amplio y puede considerarse como una enfermedad menos agresiva.

Como todo no se trata de tesis, en el plano personal incluyo una gran lista de personas que día a día me lo han puesto más fácil. Comenzando por mis amigas, Elena y Gema, sin duda nuestras vivencias en la carrera de nutrición fueron mis mejores años académicos. A la Dra. Elena Betoré por su amistad desinteresada y ánimos frente a nuevos retos académicos. Gracias Elena por un buen plan de amigas, cuando lo he necesitado. A Gema por sus consejos, confianza y fidelidad en mí. A ellas, y a Pao, Irene y Cris por los buenos ratos que vivimos juntas.

De mis años universitarios de enfermera, Andrea, con quien desde el primer día que coincidimos en prácticas, supimos que habría buena conexión. Su alegría, su cariño y sus palabras que siempre me han motivado para continuar en este camino. A Sonia que "nuestras pellas", siempre acababan en un "me quedo". Gracias a las dos por los ratos de confesiones.

A mis amigas Sonia e Isabel, por su criterio frente a sentimientos de decaimiento, que me ha permitido afrontar con una nueva visión las dificultades. Y a Belén. Gracias a ellas, por nuestros viajes, charlas y salidas, por nuestros momentos de desconexión. Y a Gala, mi fiel amiga.

A mi prima Sara que sin duda tengo plena admiración, mujer emprendedora e incansable. A quien debo mi agradecimiento en los momentos más intensos de mi carrera profesional. Quien me transmitió con su experiencia sus mejores directrices en la adversidad. A mis tíos Rosa y Juanjo y a mi prima Inés, por el mejor tándem de risas cuando más lo he necesitado. A mi prima Núria, por los libros de "nuestras personas vitamina" y sus ánimos. Y en especial, a mi abuela Pilar, ejemplo de *mujer coraje.*

Finalmente, a los pilares fundamentales que han hecho posible mi estructura, mis padres. Mi enorme cariño hacía ellos, porque nos han regalado tanto a mis hermanos, como a mí, lo mejor que disponían: herramientas de trabajo. Han sido ejemplo de incansable capacidad para hacer las cosas bien, para darnos lo mejor de sí. Y a mis hermanos, a quienes debo mi lugar en el "medio". Gracias por no ponérmelo siempre fácil y sin embargo estar siempre ahí. A mis sobrinos, por la alegría e ilusión que me transmiten siempre. Y a Rosa y Laura por su apoyo. Gracias a los que están y me han faltado por nombrar.

Si aquellas personas que pensaron que depositar obstáculos en el camino profesional significaría mi abandono, hoy, no concibo a ningún académico, no experimentar la culminación de una Tesis Doctoral. *"Hazlo. Mátate para conseguirlo, pero consíguelo. Por todos esos que confiaron en ti. Y, al margen, todos esos que nunca lo hicieron" Adp. JF. Torres.*

TESIS DOCTORAL
Compendio de publicaciones

La presente Tesis doctoral es un compendio de trabajos previamente publicados o aceptados para su publicación, que se dividen en dos grandes bloques:

- Publicaciones Principales

Trabajos que constituyen el núcleo temático de la tesis y sobre los que se profundizará en sus objetivos, metodología y conclusiones:

1. Victoria Marco-Benedí, Martín Laclaustra, Juan M. Casado-Dominguez, Rosa Villa-Pobo, Rocío Mateo-Gallego, Rosa M. Sánchez-Hernández, Marta Blanco Nuez, Emilio Ortega-Martínez de Victoria, Marta Sitges, Juan Pedro-Botet, Jose Puzo, Teresa Villarroel, Fernando Civeira. **Aortic Valvular Disease in Elderly Subjects with Heterozygous Familial Hypercholesterolemia: Impact of Lipid-Lowering Therapy.** *J Clin Med. 2019 Dec 14;8(12):2209.*

2. Victoria Marco-Benedí, Martín Laclaustra, Ana M. Bea, Manuel Suarez-Tembra, Núria Plana, Xavier Pinto, Ángel Brea, Rosa M. Sánchez-Hernández, Fernando Civeira. **Maternally inherited hypercholesterolemia does not modify the cardiovascular phenotype in familial hypercholesterolemia.** *Atherosclerosis. 2021 Mar;320:47-52.*

3. Victoria Marco-Benedí, Ana Cenarro, Martín Laclaustra, Asier Larrea-Sebal, Estíbaliz Jarauta, Itziar Lamiquiz-Moneo, Pilar Calmarza, Ana M. Bea, Núria Plana, Xavier Pintó, César Martín, Fernando Civeira. **Lipoprotein(a) in hereditary hypercholesterolemia. Influence of the genetic cause, defective gene and type of mutation.** *Atherosclerosis. 2021 Aug 23:S0021-9150(21)01270-3*

4. Victoria Marco-Benedí, Martín Laclaustra, Rosa M. Sánchez-Hernández, Emilio Ortega-Martínez de Victoria, Juan Pedro-Botet, Jose Puzo. **Cataract surgery in elderly subjects with heterozygous familial hypercholesterolemia in prolonged treatment with statins.** *J Clin Med. 2021 Aug 8;10(16):3494*

5. Victoria Marco-Benedí, Ana M Bea, Ana Cenarro, Estíbaliz Jarauta, Martín Laclaustra*, Fernando Civeira*. **Causes of death in familial hypercholesterolemia**. Remitido para publicación

- Publicaciones Secundarias

Trabajos complementarios englobados en el marco de las tesis, que se incluyen en el Anexo.

1. Victoria Marco-Benedí, Estíbaliz Jarauta, Martín Laclaustra, Fernando Civeira. **Nursing workload. Calculation of cardiovascular risk and therapeutic objectives**. Clin Investig Arterioscler. 2021 May;33 Suppl 1:10-17. doi: 10.1016/j.arteri.2020.12.006.

2. Elisenda Climent, Victoria Marco-Benedí, David Benaiges, Xavier Pintó, Manuel Suárez-Tembra, Núria Plana, Hannia Lafuente, Emilio Ortega-Martínez de Victoria, Ángel Brea-Hernando, Àlex Vila, Fernando Civeira, Juan Pedro-Botet. **Impact of statin therapy on LDL and non-HDL cholesterol levels in subjects with heterozygous familial hypercholesterolaemia.** *Nutr Metab Cardiovasc Dis. 2021 May 6;31(5):1594-1603.*

3. Ana M Bea, Esther Franco-Marín, Victoria Marco-Benedí, Estíbaliz Jarauta, Irene Gracia-Rubio, Ana Cenarro, Fernando Civeira, Itziar Lamiquiz-Moneo. **ANGPTL3 gene variants in subjects with familial combined hyperlipidemia.** *Sci Rep. 2021 Mar 26;11(1):7002*.

4. Victoria Marco-Benedí, Sofía Pérez-Calahorra , Ana M Bea, Itziar Lamiquiz-Moneo , Lucía Baila-Rueda, Ana Cenarro, Fernando Civeira, Rocío Mateo-Gallego. **High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early onset diabetic adults with overweight or obesity.** *Clin Nutr. 2020 May;39(5):1354-1363.*

5. Sofía Pérez-Calahorra, Martín Laclaustra, Victoria Marco-Benedí, Iztiar Lamiquiz-Moneo, Juan Pedro-Botet, Núria Plana, Rosa M Sánchez-Hernández, Antonio J Amor, Fátima Almagro, Francisco Fuentes, Manuel Suarez-Tembra, Fernando Civeira . **Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia**. Dyslipemia Registry of Spanish Arteriosclerosis Society. Atherosclerosis. 2019 May;284:245-252.

6. Sofía Pérez-Calahorra, Martín Laclaustra, Victoria Marco-Benedí, Xavier Pinto, Rosa M Sánchez-Hernández, Núria Plana, Emilio Ortega, Francisco Fuentes, Fernando Civeira**. Comparative efficacy between atorvastatin and rosuvastatin in the prevention of cardiovascular disease recurrence.** *Lipids Health Dis. 2019 Dec 11;18(1):216*

7. Victoria Marco-Benedí, Estíbaliz Jarauta, Sofía Pérez-Calahorra, Ana M Bea, Fernando Civeira. **Treatment of a high cardiovascular risk patient with McArdle's disease with PCSK9 inhibitors.** *Clin Investig Arterioscler. 2019 Mar Apr;31(2):89-92.*

8. Victoria Marco-Benedí, Itziar Lamiquiz-Moneo, Luis A Álvarez-Sala, Fernando Civeira. **Disappearance of recurrent pancreatitis after splenectomy in familial chylomicronemia syndrome.** *Atherosclerosis 2018 Aug;275:342-345.*

ÍNDICE

Índice de Contenidos

Índice de tablas

Índice de figuras

ABREVIATURAS

Abreviaturas

ABCA1	Member 1 of human transporter sub-family ABCA
ABCG1	Transportador dependiente de unión de G1
ABCG5	Transportador dependiente de unión de G5
ABCG8	Transportador dependiente de unión de G8
ACAT	Colesterol acil transferasa
Acetil-CoA	Acetil-coenzima A
AoVC	Calcificación válvula aorta
Apo	Apolipoproteína
ASc	Esclerosis aórtica
CETP	Proteína transferidora de ésteres de colesterol
cHDL	Colesterol HDL
cLDL	Colesterol LDL
COPII	Complejo de las proteínas de la cubierta II
CT	Colesterol total
CV	Cardiovascular
DM	Diabetes mellitus
EA	Estenosis aórtica
EGF	Epidermal Growth Factor
GEE	Ecuaciones de estimación generalizada
GPIHBP1	Glycosylphosphatidylinositol Anchored High Density Lipoprotein Binding Protein 1
HDL	Lipoproteínas de alta densidad
HF	Hipercolesterolemia familiar
HFHe	Hipercolesterolemia Familiar Heterocigota
HFHo	Hipercolesterolemia Familiar Homocigota
HMG-CoA	3-hidroxi-3-metil-glutaril-coenzima A
HTG	Hipertrigliceridemia
IDL	Lipoproteínas de densidad intermedia
IMC	Índice de masa corporal
LCAT	Lecitin colesterol acil transferasa
LDL	Lipoproteína de baja densidad
LDLr	Receptor LDL
LDLRAP1	Proteína adaptadora de LDLr
LH	Lipasa hepática
LMF1	Factor 1 de maduración de la lipasa
Lp(a)	Lipoprotein (a)
LPL	Lipoprotein Lipasa
LRP	Proteína relacionada con el receptor LDL
LXR	Liver X receptor
NPC1L1	Proteína Niemann-Pick C1-like-1
OATP1B1	Anión orgánico transportador de polipéptidos
PCSK9	Proproteína convertasa subtilisina/ kexina tipo 9
SP	Score poligénico
QM	Quilomicrón
SCAP	Proteína reguladora del metabolismo del colesterol
SCORE	Systemic Coronary Risk Estimation
SNV	Single nucletide variant (cambios de un único nucleótido)
SR-B1	Receptor scavenger clase B tipo 1
SREBP	Proteínas de unión a elementos reguladores de esteroles inactivos
STAP1	Proteína transductora de señales 1
TG	Triglicéridos
TRC	Transporte reverso de colesterol
VLDL	Lipoproteínas de muy baja densidad
Vmax	Velocidad Máxima aórtica

Fenotipo clínico de la Hipercolesterolemia Familiar en la era del tratamiento prolongado hipolipemiante del Siglo XXI

INTRODUCCIÓN

Capítulo 1

Metabolismo lípidico

1.1 Colesterol y lipoproteínas

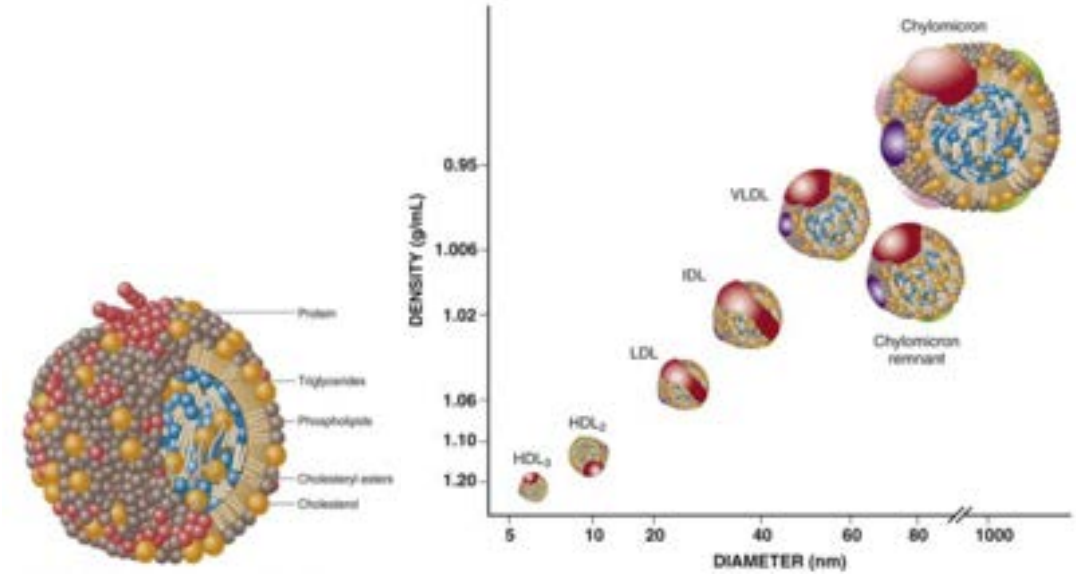
El colesterol es un componente de las membranas celulares, esencial para el mantenimiento de su integridad y función. Además, es precursor de los ácidos biliares en el hígado, de hormonas esteroideas en tejidos esteroidogénicos, de la vitamina D y de oxiosteroles (1–5). Para la regulación de colesterol en el organismo, parte es sintetizado de *novo*, donde la enzima 3-hidroxi-5-metil-glutaril-CoA-reductasa (HMG-CoA-reductasa) juega un papel muy importante (colesterol endógeno) y parte ingerido con la dieta (colesterol exógeno). Nuestro organismo obtiene de la ingesta solamente una pequeña parte del colesterol del organismo (≈400 mg/día) y la mayor parte proviene de la síntesis hepática (≈1g/día). De colesterol ingerido en la dieta aproximadamente un 50% es absorbido por vía intestinal y el sobrante eliminado por las heces (1,4,5).

El colesterol circulante en sangre, debido a su insolubilidad, está asociado a lipoproteínas, las cuales transportan otros lípidos incluyendo triglicéridos (TG) y fosfolípidos (3). Las lipoproteínas son macromoléculas esferoidales formadas por lípidos y proteínas, que se denominan apolipoproteínas. Su estructura se caracteriza por tener un núcleo hidrofóbico que contiene fosfolípidos, antioxidantes liposolubles, vitaminas y ésteres de colesterol; y una monocapa hidrofílica que contiene colesterol libre, fosfolípidos, y apolipoproteínas (apo) específicas (5–7).

Las apos tienen un papel crucial en el metabolismo lipídico y entre otras funciones, destaca la capacidad de actuar como ligandos para los receptores celulares del colesterol (7,8). Las apos son esenciales para el transporte de los lípidos en un medio acuoso, como es la sangre, y se dividen en cinco categorías según su densidad y propiedades fisicoquímicas: quilomicrones (QM), lipoproteínas de muy baja densidad o VLDL (*Very Low Density Lipoprotein*), lipoproteínas de densidad intermedia o IDL (*Intermediate Density Lipoprotein*), lipoproteínas de baja densidad o LDL (*Low Density Lipoprotein*), y lipoproteínas de alta densidad o HDL (*High Density Lipoprotein*) (5,6). (Tabla 1) (Figura 1)

A su vez, existen cinco clases principales de apos: Apo As (I-V), Apo Bs (48, 100), Apo Cs (I-III), Apo E y Apo(a), son mostradas en la Tabla 2 (6).

Figura 1. Estructura y tamaño de las lipoproteínas



Ridker et al (9).

Tabla 1. Clases de lipoproteínas

Lipoproteínas	Densidad (g/ml)	Tamaño (nm)	Lípidos principales	Apoproteínas principales
Quilomicrones	< 0,930	75-1200	Triglicéridos	Apo B-48, Apo C, Apo E, Apo A-I, A-II, A-IV
Remanentes de quilomicrones	0,930-1,006	30-80	Triglicéridos Colesterol	Apo B-48, Apo E
VLDL	0,930-1,006	30-80	Triglicéridos	Apo B-100, Apo E, Apo C
IDL	1,006-1,019	25-35	Triglicéridos Colesterol	Apo B-100, Apo E, Apo C
LDL	1,019-1,063	18-25	Colesterol	Apo B-100
HDL	1,063-1,210	5-12	Colesterol	Apo A-I, Apo A-II, Apo C, Apo E
Lp(a)	1,055-1,085	≈30	Colesterol	Apo B-100, Apo (a)

Adaptado de Feingold KR ((10))

Tabla 2. Clasificación de las apolipoproteínas

Apo	MW(Da)	Fuente principal	Lipoproteína asociada	Función
Apo A-I	28.000	Hígado, Intestino	Proteína más abundante de HDL, VLDL y QM	Estructural en HDL, Activador de LCAT
Apo A-II	17.400	Hígado, Intestino	(20%) de proteína de HDL, tras la apo-AI. QM, VLDL	Estructura de HDL, TG y el metabolismo de ácidos grasos
Apo A-IV	46.000	Hígado, Intestino	QM, HDL, libre en plasma	Metabolismo de partículas ricas en TG. Interactúa con apo-CII en LPL. Activador de LCAT
Apo A-V	41.000	Hígado	QM, VLDL, HDL	Ensamblaje de QM y VLDL. Activador de LPL
Apo B-48	264.000	Intestino	QM, remanentes de QM	Componente estructural de QM y remanentes de QM
Apo B-100	550.000	Hígado	VLDL, IDL, LDL	Componente estructural de VLDL, IDL y LDL. Ligando del receptor LDL.
Apo C-I	5.700	Hígado, Intestino	QM, VLDL, HDL	Activador de LCAT, inhibidor de LPL y CETP. Inhibe la apo E uniéndose a LRP.
Apo C-II	8.900	Hígado, Intestino	QM, VLDL, HDL	Activador de LPL: su deficiencia llevará al aumento de la HTG
Apo C-III	8.800	Hígado, Intestino	Superficie de partículas ricas en TG: QM y VLDL	Inhibidor de LPL. Desplaza la apo E de LRP.
Apo E	34.500	Hígado, Intestino, Cerebro, otros	QM, VLDL, remanentes de HDL	Proteína multifunción. Ligando del LDLr y remanentes de QM. Ligando de LRP. Modula LPL, CETP, LCAT, LH. Molécula antioxidante. Regulador de la respuesta inflamatoria
Apo(a)	187.700-80000	Hígado	Lp(a)	-

Adaptado de Dominiczaky y cols ((6)*). Abreviaturas no descritas anteriormente y que aplican en la tabla: aminoácidos (AA), Kilo Daltons (KDa), proteína transferidora de esteres de colesterol (CETP), lecitin 7 Metabolismo lipídico colesterol acil transferasa (LCAT), proteína relacionada con el LDLr (LRP), hipertrigliceridemia (HTG), Lipasa hepática (LH), lipoprotein lipasa (LPL), lipoproteína (a) (Lp(a)), receptor LDL (LDLr).*

1.2- Vía exógena del metabolismo de las lipoproteínas

La vía exógena del metabolismo de las lipoproteínas conlleva la absorción y transporte de los lípidos de la dieta, >95% son TG y el resto se trata de fosfolípidos (2-4g/día), vitaminas liposolubles, y colesterol (Figura 2) (11).

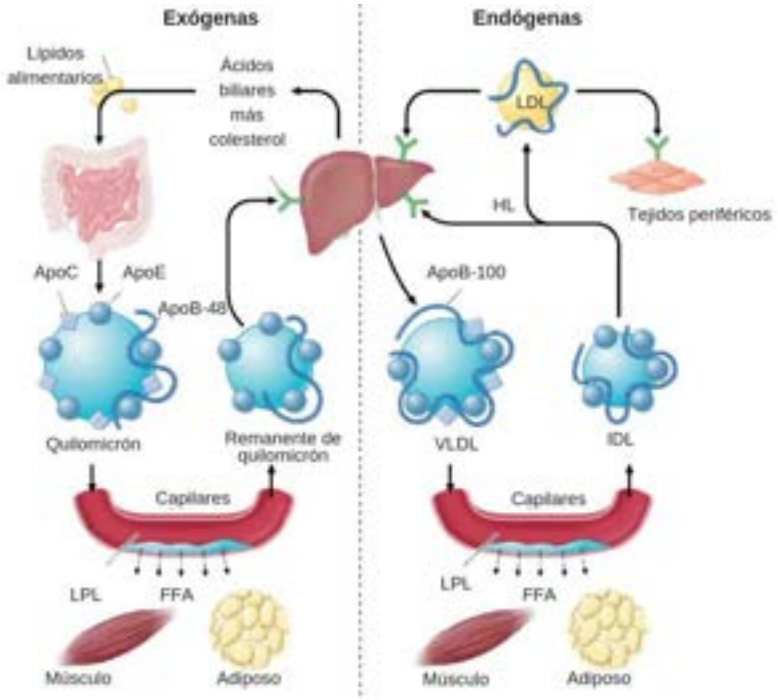
La digestión de los lípidos comienza por vía oral, donde se activan las lipasas linguales secretadas por las glándulas salivares (glándulas de Von Ebner). Las lipasas linguales hidrolizan las uniones ésteres en los TG con ácidos grasos de cadena media o corta, en la posición 3 dando 2-monoacilglicéridos (12).

En el estómago tiene lugar la emulsificación de las grasas, como resultado de las contracciones peristálticas del píloro, impulsadas por el quimo gástrico. La mucosa gástrica segrega lipasa gástrica, que se degrada rápidamente, y que produce como productos resultantes monoglicéridos y ácidos grasos de cadena larga. Son vertidos al intestino delgado donde ocurre la digestión fundamental de las grasas de forma mayoritaria gracias a las lipasas de origen pancreático (4,12–15). En la luz intestinal, los TG son hidrolizados a ácidos grasos y 2-monoglicéridos por la lipasa pancreática y emulsionados con ácidos biliares, colesterol, esteroles vegetales y vitaminas liposolubles para formar micelas y facilitar la absorción en el enterocito (por difusión o por transporte específico, como por el CD36).

La absorción de colesterol en el intestino delgado proximal representa la principal vía de entrada del colesterol a nuestro organismo. El colesterol se transporta a las células intestinales por un transportador de membrana, la proteína tipo 1 de Niemann-Pick C1-*like* (NPC1L1) (16). La importancia de *scavenger receptor class B type I* (SR-BI) todavía es cuestionable, pero se ha sugerido que desempeña un papel importante como sensor lipídico (17). Una vez en la célula intestinal, tiene lugar la esterificación del colesterol fundamentalmente mediante la acción de la enzima acil- coenzima A-colesterol-aciltransferasa (ACAT) y la enzima colesterol esterasa (con un papel menos importante) (14). En el caso de los esteroles vegetales, la formación de ésteres, ocurre con menor eficiencia puesto que son sustratos

pobres para la ACAT (18). El colesterol no esterificado, se transporta fuera de la célula intestinal, mediante la proteína dimérica ATP *binding casette* (ABCG5 y ABCG8) (3).

Figura 2. Papel de las lipoproteínas en el transporte de lípidos exógenos y endógenos.



Adaptado de Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J. 2018. (19) *LDLr: Receptor lipoproteína de baja densidad; LDL: Lipoproteína de baja densidad, FFA: Ácidos grasos libres; LPL: Lipoproteína lipasa; HL: Lipasa hepática; IDL: Lipoproteína de densidad intermedia; VLDL: Lipoproteína de muy baja densidad*.

- Formación de Quilomicrones.

Los TG hidrolizados en la luz intestinal, absorbidos como ácidos grasos y reesterificados en forma de TG, constituyen los QM. Su función es transportar los lípidos (procedentes de la ingesta) fundamentalmente al tejido muscular y tejido adiposo. Su tamaño depende de la cantidad y tipo de grasa ingerida y absorbida. Son las partículas lipoprotéicas más grandes, pero menos densas (d<1,000 g/mL). Contienen apos como: Apo B-48, Apo A-I, Apo A-IV, Apo C-II, Apo C-III, Apo C-IV y Apo E (20).

Para la formación de QM en el retículo endoplasmático de los enterocitos se necesita de la síntesis de proteína Apo B-48 (en los seres humanos, está codificada por el mismo gen que la Apo B-100 pero con secuencia truncada, ya que solo consta de un 48% de la parte N-terminal de ésta última. Ambas son codificadas por el gen *APOB*(21), y de la Proteína microsomal de transferencia de TG (MTP), que se encarga de transferir lípidos a la Apo B-48 (22–24).

- *Metabolismo de Quilomicrones.*

La mayor parte de los lípidos absorbidos por el intestino se secretan en forma de QM al sistema linfático para finalizar en el torrente circulatorio y aclararse en breve período de tiempo (5-10 min) (25). Al no enviarse directamente a la circulación portal, se facilita su transporte a los tejidos muscular y adiposo, donde se expresa en gran cantidad la lipoprotein lipasa (LPL). Las Apo A-I y Apo A-IV se pierden y la LPL, activada por el cofactor Apo C II, conlleva la hidrólisis de los QM a remanentes, liberando ácidos grasos libres (26). Parte de estos ácidos grasos son captados por los adipocitos y células musculares por las proteínas transportadoras de ácidos grasos (FATP) y CD36. Otros ácidos grasos mediante la albúmina, se conducirán a otros tejidos.

Los QM reducen su tamaño (QM residuales) se enriquecen proporcionalmente en colesterol, y al ser reconocida la Apo E que transportan, son captados por receptores hepáticos, donde son internalizados mediante la *proteína relacionada con el receptor LDL* (LRP) uniéndose a la Apo E, donde finaliza su etapa metabólica y de este modo el colesterol de la dieta llega al hígado.

1.3- Vía endógena del metabolismo de las lipoproteínas

La vía endógena se refiere a la secreción hepática de los TG y colesterol (en menor cantidad). Los ácidos grasos que no son oxidados, junto con los ésteres de colesterol constituyen las VLDL, siendo necesario para ello la síntesis de Apo B-100, Apo E, Apo C-I, Apo C-II, y Apo C-III (20,26). (Figura 2) (Figura 3)

- Metabolismo de VLDL.

Las VLDL son lipoproteínas grandes y de muy baja densidad (d <1,006 g/mL), ricas en TG. Se sintetizan exclusivamente en el hígado, en el retículo endoplasmático rugoso de los hepatocitos (27). La Apo B-100 es su proteína mayoritaria, proteína estructural para la secreción de VLDL y de las lipoproteínas que se van formando durante su catabolismo: IDL, LDL y la lipoproteína(a) (Lp(a)) (20). Los constituyentes de las VLDL son transportados al aparato de Golgi, se fusionan con Apo B -100, se glicosilan y forman vesículas que contienen las partículas nacientes de VLDL(26,28). La principal función de las VLDL es la de transportar TG y ácidos grasos a los tejidos muscular y hepático (análogamente a los QM) (20).

Las VLDL que provienen del hígado, en la circulación intercambian con las HDL: Apo C-I, Apo C-II (activador de la LPL), Apo C-III (inhibidor de la LPL) y Apo E (que modula la unión de las VLDL con receptores en la superficie celular del hepatocito) (29).

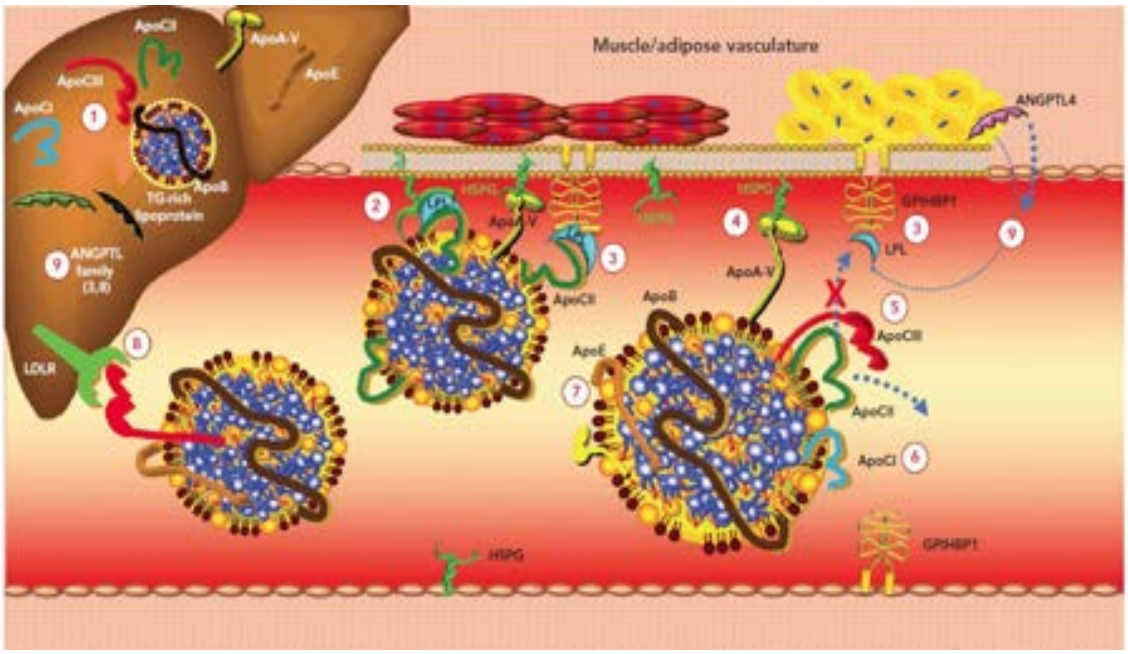
Los TG de las VLDL son hidrolizados en el tejido graso por la LPL y la partícula resultante se denomina VLDL remanente (o IDL)(30). Aproximadamente, la mitad de las VLDL remanentes son aclaradas por el receptor de las LDL (LDLr-mediated) por el hígado y otros tejidos y el resto entra en la llamada cascada lipolítica de las lipoproteínas VLDL - IDL - LDL en el plasma sanguíneo (31).

- Metabolismo de IDL.

La formación de partículas de IDL se obtiene de la deslipidación de VLDL mediante la LPL. Son partículas que contienen ésteres de colesterol y captan Apo E a partir de partículas de HDL. Se consideran de densidad intermedia (d<1,019 g/mL>1,006 g/mL). Son de menor tamaño y más densas que las VLDL.

Las IDL pueden eliminarse de la circulación por los hepatocitos (unión formada por la Apo E a los receptores de LDL y LRP), aunque se trata aproximadamente de solo un 50%. El resto de TG de las IDL son hidrolizados por la LPL, disminuyendo su tamaño y formando las partículas LDL, ricas en ésteres de colesterol y con una única molécula de a Apo B -100 (10,20).

Figura 3. Regulación del metabolismo de los triglicéridos



Adaptado de Sathiyakumar et al (32)*: (1) ApoC-III estimula la producción de lipoproteínas ricas en TG hepáticos. (2) ApoC-II Es un cofactor y activador esencial de LPL que está anclado a HSPG y GPIHBP1 (3) GPIHBP1 captura LPL en el espacio intersticial y lo transporta a la superficie luminal (4) ApoA-V se une a proteoglicanos de heparán sulfato y activa LPL. (5) ApoC-III inhibe la lipolisis mediada por LPL. (6) ApoC-I inhibe la actividad de LPL. (7) ApoE2 puede interferir con LPL. (8) ApoC-III inhibe la depuración hepática mediada por receptores de lipoproteínas ricas en TG. (9) Las proteínas similares a la angiopoyetina (ANGPTL) 3, 4 y 8 son miembros de una familia de proteínas que inhiben la LPL. Abreviaturas: ANGPTL: Angiopoietin-like proteins; Apo CII, apoproteína CII; Apo CIII, Apoproteína CIII; Apo E2: apoproteína E2; GPIHBP1: proteína 1 de unión a HDL anclada a glicosilfosfatidilinositol; HSPG, proteoglicano de heparán sulfato; LPL: lipoprotein lipasa; TG: triglicéridos.*

- Metabolismo de LDL.

Las partículas LDL son de baja densidad (d<1,063 g/mL > 1,019 g/mL), se caracterizan por transportar la mayor parte de colesterol en la circulación, hacia los tejidos periféricos y el hígado y su concentración en sangre está determinada por las tasas relativas de producción y eliminación, ambas reguladas principalmente por receptores de LDL en el hígado. Del total de las partículas un 30% se catabolizan en los tejidos periféricos y un 70% son transferidas al hígado (20,33).

- Receptor LDL.

El LDLr fue descrito por Goldstein y Brown en 1973 (34). Pertenece al grupo de glicoproteínas de membrana, llamadas receptores de lipoproteínas. Las lipoproteínas que transportan colesterol son captadas mediante dichos receptores por endocitosis por todos los tejidos. Los LDLr se encuentran en elevada concentración en el hígado, en la corteza suprarrenal y cuerpo lúteo ovárico(35).

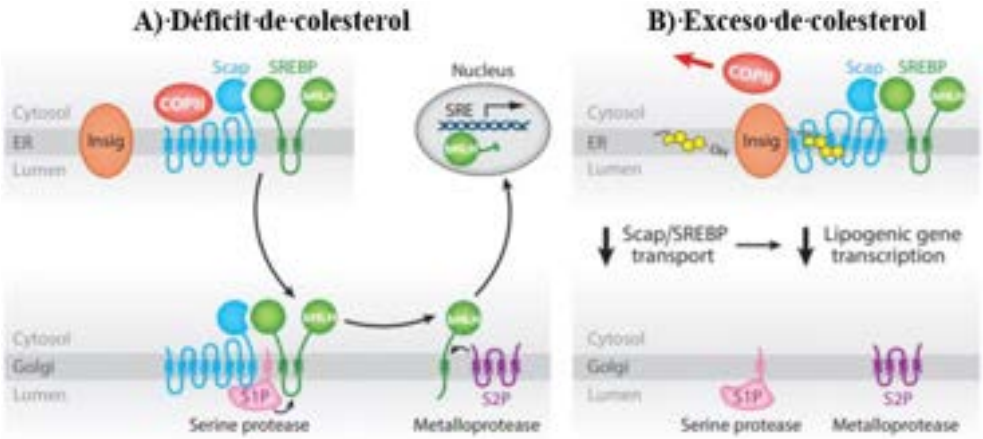
El gen que codifica el LDLr se denomina *LDLR* y se encuentra en el cromosoma 19p13.1-p13.3 (36). Contiene 18 exones y codifican para una glicoproteína transmembrana de una cadena de 839 aminoácidos (35). Se distinguen cinco dominios en la proteína LDLr: dominio citosólico, dominio transmembrana, dominio con sitios de O-glicosilación, dominio con homología al *Epidermal Growth Factor* (EGF) y dominio de unión a LDL (Apo B-100 o Apo E).

- Regulación intracelular de colesterol y LDLr

La regulación de los LDLrs en el hígado viene establecida en función del contenido de colesterol en el hepatocito. Cuando los niveles de colesterol intracelular disminuyen, las proteínas de unión a elementos reguladores de esteroles inactivos (SREBP) acompañadas de SCAP (proteína reguladora del metabolismo del colesterol, agente central del sistema de retroalimentación SREBP) se transportan en vesículas (COPII, *coat protein*) desde el retículo endoplasmático al complejo de Golgi para activarse. Se mueven al núcleo y estimulan la transcripción del *LDLR* y otros genes, incluida la 3-hidroxi-3-metil-glutaril coenzima A reductasa (HMG-CoAR) para la síntesis de colesterol (37).

Si hay aumento de los niveles de colesterol intracelular los SREBPs quedan inactivos (10,38) y la actividad de HMG-CoAR es disminuida mediante INSIG1 e INSIG2, que se unen a HMG-CoAR con ubiquitina ligasas para degradarse por distintas vías o se inactiva mediante fosforilación (39) (Figura 4).

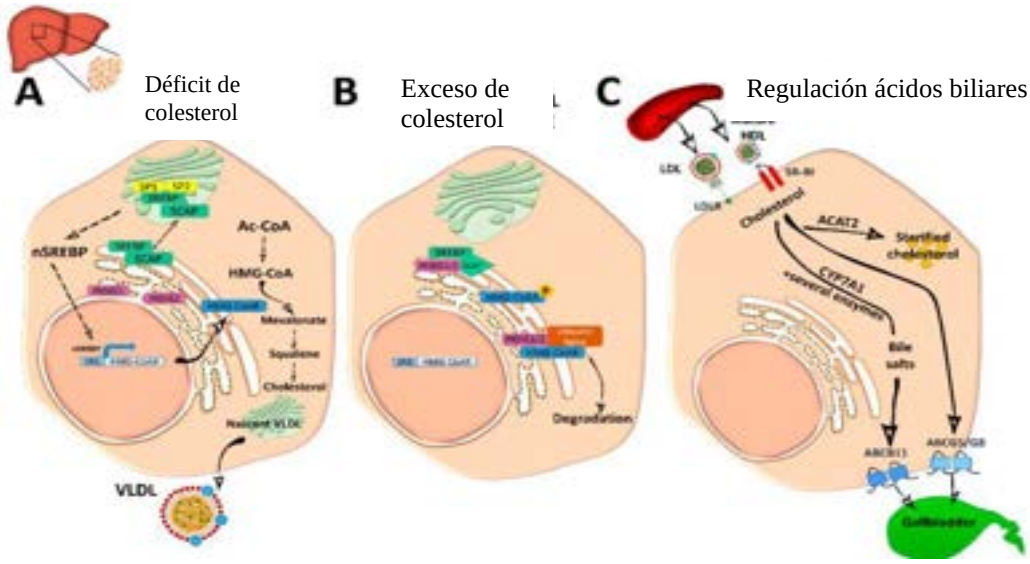
Figura 4. Vía SREBP en el metabolismo del colesterol



Adaptada de Brown, Michael S.et al(38). *Abreviaturas: bHLH, basic-helix-loop-helix zipper; COPII, Complejo de las proteínas de la cubierta II; ER:retículo endoplasmático; S1P, site-1 proteasa; S2P, site-2 proteasa; SER: elemento regulador de esterol; SREBP: Receptor scavenger clase B tipo 1*

El LDLr permite el acceso del colesterol al interior del hepatocito. También lo hace el transporte reverso de colesterol mediante SRB1. Parte de colesterol se esterifica por la *acetil coenzima A acetiltranferasa* (ACAT2) y parte forma ácidos biliares. Finalmente, el exceso de colesterol se libera a la bilis mediante ABCG5/ABCG8 (37) (Figura 5).

Figura 5. Regulación de colesterol y LDLr



Adaptada de Aguilar-Ballester M et al.(37) *Abreviaturas: SREBP: Receptor scavenger clase B tipo 1; VLDL: Lipoproteína de muy baja densidad*; *HMG-CoAR: 3-hidroxi-3-metil-glutaril coenzima A reductasa*; *acetyl coenzya A acetyltranferasa* (ACAT2)

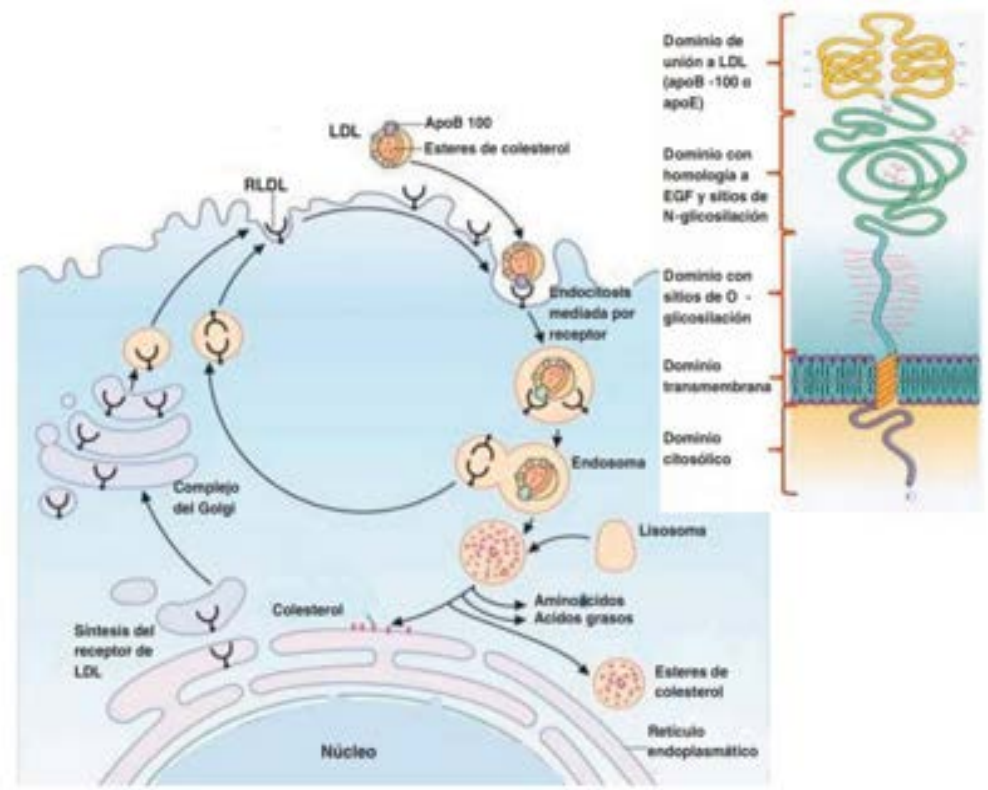
Por otro lado, *el Liver X receptor* (LXR) es también un importante regulador transcripcional de colesterol. Un exceso de colesterol en el interior de la célula y de oxiesteroles conlleva su activación, provocando la internalización de LDLr para su degradación lisosomal (40). Los transportadores de ATP *binding cassette* (ABCA1 y ABCG1) se transcriben, provocando la salida de colesterol (41).

Otro regulador de la disponibilidad de LDLr es la proproteína convertasa subtilisina/ kexina tipo 9 (PCSK9), que se encarga de conducir a LDLr al lisosoma para su degradación, de esta manera disminuye LDLr en la superficie celular y por tanto la endocitosis de LDL (42).

- Mecanismo de la endocitosis de LDL

Las partículas de LDL se unen al LDLr en la superficie de la célula, mediante un proceso que es dependiente de una secuencia NPxY (FDNPVY) en la cola citoplasmática de LDLr y que da lugar a la internalización de dichas partículas en forma de vesículas a través de hoyos recubiertos de clatrina (43). Una vez fusionadas en el interior de éstas, la disminución del pH en los endosomas facilita que la partícula LDL se separe de su receptor (disocia Apo B-100 y LDLr) Una vez disociados, el LDLr se recicla de nuevo en la superficie celular o bien se dirige a los lisosomas que conlleva un proceso de degradación. En los casos que se mantiene el LDL unido al receptor, se hidrolizan en aminoácidos y colesterol no esterificado. Dicho colesterol tendrá varias funciones: participar en la síntesis de membranas o de hormonas esteroideas, o bien quedar almacenado como reservorio celular de colesterol, en forma de ésteres de colesterol mediante la ACAT (41,44) (Figura 6).

Figura 6. Ciclo celular del receptor de LDL. LDLr: receptor de LDL



Teresa L.Errico et al(20). *Abreviaturas:* *LDL: Lipoproteína de baja densidad*; *LDLR: receptor de LDL.*

1.4 Metabolismo de HDL y transporte reverso de colesterol

El transporte reverso de colesterol (TRC) se define como el proceso mediante el cual el colesterol es transportado por las partículas HDL en la circulación desde los tejidos periféricos al hígado y es excretado por la bilis. El TRC comienza con la salida del exceso de colesterol de los tejidos periféricos, como por ejemplo de las células espumosas que se encuentran en el músculo liso o en los macrófagos. De esta manera se impide la acumulación periférica de colesterol y es una de las propiedades antiaterogénicas de la que se caracterizan las HDL (45).

Las partículas HDL son las lipoproteínas de menor tamaño y mayor densidad (d< 1,21 g/mL > 1,063 g/mL) están compuestas de 20-30% de colesterol (50% lípidos y 50% proteínas). Además de estar involucradas en el TRC, están involucradas en los procesos antiinflamatorios, antitrombóticos y de inhibición oxidativa de las LDL. Para la formación de partículas HDL son necesarios varios pasos (6,20).

El primer paso es la síntesis de la proteína Apo A-1, formada en hígado y en el intestino, que facilita que el colesterol y los fosfolípidos intracelulares se transfieran de los hepatocitos y enterocitos a las HDL nacientes, mediante la ABCA1 y ABCG1 (del casette de unión ATP). Dicho proceso es conocido como "eflujo" de colesterol. La Apo A-1 se transforma en partículas HDL nacientes o pre-β-HDL ("pre-β" hace referencia a su movilidad electroforética) unas partículas pobres en lípidos y proporcionalmente ricas en proteínas de estructura discoidal (45,46).

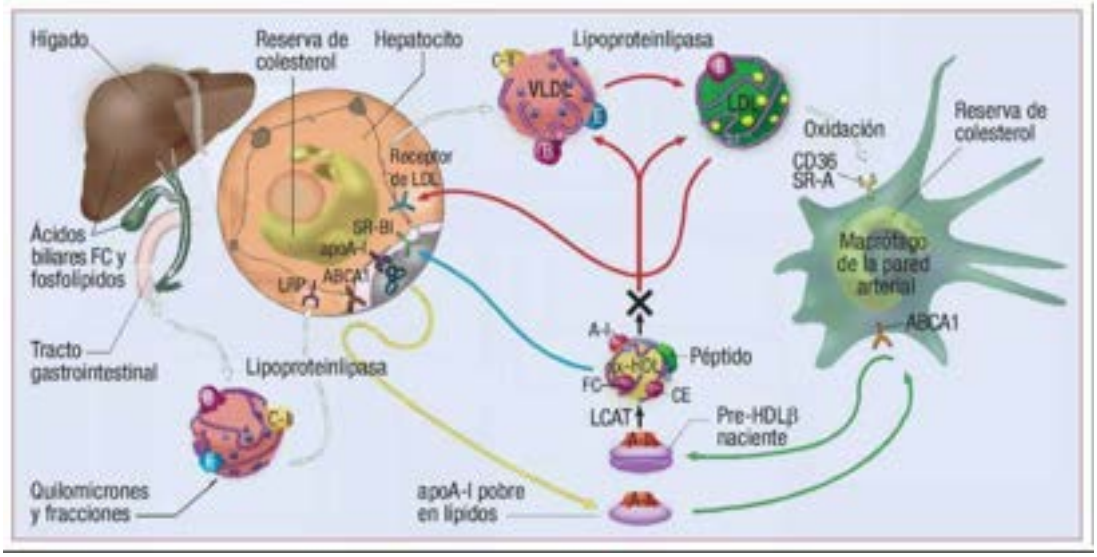
El colesterol de las HDL nacientes es esterificado mediante la enzima lecitin colesterol acil transferasa (LCAT, una enzima asociada a HDL) dando como resultado ésteres de colesterol, proceso que consiste en transferir un ácido graso de los fosfolípidos al colesterol libre, y se forman las HDL maduras α-HDL (con movilidad electroforética α). De esta manera el colesterol libre en la superficie de la partícula de HDL una vez esterificado puede dirigirse al núcleo de la partícula y aumentar progresivamente de tamaño. Conforme su aumento se van transformando desde α-HDL_3 a α-HDL_2 (45,47). En menor medida, tiene lugar un "eflujo" de colesterol de células periféricas a través de la proteína de superficie SR-B1. A nivel hepático la interacción entre SR-B1 y las HDL libera el contenido en ésteres de colesterol desde las HDL a los hepatocitos (6,48). De esta manera el exceso de colesterol puede ser eliminado por la bilis (5).

La composición de las partículas HDL también depende del catabolismo de las partículas ricas en TG (QM y VLDL) en el cual sucede una transferencia no específica de ésteres de colesterol entre diversos tipos de lipoproteínas y que es promovido por la proteína transferidora de ésteres de colesterol (CETP). Esta glicoproteína hidrofóbica se produce en el hígado y en el tejido adiposo y circula unida a las HDL. Se encarga de equilibrar la cantidad de los lípidos hidrofóbicos (ésteres de colesterol y TG) entre las partículas que contienen Apo B y las HDL (49). La lipasa hepática (LH) hidroliza los TG de las HDL recibidos por la

acción de la CETP y de los fosfolípidos, liberando Apo A-1 y, por tanto, disminuyendo su tamaño. Estas HDL podrán ser captadas por el hígado para transferir su colesterol o circular de nuevo hacia los tejidos extrahepáticos (50).

Por ello, estos procesos de lipólisis y transferencia parecen ser claves para que las partículas de HDL, puedan continuar con nuevos ciclos de transporte reverso de colesterol (Figura 7).

Figura 7. Representación del metabolismo de HDL y transporte reverso de colesterol

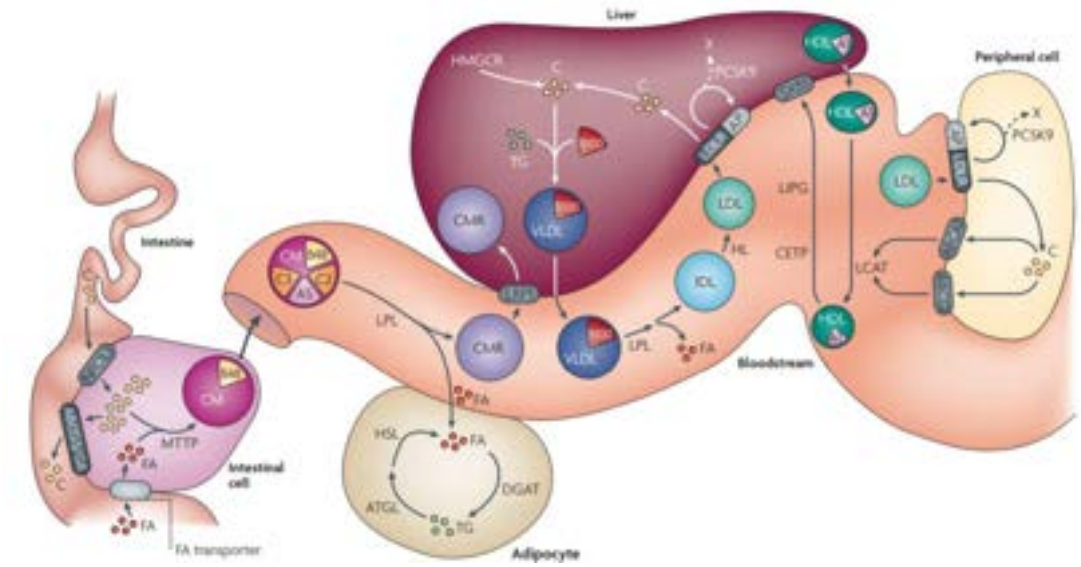


Adaptado de Brewer et al(51) *LDL: Lipoproteína de baja densidad*; *LDLR: receptor de LDL. SREBI: Receptor scavenger clase B tipo; HDL: Lipoproteína de alta densidad; LCAT: Lecitin colesterol acil transferasa.*

1.5 Metabolismo integrado de las lipoproteínas

Una visión global del metabolismo lipoproteico nos muestra la alta conexión entre las distintas lipoproteínas. Por un lado, como se comportan los QM y VLDL en relación a la síntesis de HDL. Por otro lado, la asociación entre las vías metabólicas y funcionales, ya comentadas anteriormente, y que son controladas por factores de transcripción que se activan mediante ligandos, como los receptores nucleares que regulan los genes del metabolismo (PPAR alfa, PPAR delta, PPAR gamma y LXR). Por último, la intervención de lipasas o proteínas como la CETP (20). (Figura 8).

Figura 8. Representación global del metabolismo de las lipoproteínas**.**



Hegele y cols(5). Abreviaturas: FA:ácido graso; HLS: Lipasa sensible a hormona proteína adaptadora (AP); CT: colesterol; ATGL: lipasa adiposa de TG; SRB: receptor scavenger clase B tipo 1.

1.6 Lipoproteína(a)

La lipoproteína(a) (Lp(a)) fue descrita por primera vez por Berg en 1963. Se trata de una partícula similar a la de LDL, sintetizada por el hígado, en la que la ApoB-100 establece una unión covalente por un enlace de disulfuro a una apoliproteína(a) (Apo(a))(52). Su conjunto consiste en un complejo molecular esférico con una densidad entre 1,05 y 1,12 g/mL)(52–54). La Apo(a) ha evolucionado del gen del plasminógeno mediante duplicación y remodelación, aunque su significado se desconoce. El plasminógeno (enzima fibrinolítica) tiene 5 Kringles (KI a KV) y un dominio de proteasa. La Apo(a), sin embargo, contiene 10 subtipos de KIV, 9 de ellos (KIV_1 y $KIV_{3–10}$) presentes en 1 sola copia, y el KIV_2 presente entre 1 a >40 copias, 1 copia de KV, y un dominio inactivo proteasa. El gen de la apo(a),

LPA, parece ser responsable de más del 90% de la variación de la cuantía circulante de la lipoprotein(a) (Lp(a)) en la población (52).

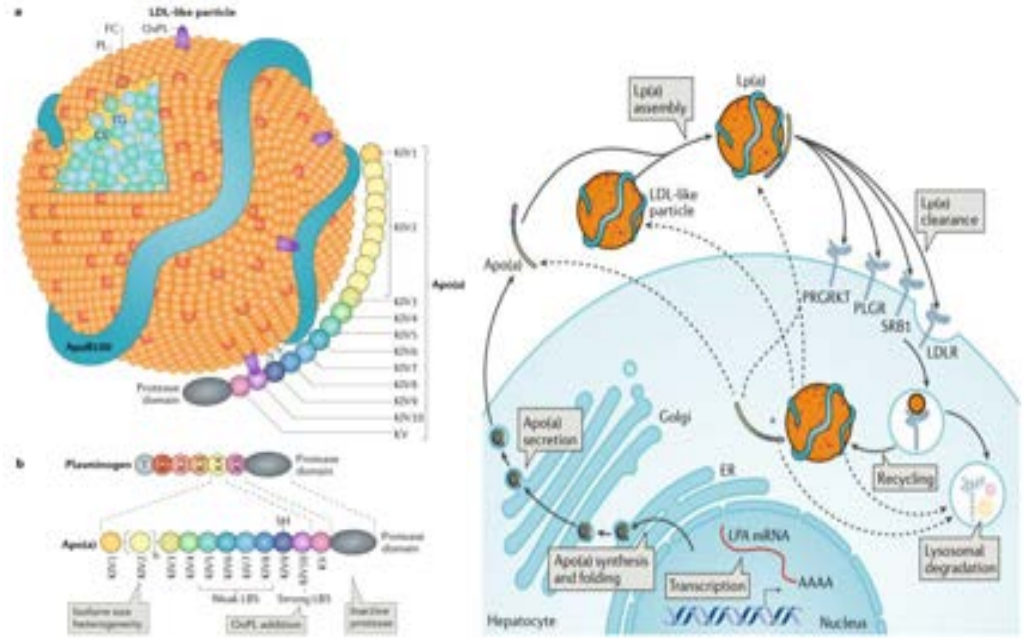
La Apo(a) es sintetizada en el hígado (hepatocitos), pero el lugar donde se ensambla a la Lp(a) todavía es desconocido. A pesar de la gran similitud con el LDL, son partículas metabólicamente distintas. Aunque varios estudios han identificado al LDLr como un elemento clave en el catabolismo de la Lp(a), no está claro si participa en su catabolismo(55).

La Lp(a) contiene Apo B que es un ligando de los LDLr, lo que sugeriría que tiene la capacidad de unirse a ellos. En estudios en los que se compara con LDL, se observa que la afinidad de los LDLr para la Lp(a) es menor que para LDL *in vitro*, pero esto es más dudoso en estudios *in vivo* como los realizados en la Hipercolesterolemia Familiar (HF) (54).

Se han observado variaciones en la concentración de Lp(a) entre poblaciones, en parte dependiente de la raza y sexo, y se sabe que la concentración de Lp(a) es de carácter hereditario, y no modificable en el tiempo, pero ligeramente modulada por la edad, la dieta y el ejercicio (56).

Estudios epidemiológicos han asociado la enfermedad coronaria con una elevada concentración de Lp(a). Igualmente, la Apo(a) se ha asociado a un efecto aterogénico (57). En humanos, los tratamientos farmacológicos hipolipemiantes, han demostrado no tener importancia en la concentración de Lp(a), incluyendo los anticuerpos monoclonales anti-PCSK9. En pacientes con HF, una población de muy alto riesgo cardiovascular (CV), los niveles de Lp(a) han sido independientemente asociados con el incremento de dicho riesgo. Pero todavía no hay estudios que confirmen el efecto de la reducción de Lp(a) en la enfermedad coronaria (58) (Figura 9).

Figura 9. Estructura de la Lp(a)



Adaptado de Boffa M.B and Koschincky. M (59).Estructura y regulación de la lipoproteína (a)

Capítulo 2

Clasificación de los trastornos del metabolismo lipídico

2. Dislipoproteinemia

El concepto de dislipoproteinemia se define como aquella alteración en las concentraciones plasmáticas o en la composición de las lipoproteínas. Cuando las alteraciones elevan las cifras de dichas partículas, se denomina "hiperlipoproteinemia", si al contrario es por una disminución o defecto, se nombra como "hipolipoproteinemia". Dichas alteraciones son muy relevantes clínicamente puesto que se asocian con el desarrollo de arteriosclerosis. En las fases iniciales de las hiperlipoproteinemias, la enfermedad es asintomática, sin embargo, en fases avanzadas se relaciona con una acumulación de colesterol en forma de placa de ateroma conocido como arteriosclerosis con un incremento del riesgo CV (60,61).

2.1. Clasificación de las dislipemias

Desde que Fredrickson clasificó en 1970, las hiperlipoproteinemias en función del fenotipo lipídico se ha utilizado dicha clasificación durante años (ver Tabla 3).

La clasificación de las dislipemias (alteraciones cuantitativas o cualitativas de colesterol y TG en sangre) según el fenotipo, se dividen en:

- *Hipercolesterolemia aislada*: con aumento de la concentración de colesterol,
- *Hipertrigliceridemia aislada*: con aumento de la concentración de TG,
- *Dislipemias mixtas*: manifestadas con aumento de concentraciones en colesterol y TG.
- *Hipolipidemias*: disminución de concentración en el plasma de los lípidos, generalmente conocidas como hipocolesterolemia, cuando existe una disminución de colesterol o

hipoalfalipoproteinemia, cuando se debe a una disminución de la concentración del colesterol HDL (cHDL) (60,62).

Actualmente, se utiliza desde un punto de vista etiológico, una clasificación más práctica, donde se incluyen factores ambientales, fármacos o distintas patologías. La clasificación consiste en denominarlas según su origen, sean por causas genéticas o primarias, o bien por causas secundarias (ver tabla 3).

Tabla 3. Clasificación fenotípica de las hiperlipoproteinemias según Fredrickson (Organización Mundial de la Salud, 1970)

Fenotipo	**Triglicéridos**	**Colesterol total**	**Lipoproteínas aumentadas**	**Aterogénesis**
I	↑ ↑ ↑ ↑	Normal o ↑	Quilomicrones	Ninguna observada
IIa	Normal	↑ ↑ ↑	LDL	+++
IIb	↑	↑ ↑ ↑	VLDL	+++
III	↑ ↑	↑ ↑	β-VLDL (↑ IDL)	+++
IV	↑ ↑ ↑	Normal o ↑	VLDL	++
V	↑ ↑ ↑ ↑	↑	Quilomicrones VLDL	+

Adaptado de J L Beaumont et al(60). *LDL: Lipoproteína de baja densidad; IDL: Lipoproteína de densidad intermedia; V*

: Lipoproteína de muy baja densidad.

2.2 Dislipemias familiares

La expresión más grave o extrema de hipercolesterolemia, es aquella determinada en mayor medida por los factores genéticos. La HF es la más característica y está mayormente asociada a la enfermedad CV. (Ver tabla 4).

2.3 Hipercolesterolemias primarias

Las hipercolesterolemias primarias son trastornos del metabolismo lipídico que se caracterizan por aumento en las concentraciones plasmáticas de colesterol, habitualmente de origen genético sin existir una causa secundaria productora. Afectan por aumento de las LDL, HDL o Lp(a).

Tabla 4. Clasificación de las dislipemias

Dislipidemias que afectan al colesterol	**Primarias:** • Aumentan el cLDL: – Hipercolesterolemias monogénicas dominantes (heterocigota/homocigota): - Por mutaciones en el gen *LDLR* - apo B100 defectuosa familiar - Por mutaciones en el gen *PCSK9* – Hipercolesterolemia autosómica recesiva – Hiperlipoproteinemia(a) • Disminuyen el cLDL: – Abetalipoproteinemia – Hipobetalipoproteinemia • Aumentan el cHDL: – Deficiencia de CETP – Disfunción/déficit de apolipoproteína CIII • Disminuyen el cHDL: – Analfalipoproteinemia – Hipoalfalipoproteinemia familiar – Deficiencia familiar de LCAT • Otras formas primarias de hipercolesterolemia: – Sitosterolemia – Deficiencia de colesterol 7-alfa-hidroxilasa
	Secundarias: • Dietéticas • Por fármacos • Por comorbilidades
Hipertrigliceridemias graves	**Primarias:** • Deficiencia de LPL • Deficiencia de apo C2 • Inhibidor familiar de la LPL • Deficiencia de apo A5 • Deficiencia de LMF1 • Deficiencia de GPIHBP1 • Hipertrigliceridemias multigénicas
	Secundarias: • Factores exógenos
Hipertrigliceridemias moderadas	**Primarias:** • Hipertrigliceridemia familiar • Hiperlipidemia familiar combinada • Disbetalipoproteinemia
	Secundarias: • Dislipidemia aterogénica (diabetes mellitus, síndrome metabólico, enfermedad renal crónica, síndrome del ovario poliquístico, obesidad) • Factores exógenos
Dislipidemias mixtas	**Primarias:** • Hiperlipidemia mixta esporádica • Hiperlipidemia familiar combinada • Disbetalipoproteinemia • Deficiencia de lipasa hepática • Deficiencia de lipasa ácida lisosomal
	Secundarias: • Dietéticas • Por fármacos • Por comorbilidades

LCAT: lecitina-colesterol-acil-transferasa; LPL: lipoproteinlipasa.

Alicia Taboada Duro et al (63)

2.1 Hipercolesterolemias monogénicas que aumentan el colesterol LDL

2.1.1- Hipercolesterolemia Familiar Heterocigótica

La Hipercolesterolemia Familiar Heterocigota (HFHe) es un trastorno autosómico codominante del metabolismo de las lipoproteínas, que se caracteriza por unas concentraciones muy elevadas en el plasma de cLDL. La HFHe es causada por mutaciones en los genes que codifican para *LDLR, APOB, PCSK9 o APOE* (64,65).

Las características clínicas que derivan de dicha enfermedad son:

- la presencia de xantomas tendinosos (casi patognomónicos de la HF)
- xantomas cutáneos
- xantelasmas: acumulación de depósitos de colesterol en la piel
- depósito de colesterol en la córnea dando el arco corneal (no patognomónico) (2,35).

La implicación clínica más destacable en estos pacientes es el desarrollo de la enfermedad coronaria precoz. Los sujetos con HFHe sin tratamiento, antes de los 55 años en el caso de los varones y 60 en las mujeres sufrirán un evento CV, un riesgo que está aumentado hasta 10 veces, en el caso de pacientes con HFHe definida o probable.

La frecuencia de la enfermedad se ha estimado entre 1/200 y 1/250 (14 y 34 millones de personas en el mundo), incluso recientes estudios indican que en algunas poblaciones aumentaría hasta 1/137 (66,67).

2.1.2 Hipercolesterolemia Familiar autosómica recesiva

La hipercolesterolemia autosómica recesiva es una enfermedad muy poco frecuente <1:1.000.000, representa menos del 1% de las HF. El gen *LDLRAP1*, en el cromosoma 1 (1p36.11) codifica la proteína adaptadora de LDLr (LDLRAP1), que está involucrada en la unión de Apo B-100-LDLr de manera que facilita su endocitosis, y por tanto su defecto causa el aumento de cLDL. Se han descrito más de 10 mutaciones en este gen, con la característica de favorecer la enfermedad CV prematura presentando cifras similares a las de HF homocigótica (68).

2.1.3 Hipercolesterolemia Familiar Homocigota

La Hipercolesterolemia Familiar Homocigota (HFHo), es una enfermedad hereditaria que presenta habitualmente variantes patogénicas en los alelos del gen *LDLR.* Se produce por mutaciones en ambos alelos de este gen y conduce a una reducción del reciclaje y aclaramiento de cLDL. Puede deberse a *verdaderos homocigotos* (con misma mutación en ambos alelos de *LDLR*) *heterocigotos compuestos* (que presentan diferentes mutaciones en cada alelo de *LDLR*), o bien heterocigotos dobles (con dos variantes patogénicas en dos genes diferentes asociados a HF: *LDLR, APOB, PCSK9 y APOE*). Presentan una clínica similar a la HFHe, pero más grave, ya que la concentración de cLDL suele superar los 500 mg/dL y desarrollan una enfermedad precoz coronaria en las primeras décadas de la vida. Además se ha observado un desarrollo más avanzado y precoz de estenosis aórtica (69,70).

2.1.4 Hiperlipoproteínemia(a)

La hiperLp(a) se transmite con carácter codominante, y se han descrito hasta 30 isoformas de Apo(a). La concentración de Lp(a) es muy variable entre sujetos entre 0,1-300 mg/dL, derivada de las variaciones en el gen *LPA*, que codifica la Apo(a) (71).

Aproximadamente en un 25% de los sujetos con una hipercolesterolemia diagnosticada clínicamente de HFHe, ésta se asocia a un aumento de la Lp(a). En el caso de la HF, los sujetos presentan valores más elevados de Lp(a) que la población general, sin saber la causa que justificaría este aumento, parece ser que se trata de un mecanismo que influye tanto la síntesis como el catabolismo de la Lp(a) (72).

Se ha observado que las concentraciones Lp(a) superiores a 30 mg/dL se relacionan independientemente con un aumento de riesgo CV. La relación de tamaño de la molécula de Apo(a) y sus repeticiones de KIV-2, tiene que ver con el riesgo de enfermedad CV. A menor número de repeticiones, menor tamaño de la partícula, mayor concentración en sangre y mayor riesgo CV (73). Hasta la fecha no existe un tratamiento eficaz para conseguir reducir drásticamente la Lp(a), aunque los inhibidores de PCSK9 (iPCSK9) la disminuyen en torno a un 20-25% independientemente de la concentración de cLDL(74–76).

2.2. Hipercolesterolemias monogénicas que aumentan el cHDL

2.2.1 Hiperalfalipoproteínemia-déficit de CETP

Esta condición de hipercolesterolemia se diferencia por una elevada concentración de cHDL, mayor del percentil 90 en la población general. En algunos casos considerados como moderados, los niveles se concentran entre 80 mg/dL y 100 mg/dL y severo como >100 mg/dL. Las mutaciones que intervienen son producidas fundamentalmente por aquellos genes que codifican a la proteína transportadora de ésteres de colesterol (CETP), lipasa hepática (LIPC), apolipoproteína C-III (ApoC3) y receptor SRB1 (scarvenger receptor clase B tipo I) (77). Se ha observado que dificultan el transporte reverso de colesterol (TRC) y en algunos estudios epidemiológicos, a pesar de que pudiera parecer contradictorio, han asociado una relación directa entre concentraciones muy altas de cHDL y riesgo de desarrollar enfermedad CV aterosclerótica (78).

2.3. Otras formas primarias de hipercolesterolemia

2.3.1 Sitosterolemia

Se caracteriza por ser una enfermedad rara autosómica recesiva y que se presenta en edades iniciales de la vida. Se concentran altos niveles de esteroles vegetales o fitoesteroles en sangre (79). Es una enfermedad infradiagnosticada ya que no existe un cuadro clínico característico, con síntomas similares a la HF (xantomas, arco corneal, enfermedad coronaria precoz) (80). Las mutaciones responsables aparecen en el transportador ABCG5/ABCG8. En el mundo se han estudiado en torno a 100 casos, aunque se estima que la prevalencia podría llegar a ser 1:50.000 (81).

2.3.2 Deficiencia de colesterol -7-alfa-hidroxilasa.

Las mutaciones descritas en el gen *CYP7A1*, codifican a la enzima CYO7A1 (colesterol-7-α-hidroxilasa). Se trata de una enfermedad autosómica dominante, con prevalencia de <1:1.000.000. Dicha enzima está asociada al citocromo P450, encargado de regular la

circulación del colesterol. Los niveles inferiores de esta enzima, provocan un aumento de la concentración en plasma de cLDL, derivada de una menor síntesis de LDLr (82).

2.4. Hiperlipidemias mixtas primarias

2.4.1 Hiperlipidemia mixta esporádica

En dicho trastorno, de etiología desconocida, los valores de CT y TG aumentan, derivados del aumento de las partículas VLDL y LDL. De carácter poligénico, se ha asociado con diferentes variantes en múltiples genes. Aparece en edad adulta, y estaría favorecida su expresión por la obesidad abdominal, la resistencia a la insulina y síndrome metabólico, por tanto, con un elevado riesgo CV. Presentan valores de CT ≥250 mg/dL, cLDL ≥160 mg/dL y TG≥200 mg/dL, además un cHDL bajo y Apo B elevada (63).

2.4.2 Hiperlipemia familiar combinada

Es el trastorno hereditario más frecuente del metabolismo lipídico que afecta al 1-3% de la población. Tiene un carácter poligénico. La región cromosómica 1q21-1q23 se ha descrito como fuertemente asociada a este trastorno. En torno a 1 millón de personas en España podrían estar afectadas de esta enfermedad (83). Fue descrita por Goldstein et al, Hazzard et al., and Kwiterovich et al, simultáneamente, cuando se estudiaron pacientes con enfermedad CV (84). Cerca del 20-38% de los pacientes tienen antecedentes personales de infarto de miocardio (IM)(83). Son varios los genes cuyas mutaciones intervienen en su génesis. Se asocia a la cardiopatía isquémica familiar. Además, va relacionada con las enfermedades como diabetes mellitus (DM) y obesidad, de ahí la variedad en el fenotipo en individuos de la misma familia. Se diferencia del cuadro anterior porque en la hiperlipemia familiar combinada existe un marcado componente familiar con varios miembros afectos.

Dicha enfermedad, está caracterizada por fluctuaciones en el perfil lipídico, debido a un tejido adiposo disfuncional, que conlleva un aumento de los niveles de ácidos grasos libres. Se produce una sobreproducción de partículas VLDL y TG por retraso en el aclaramiento de éstos. También la Apo B se ve alterada, con mayor concentración >120 mg/dL en plasma, con predomino de partículas aterogénicas de LDL, y un cHDL disminuido(85).

Los criterios diagnósticos no están claros, para ello hay que valorar la presencia de dislipemia y/o los antecedentes familiares de enfermedad CV en los familiares de primer grado (<55 años en hombres y <60 en mujeres) y analizar si hubiera causas secundarias. La hipercolesterolemia y/o hipertrigliceridemia moderada están presentes en estos sujetos. No muestran signos de xantomas tendinosos, pero sí algunos casos muestran arco corneal y xantelasmas (Ver tabla 5).

Tabla 5. Criterios diagnósticos de Hiperlipemia familiar combinada

Persona afectada: • En adultos: CT >240 mg/dL (>6,2 mmol/L) o cLDL >160 mg/dL (>4,1 mmol/L) y/o TG >200 mg/dL (>2,3 mmol/L) • En menores de 20 años: CT >200 mg/dL (>5,2 mmol/L) o cLDL >130 mg/dL(>3,4 mmol/L) y/o TG >120 mg/dL (>1,4 mmol/L) • Descartar causas secundarias: índice de masa corporal >35 kg/m^2, hemoglobina glucosilada >10%, hipotiroidismo no controlado y etilismo (>40 g de alcohol/día)
Familia afectada: • Dos o más miembros de primer grado (padres, hermanos, hijos) afectados de hiperlipidemia mixta, o de combinaciones de los fenotipos hipercolesterolemia pura, hiperlipidemia mixta o hipertrigliceridemia • Se excluyen las familias con xantomas tendinosos y/o cifras de cLDL >300 mg/dL (>7,8 mmol/L) en dos o más familiares de primer grado con hipercolesterolemia

Red Temática de Investigación Cardiovascular en Hiperlipidemias Genéticas (Instituto de Salud Carlos III). cLDL: colesterol unido a lipoproteínas de baja densidad; CT: colesterol total; TG: triglicéridos.

2.4.3 Disbetalipoproteínemia

La disbetalipoproteínemia familiar o hiperlipoproteinemia tipo III, es una enfermedad grave que presenta mutaciones en el gen de *APOE*, hiperlipidemia mixta y un alto riesgo de enfermedad CV. Está caracterizada por presentar homocigosis para el alelo E2 en el gen Apo E. Hay otras variantes de *APOE* más raras como la *APOE* Arg136Ser, que parece frecuente en la población española y tiene un carácter codominante. La prevalencia de la disbetalipoproteinemia es alrededor de 0,1% (1:1000) (86).

La primera descripción de la enfermedad se relata en 1952, McGinley et al. describieron 14 pacientes con "xantomas tuberosos" (87). En 1967 Fredickson et al. la clasificaron como

hiperlipoproteinemia tipo III (88). Utermann et al., observaron que se trataba de una variante genética de Apo E (89).

La Apo E defectuosa no se une al LDLr y a la proteína relacionada con el receptor LDL (LRP), lo que provoca un incremento de VLDL y de lipoproteínas de densidad intermedia (IDL) en la sangre. Si además se acompañan de obesidad o diabetes, la manifestación de la enfermedad se potencia. Los niveles de CT y TG son entre 300-600 mg/dL (90).

Se expresa con dislipemia mixta, xantomatosis y enfermedad CV. Hasta la edad adulta no suelen mostrar manifestaciones. Estas ocurren antes en los hombres. Los xantomas cutáneos afectan a un 60% de los pacientes no tratados; los lípidos generalmente se depositan en los pliegues de las manos (*xantoma striata plamaris*). El 50% presentan xantomas tuberosos, en codos, rodillas y nalgas. En otros casos también aparecen xantelasmas (86,91).

2.4.4 Deficiencia de lipasa hepática

La lipasa hepática (LH) es una enzima sintetizada en el hígado y que actúa en la superficie de los hepatocitos. Está codificada por el gen *LIPC* que se localiza en el cromosoma 15 (15q21.3). Puede hidrolizar TG y fosfolípidos y fundamentalmente se ve involucrada en la conversión de las IDL a LDL, además interviene en el aclaramiento de los QM y de las HDL. El déficit de LH es muy raro, se trata de una enfermedad autosómica recesiva y su descubrimiento es relativamente reciente, en los años 80 (92).

La clínica suele estar asociada a una enfermedad prematura coronaria y a incremento de arteriosclerosis (93). Sin embargo, otras investigaciones como la realizada por Cohen et al., no mostraron diferencias en pacientes con enfermedad CV en la actividad de LH. Todavía se cuestiona si la actividad de la LH está en relacionada con el riesgo CV ya que aumenta tanto la Apo B, como las HDL. Por ello, independientemente del cambio lipídico producido, puede que el riesgo aterogénico quede modulado (94).

2.4.5 Deficiencia de lipasa ácida lisosomal (DLAL)

Es una enfermedad muy rara, de carácter autosómico recesivo, crónica y progresiva que afecta al metabolismo lipídico. Se trata de mutaciones en el gen de *LIPA*, en el cromosoma 10 (10q23.31), con una disminución de la actividad de dicha enzima (95). Su frecuencia es

de 1:130.000 habitantes (96). En 1956 se hizo referencia a ella por primera vez, y se observó una actividad de LAL inferior a 1% (97).

Su mecanismo consiste en acumular progresivamente ésteres de colesterol y TG en los hepatocitos y macrófagos (98). Se trata de una clínica muy grave, que afecta con una mortalidad precoz en los primeros meses de vida, en la forma más grave, llamada enfermedad de Wolman caracterizada por vómitos, diarreas, hepatoesplenomegalia, y fallo multiorgánico. Además retraso mental y generalmente muerte prematura (99). En la forma del adulto, los síntomas suelen aparecer en la tercera o cuarta décadas de la vida. En las formas tardías se presenta elevación de las enzimas hepáticas en 2-3 veces los niveles de límite de normalidad. Puede derivar en cirrosis hepática y puede pasar inadvertida hasta más allá de los 30 años de edad (100).

2.5. Hipertrigliceridemias moderadas y graves.

La hipertrigliceridemia (HTG) se caracteriza por aumento de las partículas que llevan predominantemente TG, es decir QM y/o VLDL y se define por TG elevados en ayunas >200 mg/dL. Las formas severas presentan cifras superiores a 900 mg/dL y suelen asociarse a un defecto monogénico, con riesgo de pancreatitis aguda (101).

2.5.1 Hipertrigliceridemia familiar

Es una enfermedad común hereditaria autosómica dominante, afecta al 1% de la población y se manifiesta con frecuencia en la edad adulta. Los estudios de asociación del genoma completo (GWAS, genoma-wide association study) han reconocido 45 loci relacionados con alteraciones en las concentraciones de TG, las variaciones genéticas más frecuentemente encontradas se encuentran en los genes *APOC2, APOC3, APOA4 y APOA5* (102).

Aproximadamente la mitad de los familiares de primer grado presentan cifras de 250 a 1000 mg/dL, pero sin cifras elevadas de cLDL. La síntesis de TG está aumentada y por lo tanto la de VLDL. Las partículas de HDL aparecen disminuidas en la mayoría de los pacientes con HTG. A pesar de esta alteración, no está clara las asociación con la enfermedad CV prematura (101,103). Con frecuencia coexisten factores como consumo excesivo de alcohol, diabetes mal controlada, consumo de fármacos como antitiroideos y estrógenos, que

pueden favorecer la enfermedad y derivar en la forma más grave, conocida como hiperquilomicronemia o fenotipo tipo V (104).

Para su diagnóstico se debe tener en cuenta la determinación de Apo B, que es normal al contrario que en la hiperlipemia familiar combinada (105). Existe una clara asociación de las partículas ricas en TG más pequeñas y el riesgo de arteriosclerosis (106).

La clínica que presentan estos pacientes, se orienta a la valoración de presencia de rasgos de síndrome metabólico, comprobar la existencia de xantomas eruptivos (en las formas más graves) y la sospecha de esplenomegalia (limitado a las enfermedades raras) y hepatomegalia(107,108).

2.5.2 Síndrome de quilomicronemia familiar

El síndrome de quilomicronemia familiar es autosómico recesivo, se caracteriza por presentar unos niveles de TG muy elevados (>1500 mg/dL) en ayunas de 12-14 horas. La primera referencia en la literatura científica se muestra en 1981. Se observa una clínica muy llamativa que se identifica en la niñez o edad adulta joven, y en la que aparecen marcados niveles muy elevados de TG y con características clínicas como dolor abdominal, hábito delgado y con o sin pancreatitis aguda, xantomas eruptivos, *lipemia retinalis*, confusión mental, pérdida de memoria, hepatomegalia o esplenomegalia (109). La prevalencia de este trastorno es de 1:1.000.000 de habitantes (110).

La causa principal de esta enfermedad es una alteración en el gen que codifica la enzima lipoproteína lipasa (*LPL*). Defectos genéticos provocan el descenso de la actividad lipasa (hepática, pancreática, y endotelial) lo que conlleva una menor actividad lipolítica que deriva en altas concentraciones de QM y VLDL (67). Entre el 10-20% de los casos se debe a afectaciones en uno de los cuatro genes que interactúan con lipoprotein lipasa, incluyendo *APOC2*, *GPIHBP1*, *APOA5* y *LMF1* (111). Aunque son mutaciones extremadamente raras, quienes padecen esta enfermedad tienden a agruparse en grupos de hermanos y no en transmisión vertical (son necesarios dos alelos defectuosos).

El tratamiento de estos pacientes es fundamentalmente la restricción de grasas < 15% o incluso <5-10% en los casos más severos. La dieta se debe componer de TG de cadena media, ya que no se absorben con los QM, además de suplementos vitamínicos de vitaminas

liposolubles. La plasmaféresis puede contemplarse en los casos de pancreatitis recurrentes (112–114).

2.6. Hipercolesterolemia poligénica

La hipercolesterolemia poligénica se caracteriza por una elevación de las partículas de LDL, como consecuencia de variaciones comunes en la secuencia de múltiples genes.

En 2011, Talmud y cols. observaron que una proporción alta de pacientes con HF diagnosticada con criterios clínicos pero sin mutaciones en los genes de *LDLR, APOB y PCSK9*, acumulaban alelos asociados a concentraciones más altas de cLDL y por ello se trataba de una causa poligénica y no de una causa monogénica (115). Futema et al. demostraron la solidez de este análisis, simplificándolo a 6 *Single nucleotide variant* (SNV), en muestras de seis países europeos (116). Estos sujetos pueden presentar fenotipo clínico similar al de la HF, sin embargo, a diferencia de éstos, no suelen acumular depósitos de colesterol superficiales como los xantomas tendinosos. El cribado diagnóstico en cascada familiar que se recomienda en aquellos casos con sospecha de causa monogénica no tendría tanta importancia en las formas poligénicas (117,118). Se plantea que todavía son necesarios más estudios de genotipado y secuenciación masiva para determinar con mayor precisión e indagar en el conocimiento de esta enfermedad.

Capítulo 3

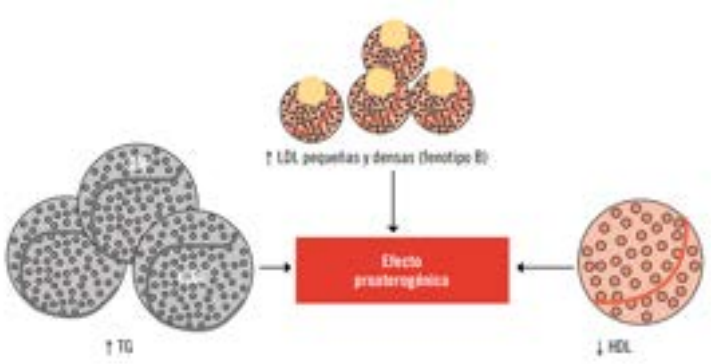
Lipidos y arteriosclerosis

3. Dislipemia aterogénica

La dislipemia aterogénica se define como un aumento de los niveles de TG en sangre, partículas que contienen Apo B, y disminución de HDL. Las LDL son predominantemente pequeñas y densas (119). Esta alteración está relacionada con mayor riesgo CV (119).

Se ha denominado como la "triada lipídica", "fenotipo lipoprotéico B", "fenotipo lipídico aterogénico" o "hiperapobetalipoproteinemia". Esta alteración es consecuencia de enfermedades como síndrome metabólico, resistencia periférica a la insulina, obesidad central e hiperlipemia familiar combinada (105,120,121). En España se muestra en un 35% de la población diabética a pesar de estar bajo tratamiento. Si se refiere a prevención secundaria, la prevalencia supera el 51% (120). Además de los pacientes diabéticos, se presenta en pacientes con enfermedad coronaria, síndrome de ovario poliquístico o enfermedad renal crónica (122).(Figura 10) (Tabla 6).

Figura 10. Fenotipo lipoproteico de la dislipemia aterogénica.



Alicia Taobada et al(63). *LDL: lipoproteínas de baja densidad; CT: colesterol total; TG: triglicéridos; HDL: lipoproteínas de alta densidad*

Tabla 6. Parámetros lipídicos alterados en la dislipemia aterogénica.

Principales:
- Concentraciones plasmáticas moderadamente elevadas de cLDL (>100 mg/dL [>2,6 mmol/L]), con predominio de partículas LDL pequeñas y densas (LDLpd)
- Concentraciones plasmáticas altas de TG (>150 mg/dL [>1,7 mmol/L])
- Concentraciones plasmáticas bajas de cHDL (<40 mg/dL [<1 mmol/L] en hombres; <45 mg/dL [<1,2 mmol/L] en mujeres), con predominio de partículas de HDL pequeñas y densas

Otros:
- Concentraciones plasmáticas altas de colesterol no HDL (>130 mg/dL [>1,7 mmol/L])
- Concentraciones plasmáticas altas de remanentes de lipoproteínas ricas en TG, como las lipoproteínas de muy baja densidad (VLDL) y de densidad intermedia (IDL)
- Concentraciones plasmáticas altas de partículas LDLpd (TG/cHDL >2)
- Niveles altos del cociente colesterol total/cHDL (>5 en hombres; >4,5 en mujeres)
- Aumento de apolipoproteína B
- Aumento del cociente apolipoproteína B/colesterol total
- Descenso de apolipoproteína A-I
- Aumento de apolipoproteína CIII
- Aumento de la actividad de la proteína transferidora de ésteres de colesterol (CETP)
- Aumento de la actividad de la lipasa hepática (LH)
- Descenso de la actividad de la lipoproteinlipasa (LPL)

Alicia Taobada et al(63).*LDL: lipoproteínas de baja densidad; CT: colesterol total; TG: triglicéridos; HDL: lipoproteínas de alta densidad*

3.1 Relación entre el colesterol y la arteriosclerosis

En el año 2015 los profesores Joseph Goldstein y Michael Brown, trabajaron en un documento donde plasmaron e identificaron los mayores acontecimientos científicos de la historia de la asociación del colesterol con la arteriosclerosis (34). Están recogidos en la figura que se muestra a continuación (Tabla 7).

Tabla 7. Historia del colesterol y enfermedad cardiovascular

Primera mitad del siglo xx: la era del colesterol	
1910	Las placas ateroscleróticas humanas contienen colesterol
1911	La dieta rica en colesterol causa aterosclerosis en conejos
1938	Se describe la hipercolesterolemia familiar
1950	Se dilucida la vía biosintética del colesterol
1951	Las dietas altas en grasas aumentan el colesterol plasmático en humanos
1953	Concepto de factor de riesgo
Segunda mitad del siglo xx: la era del LDL	
1955	Se identifica el cLDL como factor de riesgo de enfermedad coronaria
1973	Descripción del receptor de las LDL
1976	Descubrimiento de los inhibidores de la HMG CoA reductasa (estatinas)
1984	La reducción del cLDL reduce la incidencia de enfermedad coronaria
1985	Recomendación de reducir el cLDL del National Cholesterol Education Program (NCEP)
1987	Primera estatina (lovastatina) aprobada para uso humano
1994	Las estatinas disminuyen los infartos de miocardio y prolongan la vida
2006	Se descubre la PCSK9: regulador de los receptores de las LDL

Tabla elaborada con datos de Goldstein et al.(34)

La primera noción que hizo referencia a las placas de ateroma fue en 1910, por el químico Adolf Windaus que observó que en las aortas humanas se concentraba 25 veces más colesterol que las aortas sin enfermedad. En 1954, John Gofman estudió que las partículas LDL en aquellos pacientes con enfermedad CV estaban aumentadas, era la primera asociación que se establecía entre cLDL y la enfermedad coronaria (123). Posteriormente en el estudio "*Lipid Research Clinics Coronary Primary Prevention Trial*"(LRC-CPPT) multicéntrico, aleatorizado, doble ciego, donde se estudiaron a 3.806 varones con alto riesgo CV, el tratamiento con colestiramina fue beneficioso y se pudo concluir que un menor cLDL se asociaba a una menor incidencia de morbilidad y mortalidad CV(124).

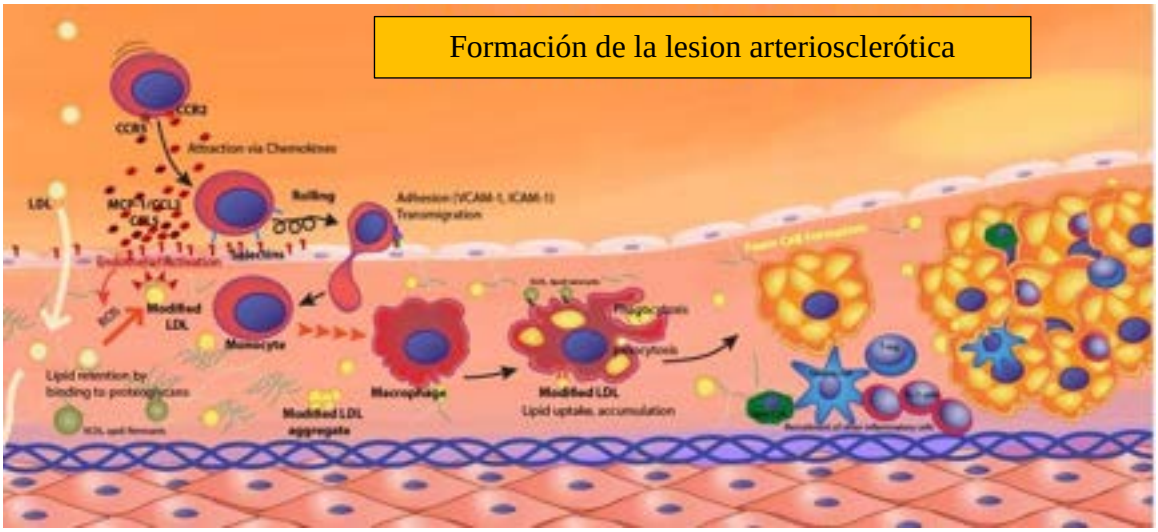
3.2. Remanentes de lipoproteínas y arteriosclerosis

Los remanentes de TG han demostrado tener un impacto en los mecanismos fisiopatológicos que conllevan a la aterogénesis. Si los niveles están aumentados en exceso, los remanentes pueden infiltrarse a nivel de la barrera endotelial y acceder al espacio subendotelial. El acceso a través de la pared arterial es influido por varios factores como la permeabilidad de la pared, el tamaño y concentración de la partícula, mediante un proceso de transcitosis. Un acceso muy similar al que tienen lugar las partículas de LDL al interior de la íntima (125–127).

3.2.1 Mecanismos patogénicos del cLDL en el desarrollo de la arteriosclerosis

Las partículas de LDL, junto con las remanentes y Lp(a), tienen un tamaño inferior a 70 nm, por ello tienen la capacidad de atravesar al espacio subendotelial. En un principio, se planteó la hipótesis de que el paso de las partículas LDL y otras lipoproteínas aterogénicas se desarrollaba mediante filtración pasiva, dependiendo del tamaño en ambos lados de la capa íntima. Recientemente se ha analizado que este proceso tiene mayor complejidad y que consiste en un proceso de transporte transcelular o "transcitosis", que combina endocitosis y exocitosis, generando vesículas donde son transportadas. Este proceso es favorecido por la afinidad de las partículas LDL por los receptores de las LDL y posiblemente SR-B1 (128,129). (Figura 11).

Figura 11. El papel del LDL en la arteriosclerosis.



Adaptado de MacRae F Linton et al(130) *LDL: lipoproteínas de baja densidad*

La arteriosclerosis comienza en el momento en que las lipoproteínas pequeñas que contienen Apo B (LDLy remanentes de VLDL) quedan retenidas y unidas a proteoglicanos en el espacio subendotelial.

A partir de este momento, por un lado, comienzan los procesos de oxidación y glicación que activan las células endoteliales. Estas células una vez activadas, comienzan a interaccionar y adherirse a monocitos (selectinas, VCAM-1) y quimioatrayentes (MCP-1). De esta manera los monocitos se conducen al espacio subíntimal. Por otro lado, se activan también células inmunes, incluidas las células dendríticas, los mastocitos, las células T reguladoras (T-reg) y las células T auxiliares 1 (Th-1). Los monocitos cambian a macrófagos y éstos a células espumosas. Así se activan vías de señalización inflamatoria con una modificación de las LDL.

3.2.2 Mecanismos patogénicos de Lp(a) en el desarrollo de la arteriosclerosis

La Lp(a) también se ha relacionado como factor independiente de riesgo CV, su intervención en este proceso es desconocido, pero se ha asociado por un lado con la capacidad de transportar colesterol al espacio subendotelial, y por el transporte de fosfolípidos oxidados con alta actividad proinflamatoria y proaterogénica(71,129,131). Su potencial efecto protrombótico y antifibrinolítico es más discutible.

Niveles elevados de fosfolípidos oxidados transportados en la Lp(a) han sido mostrados en pacientes con enfermedad inflamatoria intestinal, artritis reumatoide, lupus eritematoso, inmunodeficiencia adquirida, enfermedad crónica renal e hipertensión pulmonar. Este

incremento pudiera explicar, al menos en parte, el mayor riesgo cardiovascular de estas entidades (132,133).

3.3. Reacción inflamatoria de las partículas LDL

Una vez en la íntima, las partículas LDL se unen a los proteoglicanos de la pared arterial a través de los aminoácidos arginina y lisina de la Apo B. Aquellas zonas de la pared con mayor densidad de proteoglicanos será las zonas más vulnerables de acumulación LDL tendrán mayor hiperplasia y acumulación de células musculares. La reacción inflamatoria que tiene lugar se describe en una revisión de expertos de la *European Atherosclerosis Society*, dividiendo el proceso en etapas:

1. Oxidación de lípidos (fosfolípidos, ésteres de colesterol) por la actividad oxidativa de las células endoteliales activadas o por los macrófagos.
2. Formación de las células espumosas y liberación de los lípidos proinflamatorios.
3. Desnaturalización de partículas lipídicas y acumulación de depósitos extracelulares.
4. Formación del núcleo de la placa: los lípidos que se han modificado provocan una respuesta inmunitaria innata, se promueve la inflamación local con la liberación de citocinas proinflamatorias.
5. Activación de las células T que conduce a una respuesta inmunitaria adaptativa. La actividad de los antígenos específicos procede a la inflamación y muerte celular.
6. Eferocitosis donde las células fagocíticas se encargan de eliminar las células muertas antes de que se rompa la membrana y se eliminen componentes proinflamatorios vertiéndose al espacio extracelular (129,134,135).

3.4. Diagnóstico de la dislipemia

En el estudio Framingham se planteó la necesidad de elaborar un programa de prevención de la enfermedad cardiovascular. Se estudiaron a 4.469 personas en edades comprendidas entre 30 y 59 años, y se observó que la elevación del CT $\geq$ 245 mg/dL estaba relacionado con un riesgo 3 veces superior de sufrir un evento CV en hombres entre 40-59 años (136).

Posteriormente múltiples estudios epidemiológicos, de aleatorización mendeliana y de intervención han confirmado la asociación.

En la actualidad, las guías Europeas de la Sociedad Europea de Arteriosclerosis (EAS, en inglés) recomiendan una valoración de la dislipemia en los adultos varones >40 años y en mujeres > de 50 años o posmenopáusicas, fundamentalmente en aquellos sujetos con alto riesgo CV(137). Para la valoración del diagnóstico de dislipemias se precisa el cálculo de niveles de cLDL estimados con la fórmula de Friedewald (cLDL =(TC-TG/5)- cHDL)). En el caso de los pacientes con TG muy elevados (>400 mg/dL) o muy bajos niveles de cLDL se pueden calcular mediante el método de Martin/Hopkins (TG> 150 mg/dL o cLDL <70 mg/dL) o bien utilizar el colesterol noHDL (no-cHDL), que es la suma del CT vehiculizado por partículas aterogénicas (VLDL + LDL + IDL) y otros remanentes. El no-cHDL se calcula con la siguiente fórmula: c-no-HDL= CT – cHDL (63,138).

Las ecuaciones de riesgo siguen siendo de referencia para calcular el riesgo CV mediante el uso de *Systemic Coronary Risk Estimation (SCORE)* de las guías EAS, o bien el *pooled cohort equations (PCEs)* en las Guías Americanas de American College of Cardiology/American Heart Association (ACC/AHA) (139).

Además de la estimación de cLDL, actualmente se recogen en ambas guías la valoración de Lp(a), siendo de alto riesgo cifras ≥50 mg/dL y de muy alto riesgo CV valores superiores a 180 mg/dL. La EAS incluye también recomendaciones de medición de placas carótidas y femorales como otro factor de riesgo CV(140). La evidencia de enfermedad arterial coronaria o cerebrovascular mediante imagen, como angiografía por tomografía computarizada (TAC) o ecografía de ultrasonidos en carótidas se considera en el documento de la EAS con un riesgo semejante a la enfermedad clínica (139).

Los objetivos terapéuticos de cLDL en pacientes considerados como “muy alto riesgo CV” se han establecido en unos valores más agresivos desde la última revisión de las guías Europeas, siendo (<55 mg/dL y una reducción de ≥50% o <40 mg/dL para pacientes con enfermedad aterosclerótica cardiovascular recurrente en 2 años a pesar del tratamiento con estatinas máximo tolerado). Las guías recomiendan un uso escalonado de fármacos iniciando con estatinas potentes para después en caso de no alcanzar objetivos asociar ezetimiba y

posteriormente iPCSK9 en base a los ensayos clínicos publicados en los últimos años (75,76,141).

En unidades especializadas de lípidos, el análisis de Apo B y de Apo A1, se consideran de especial interés para identificar a los sujetos de alto riesgo CV, mediante el cociente de Apo B /Apo A1. En concreto, las partículas que tienen Apo B son aterogénicas y la Apo A1 componente de las HDL como principal apo contenida en éstas e involucrada en su metabolismo. Otra medida de predicción de riesgo CV, consiste en el cociente CT/ cHDL en hombres y >4,5 en mujeres. Ambos cocientes muy similares al calculado por no-cHDL. Todavía las guías de recomendaciones de dislipemias no incluyen estos parámetros como objetivos terapéuticos, limitándose a las concentraciones de cLDL (142).

3.5. Otros índices de riesgo aterogénico

Otra medida de riesgo aterogénico se basa en el cálculo de los cocientes CT/cHDL, cLDL/cHDL y Apo B/Apo A1, con valores altos en estos cocientes se puede establecer un desequilibrio entre las lipoproteínas aterogénicas y las lipoproteínas protectoras, incrementando el riesgo CV. Para una mayor precisión de aquellos casos con hipertrigliceridemia (HTG) se recomienda el uso del cociente CT/cHDL y del índice de Mayurama (TG/cHDL >2), cuando se acompaña además de un cHDL disminuido (143).

3.6. Evidencia de los efectos de los lípidos y lipoproteínas en la enfermedad aterosclerótica cardiovascular.

3.6.1. cLDL y riesgo de aterosclerosis

Las partículas de cLDL se caracterizan por ser muy heterogéneas, tanto en densidad como contenido lipídico y comportamiento metabólico y su tamaño está afectado por la concentración de partículas VLDL y TG.

En 1950 el investigador John Gofman et al. clasificaron las lipoproteínas en subtipos. Fueron clasificadas en grandes, medianas y pequeñas (grandes LDL-I, densidad: 1.019–1.023 g/L; medianas LDL -IIa, densidad: 1.023–1.028 g/L; medianas LDL-IIb, densidad: 1.028–1.034 g/L; pequeñas LDL-IIIa, densidad: 1.034–1.041 g/L; pequeñas IIIb, densidad:

1.041–1.044 g/L; muy pequeñas LDL- IVa, densidad: 1.044–1.051 g/L y muy pequeñas LDL- IVb, densidad: 1.051–1.06 g/L g/L)(144,145).

En el caso de las partículas de cLDL pequeñas y densas (fenotipo B), presentan mejor afinidad por los proteoglicanos vasculares y por tanto representan una mayor aterogenicidad y riesgo de enfermedad coronaria. La presencia de este tipo de partículas se muestra mayoritariamente en pacientes con hipertrigliceridemia. Estos sujetos presentan un fenotipo que puede incluir: resistencia a la insulina, concentraciones altas de Apo B, niveles elevados de VLDL y TG y una concentración de cHDL baja (146).

Estudios de randomización mendeliana y ensayos de randomización controlados han demostrado la asociación entre los niveles de cLDL y su influencia en el riesgo CV (147,148). De manera que una exposición larga en el tiempo a unos valores de cLDL bajos se relaciona con un menor riesgo de eventos CV. Es decir, tanto la magnitud como la duración en el tiempo parecen afectar (149).

3.6.2. TG y riesgo de aterosclerosis

Los niveles elevados de TG están asociados independientemente con mayor morbimortalidad CV, en concreto cuando se asocian con partículas LDL pequeñas y densas, y el cHDL está disminuido. Algunos estudios parecen establecer una relación más bien causal con la enfermedad CV, pero todavía no se ha concretado está afirmación, ya que la asociación disminuye cuando se controla por cHDL, cLDL o Lp(a) (150). Recientemente se observó que la concentración de Apo B explica gran parte del riesgo atribuido a los TG, por lo tanto, el efecto de los TG y sus remanentes en dicho riesgo viene determinado por las partículas de Apo B contenidas, más que por los TG en sí mismos (140,151).

3.6.3. cHDL y riesgo de aterosclerosis

Las concentraciones bajas de cHDL son un factor independiente de riesgo CV. En el hombre es mayor con concentraciones cHDL <40 mg/dL y de 46 mg/dL en las mujeres. La inversa relación con el riesgo parece estar bien estudiada en estudios observacionales. No está claro la protección que plantea el cHDL frente al desarrollo de aterosclerosis, pero parece estar asociado con la capacidad antioxidante y antiinflamatoria que proporcionan las

partículas que contienen Apo A1 y sus enzimas asociadas. Datos recientes han demostrado que el aumento de 10 mg/dL de cHDL disminuye un 14% el riesgo CV (63,140,152). Sin embargo, no hay evidencia de ensayos randomizados en los que el aumento de cHDL produzca dicho efecto. Por ello el efecto que proporciona esta partícula, necesita futuros estudios que clarifiquen dicha asociación (153).

3.6.4. Lp(a) y riesgo de aterosclerosis

Las altas concentraciones en el plasma de esta partícula han demostrado una asociación con la enfermedad CV. Los estudios de randomización mendeliana y los ensayos randomizados parecen contradecirse, mientras que los primeros han concluido una fuerte asociación, las terapias que han sido utilizadas para disminuir la partícula Lp(a), incluyendo niacina y los inhibidores CETP, no habían mostrado este efecto. En la actualidad el uso de iPCSK9, parece reorientar a una dirección optimista de los tratamientos frente a esta partícula, ya que han supuesto una disminución de hasta un 30-40% con beneficio independiente del cLDL en la reducción de eventos (57,154,155).

3.6.5. No-cHDL y riesgo de aterosclerosis

Las concentraciones de no-cHDL en el metaanálisis de Boekholdt et al., demostraron ser el parámetro más adecuado, para valorar el riesgo aterogénico asociado a los lípidos, puesto que representaban una mejor correlación con el riesgo CV que el cLDL (156). La determinación de Apo B se ha incluido también en dicho análisis, pero siendo un parámetro menos fiable y económico que la medición de no-cHDL viene quedándose en un segundo plano (63).

Capítulo 4

Hipercolesterolemia Familiar

4.1 Definición e historia

La Hipercolesterolemia Familiar (HF) es una enfermedad hereditaria, autosómica dominante, que cursa con elevadas concentraciones de LDL, consecuencia de alteraciones en el metabolismo lipídico. Se caracteriza por presentar enfermedad prematura cardiovascular y una clínica representativa que muestra depósitos en tendones denominados xantomas tendinosos y arco corneal (35,67,157).

En 1938 el científico Carl Müller describió la enfermedad al identificar varios sujetos con xantomas tendinosos, colesterol muy elevado y lesiones coronarias (158).

En 1964 el fenotipo fue diferenciado en HFHe menos severa y en la forma grave HFHo por Khachadurian, en la Universidad Americana en Beirut, en una amplia familia libanesa (159). Por entonces, tres años más tarde, en 1967, Fredrickson y Levy relacionaban la enfermedad con el metabolismo de las partículas de LDL (88). A partir de 1972, Brown y Goldstein comenzaron a profundizar en esta enfermedad, observando que estos pacientes mostraban concentraciones altas en sangre de cLDL y sufrían infartos en edades precoces. La clave fundamental fue en 1973, cuando describieron el mecanismo del LDLr, y observaron que la actividad de la enzima HMG CoA reductasa, en las células de pacientes con HFHo, era de 50 a 100 veces por encima de lo normal (160). Posteriormente, en el año 1985, Goldstein y Brown reciben el Premio Nobel por su descubrimiento en el análisis del LDLr y la asociación de la HF a distintas mutaciones en *LDLR* en 110 sujetos (2). En 1983, habían determinado la prevalencia de la enfermedad de 1:500 en HFHe y 1:1.000.000 en HFHo (2,35).

4.2 Epidemiología

Desde que Goldstein y Brown definieron la prevalencia de la HF en 1983, se aceptó 1:500 como una estimación de la enfermedad. En poblaciones, con alta consanguinidad, como canadienses franceses (161), libaneses cristianos (162), sudafricanos Afrikaner (163) o judíos Ashkenazi (164), puede aumentar a una prevalencia de 1:100 personas, dado su aislamiento genético en la historia de estos grupos étnicos (165,166). Recientemente *The Copenhagen General Population Study* y la EAS han sugerido que se trata de una enfermedad infradiagnosticada e infratratada y que su prevalencia podría ser mayor con una afectación de 1:200 y en el caso de la HF definida molecularmente, de 1:244 personas. Se calcula que entre 14 y 34 millones de personas en el mundo podrían sufrir de HF en la actualidad y un porcentaje de entre 20-25% pertenecería a niños y adolescentes (67,157,167,168). En el caso de la HFHo esta estimación también sería mayor de la diagnosticada hasta ahora, siendo 1:160.000 a 300.000 personas (169). En España, solamente el 6% de los pacientes están diagnosticados genéticamente, cuando la prevalencia esperada estaría en 180.000 personas (170).

Dada la morbilidad y mortalidad que supone esta enfermedad, sería necesario establecer estrategias en la población para un diagnóstico más preciso de la HF.

4.3 Etiología y patogenia

La HF con un patrón autosómico dominante, viene determinada por mutaciones en los genes *LDLR*, *APOB*, *APOE* (171) y en *PSCK9*. También se han localizado otros genes con menor frecuencia como el gen de *STAP1* (172). En la actualidad, se han analizado 1.700 mutaciones en *LDLR*, de las cuales 1.200 han sido expresadas como HF severa. Todas las mutaciones, a excepción de las afectadas en el gen de *LDLRAP1* , causan un patrón autosómico dominante (69,173–176).

La penetrancia de esta enfermedad es de más de 90%, afecta a ambos sexos indistintamente y aparecen las primeras manifestaciones en la infancia. La mitad de los descendientes mostrarán la enfermedad dada la alta probabilidad de expresar este fenotipo (177). La herencia puede ser de tipo autosómico dominante, codominante o recesivo. En el

caso de la HFHe la herencia transmite uno de los alelos con mutación y el otro normal. En la HFHo los 2 alelos que se han heredado muestran mutación. Los *heterocigotos compuestos* se denominan aquellos que heredan mutaciones diferentes en los dos alelos; a diferencia de los *heterocigotos dobles* que presentan en dos genes distintos, mutaciones diferentes. Generalmente se trata de una mutación de *LDLR* y el otro alelo presenta mutaciones en los genes *APOB o PCSK9* (63,178).

4.3.1- Vías moleculares que causan hipercolesterolemia familiar

1- Proteínas que afectan a la función del LDLr

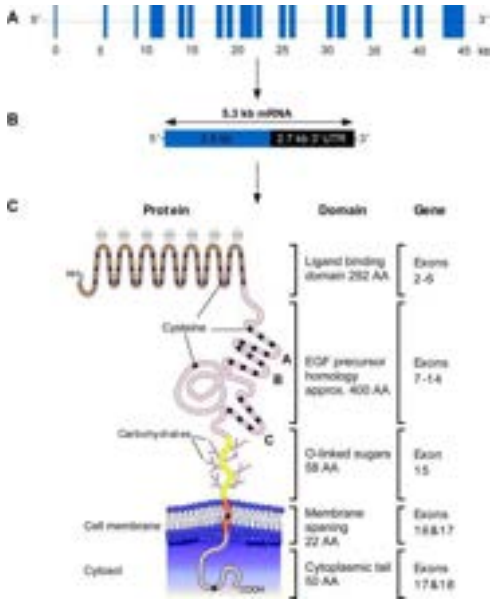
El LDLr se caracteriza por ser una proteína quimérica, en su forma madura, consta de 839 aminoácidos y se sintetiza como precursor de 120 kDa (179). Se origina en el retículo endoplasmático y se desplaza hasta el aparato de Golgi para dirigirse a la membrana celular. En dicha membrana se concentra en invaginaciones de clatrina. El LDLr une Apo B-100 de las LDL y a la Apo E de las partículas remanentes. En esta unión tiene un papel importante la proteína adaptadora 1 del LDLr (LDLRAP1) que permite su endocitosis. Los endosomas transportan el complejo y por su medio acidificado, tiene lugar una disociación donde se divide por un lado la LDL que se metaboliza y por otro el LDLr que vuelve a la superficie celular (64,177,180). En dicho proceso la proteína PCSK9, también puede interactuar con el LDLr inhibiendo su reciclado y como consecuencia se degradará, pero no se reciclará (181). Todo ello dependerá de la demanda de las concentraciones de esteroles y colesterol en el interior de la célula, capaces de interactuar con el factor de transcripción *sterol regulatory element-binding proteins* (SREBP-2), sensible a los esteroles que modula la expresión de múltiples genes del metabolismo del colesterol intracelular incluidos los que codifican al LDLr y HMGCoA reductasa (177).

2- Gen del *LDLR* y mutaciones en el *LDLR*

La causa de la HFHe son mutaciones en el gen del *LDLR,* que tiene su localización en el cromosoma 19 (19p13) (182). Se han estudiado más de 1700 mutaciones en este gen, como se indica anteriormente (176,183). El gen que codifica este receptor contiene 18 exones. El exón 1 codifica 21 aminoácidos o péptido señal y es necesario para el transporte celular. Un

4,5 % de las mutaciones tienen lugar en esta región. Los exones 2 al 6 median la interacción de receptor y lipoproteínas gracias a la unión de Apo B. Constituyen una secuencia de 7 repeticiones de 40 aminoácidos. En torno al 40% de las mutaciones de HF ocurren en este domino. Los exones 7 al 14 codifican 411 aminoácidos, y tienen un 33% de homología con el factor de crecimiento epidérmico (EGF). El exón 15 su actividad funcional es desconocida, es una zona de glucosilación, podría tratarse de una función estabilizadora del receptor. El exón 16 está involucrado en el anclaje del LDLr a la membrana celular. Los exones 17 y 18, codifican el dominio citosólico (177,183–188). (Figura 12).

Figura 12. Estructura del *LDLR***.**



Al-Allaf et al.(189). *A) Representación esquemática del gen LDLR; B) Región 3'UTR; C) Dominio de la proteína.*

La actividad residual del *LDLR*, nos condiciona su clasificación en:

- *Mutaciones de alelo nulo o mutaciones de clase 1*, con una actividad inferior al 2%. Afectan a la síntesis del receptor e implican pérdida del promotor, afectan al ajuste, generan codones de parada o sin sentido o bloquean al ARN mensajero. Estas mutaciones son la forma grave de la enfermedad, asociada a una mayor concentración de cLDL y consecuente aterosclerosis (190),

- Mutaciones *de alelo defectuoso* o de disminución de función en el gen *LDLR* con una actividad entre 2-25%. En este caso se pueden afectar las síntesis en el retículo endoplasmático, su maduración en el aparato de Golgi, su traslocación a la membrana celular, su unión a Apo B o su reciclado. Se clasifican en (Figura 13):

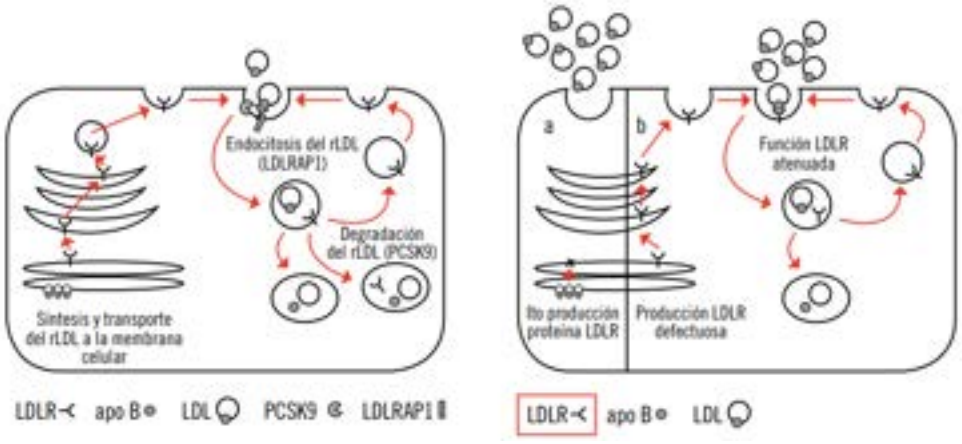
1. Mutaciones de clase 2. Las proteínas que son codificadas quedan bloqueadas total o parcialmente, al no presentar una estructura tridimensional necesaria para su correcto funcionamiento. Se trata de deleciones en el *LDLR*, en los exones para el dominio de unión al ligando o dominio a EGF.

2. Mutaciones de clase 3. Los alelos defectuosos no pueden unirse a las partículas LDL, consecuencia de distintos reordenamientos de residuos de cisteína o deleciones en dominio EGF (191).

3. Mutaciones de clase 4. Los dominios citoplasmático y dominio transmembrana se ven afectados por defectos en los alelos que impiden que los LDLr no puedan internalizarse en las fosas de clatrina (192).

4. Mutaciones de clase 5. Afectan al dominio EGF, son mutaciones "sin sentido" e impiden que el LDLr pueda reciclarse (193).

Figura 13. Proceso del LDLr y proteínas que afectan a su función. Alteraciones del receptor LDL.



Ascaso et al(178) *Abreviaturas: LDLRAP1: proteína adaptadora 1 del LDLr; PCSK9:Proproteína convertasa subtilisina/ kexina tipo 9; LDL: lipoproteínas de baja densidad LDLR: Receptor de lipoproteínas de baja densidad*

3. ApoB -100 defectuosa familiar

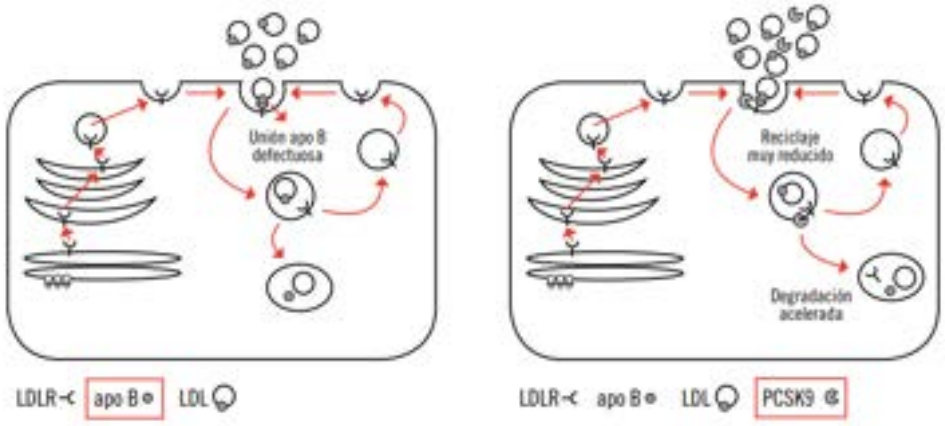
La HF por defecto de Apo B-100 es una enfermedad genética que se caracteriza por la incapacidad de las partículas LDL de establecer unión con el LDLr. APOB está localizado en el cromosoma 2 (2p24-p23). Existen muy pocas mutaciones en *APOB* causales de HF pero la más conocida es aquella que sustituye un aminoácido en posición 3500, glutamina por arginina (R3500Q). Es poco frecuente y representa un 5 % de las HF. Dichos pacientes son buenos respondedores del tratamiento a estatinas en su respuesta con disminución de cLDL (194) (Figura 14).

4. Mutaciones en el gen *PCSK9*

El gen de *PCSK9*, se encuentra en el cromosoma 1(1p32.3). Dicho gen codifica a la proteína sérica PCSK9, que se sintetiza en hígado y se encarga de regular los LDLr en la superficie de la célula y en el catabolismo del LDL. La unión con el LDLr evita que se recicle a nivel intracelular. Hay distintas mutaciones con ganancia de función que disminuyen el número de LDLr en superficie celular y que suponen un 1% de las HF (195) (Figura 14). En las mutaciones con ganancia de función la actividad de PCSK9 se ve alterada. El cLDL aumenta, debido a que el gen *PCSK9* codifica a una proteína hiperactiva de PCSK9 que provoca que el LDLr se degrade a mayor intensidad y por tanto una presencia cada vez menor en la superficie celular. Algunos estudios han sugerido que el PCSK9 podría unirse al LDLr en un estado catalíticamente inactivo que al acidificarse en el medio provocaría su degradación (181).

- *Mutaciones con pérdida de función*: el LDLr se ve incrementado y conlleva un aumento de aclaramiento de cLDL en sangre (195,196). Además, se observaron en estudios entre africanos- americanos y caucásicos (mutaciones en Y142X -C679X y R46L, respectivamente) relacionados con un efecto protector frente a enfermedades cardiovasculares (197,198). Estas aportaciones plantearon un beneficio mayor en el desarrollo de arteriosclerosis con una intervención temprana de la terapia hipolipemiante (199).

Figura 14. Unión defectuosa de la apolipoproteína B. Ganancia de función de PCSK9.



Ascaso et al.(178) *Abreviaturas: PCSK9:Proproteína convertasa subtilisina/ kexina tipo 9; LDL: lipoproteínas de baja densidad LDLR: Receptor de lipoproteínas de baja densidad.*

4.4 Manifestaciones clínicas de la Hipercolesterolemia Familiar.

4.4.1 Manifestaciones clínicas de la Hipercolesterolemia Familiar Heterocigota

- Aumento del cLDL

Los pacientes con HFHe muestran cifras de cLDL de dos a tres veces por encima de los niveles normales, es decir, cifras superiores a ≥190–400 mg/dL (200). Se han encontrado mayores cifras de cLDL en aquellos pacientes con mutación en el gen de *LDLR*, en concreto en los sujetos con alelos *LDLR* negativos (201). El nivel de TG está frecuentemente dentro de la normalidad, y en caso de tener cifras elevadas podría ser explicado por la interacción de otros genes (genotipo E2/E2) o factores ambientales (alcohol, sobrepeso, y DM) (202).

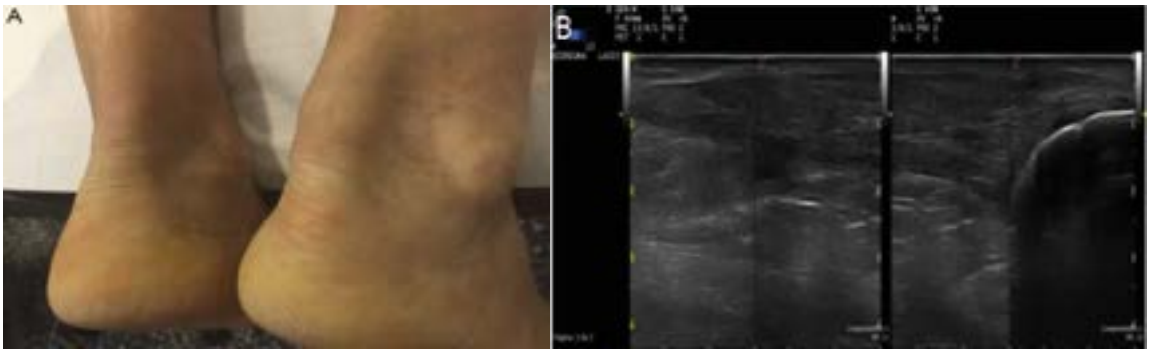
- Depósitos extravasculares de colesterol

Los xantomas constituyen una agrupación de células espumosas en el tejido conectivo de la piel, tendones y fascias, incluso con menor frecuencia en periostio. Son formaciones consecuencia de una acumulación de lípidos, a partir de los macrófagos. Su presentación

clínica es variable, de máculas cutáneas blandas o semisólidas hasta pápulas o nódulos de gran tamaño, su apariencia es de color amarillento, debido al caroteno que almacenan los lípidos (203).

- *Xantomas tendinosos:* se caracterizan por ser una acumulación de depósitos de colesterol en los tendones. Pueden verse afectados, además, inserciones de tendones, fascia y periostio. Forman nódulos móviles en las zonas de tendón de Aquiles, codos, dorso de manos, rodillas y talones. Un tercio de los pacientes con HFHe presentarán esta clínica en la cuarta década de su vida. El riesgo de CV prematura se relaciona con la aparición de dichos xantomas, demostrado por Civeira et al. y consolidado por numerosos estudios posteriores. Por ello se trata de un buen marcador para una intervención precoz y la prescripción de un tratamiento hipolipemiante de alta intensidad (202–204).(Figura 15).

Figura 15. Xantomas tendinosos en los tendones de Aquiles



Delia Reina(205). *A. Xantomas tendinosos en los tendones de Aquiles. B. Ecografía del mismo paciente, del tendón de Aquiles: en un corte longitudinal se ve un tendón engrosado, de una ecotextura heterogénea.*

- *Xantelasmas:* Se definen como placas blandas o semisólidas en los párpados superiores (70% de los casos) e inferiores. En edades tempranas de la vida, es un evidente signo de hipercolesterolemia pero generalmente es a partir de los 50 años cuando suele presentarse (202,206,207) (Figura 16,A).

Figura 16. A.Xantelasmas B. Arco córneal

A. Civeira et al (208) *B. A.Fernández* (209)

- *Arco corneal:* Se define como un depósito de colesterol que se acumula en la periferia de la córnea, aparece como una opacidad gris, blanca o amarillenta de 1-1,5 mm de ancho y separado por una zona corneal clara de 0.3-1mm que se denomina *intervalo lúcido de Vogt*. Se presenta mayoritariamente en ambos ojos. Se ha considerado que el cLDL está presente en el arco corneal. Aparece en el 14 % de los casos en la cuarta década de la vida. La asociación de arco corneal y altos niveles de cLDL se da con frecuencia en la HF en variedad de estudios (202,209–211). (Figura 16,B).

- Enfermedad cardiovascular

La enfermedad CV aterosclerótica, es causa de la muerte de >4 millones de personas en Europa anualmente (212). La elevación de las cifras de cLDL desde el nacimiento en pacientes con HF, conlleva el ser factor de riesgo de enfermedad aterosclerótica y mortalidad. Los estudios hasta la fecha, han analizado que sin tratamiento un 50% de los varones y un 33% de las mujeres desarrollará enfermedad CV antes de los 55 años y 60 años respectivamente (67,140,213,214). La HF representa entre 1-2% de todos los infartos de miocardio prematuros en la mayoría de países y en concreto en Alemania y Finlandia ascendería a 9% la enfermedad cardiovascular isquémica muy prematura (215,216). Se estima que el incremento de riesgo CV en estos pacientes es de al menos 10 veces superior al de la población general (140).

Actualmente lo datos analizados tras años de tratamiento hipolipemiante con estatinas desde 1980, son esperanzadores. En el metaanálisis más reciente de *Clinical treatment trialists* (CCT) de 27 estudios randomizados, se concluyó que la disminución de 1.0 mmol/L, con tratamiento con estatinas durante 5 años, reduce un 10%, el riesgo de mortalidad (217). Y en el estudio *The West of Scotland coronary prevention study (WOSCOPS),* se analizó, que el seguimiento durante 20 años de este tratamiento descendía en un 18% (218).

En el estudio del Registro Español de Dislipemias, se identificó que solamente un 15,1 % de pacientes con HFHe tenía enfermedad CV. En línea con estos datos el registro SAFEHEART mostró que un 21,9% presentaba la enfermedad (219,220). Diferentes cohortes de Reino Unido (221), Noruega (222), Dinamarca (223), y Holanda (224) han referido que los pacientes con HF tratados en el tiempo, presentan una mejora substancial en la enfermedad CV con respecto a décadas pasadas. El tipo de mutación también parece tener alguna asociación, ya que en aquellos pacientes con mutación nula, se observó en una cohorte española con HF que las enfermedades CV y la recurrencia de los eventos CV fue de 1,7 veces superior (201).

Por otro lado, la Lp(a) se ha asociado como factor de riesgo cardiovascular independiente en la HF. Hasta la fecha, los pacientes con HFHo que presentaban dos alelos en *LDLR* nulos, han mostrado tener una concentración de Lp(a) hasta dos veces superior. Sin embargo, los datos referentes a los pacientes heterocigotos son todavía inconcluyentes (225). El análisis de la asociación entre Lp(a) y calcificación aortica valvular ha sido contemplado por varios estudios, refiriéndose a esta partícula como un conocido e independiente factor de riesgo CV (226). Además, se han analizado en diversos estudios el estrechamiento de la válvula aórtica y su calcificación en este tipo de pacientes hipercolesterolémicos. Ten Kate, mostró en aquellos pacientes con mutación patogénica una alta prevalencia de enfermedad valvular aórtica, hasta un 41% respecto a 21% en la población en general) (227). Sin embargo, en el *Cardiovascular Health Study*, esta asociación no pudo ser demostrada(228). (Figura 17)

Figura 17. Fisiopatología de la enfermedad HFHe

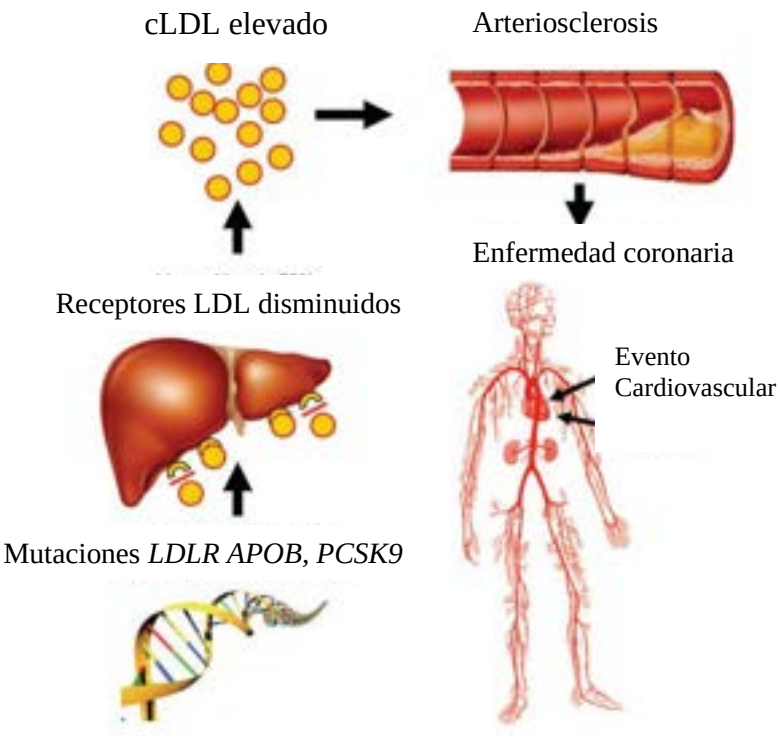


Figura Adaptado de Nordestgaard (67).

4.5 Diagnóstico

La detección precoz de la enfermedad de HF es fundamental para evitar la enfermedad prematura cardiovascular y clínica derivada lo más pronto posible. Dado que se trata de un problema de salud muy importante, el diagnóstico y correcto tratamiento es clave para un buen control de la enfermedad. Hasta la actualidad el diagnóstico se ha basado en el perfil lipídico de la familia, la presencia de depósitos de colesterol y antecedentes personales y familiares de enfermedad coronaria prematura. Los criterios del *Dutch Lipid Clinic Network* se muestran en la siguiente Tabla 8.

Tabla 8. Criterios Dutch Lipid Clinic Network

Historia familiar:	
• Familiar de primer grado con enfermedad coronaria prematura (hombres <55 años y mujeres <60 años) • Familiar de primer grado con niveles de cLDL >210 mg/dL (>5,4 mmol/L) o >p95	1
• Familiar de primer grado con xantomas tendinosos y/o arco corneal <45 años o • Familiar <18 años con cLDL ≥150 mg/dL (≥3,9 mmol/L) o >p95	2
Antecedentes personales:	
• Paciente con enfermedad coronaria prematura (hombres <55 años; mujeres <60 años) • Paciente con enfermedad cerebrovascular prematura (hombres <55 años; mujeres <60 años) o enfermedad arterial periférica	2 1
Examen físico:	
• Xantomas tendinosos • Arco corneal en personas <45 años	6 4
Análisis de laboratorio:	
• cLDL ≥330 mg/dL (≥8,5 mmol/L) • cLDL 250-329 mg/dL (6,5-8,5 mmol/L) • cLDL 190-249 mg/dL (4,9-6,5 mmol/L) • cLDL 155-189 mg/dL (4,0-4,9 mmol/L)	8 5 3 1
Análisis genético:	
Mutación funcional en los genes *LDLR, APOB, PCSK9* o *LDLRAP1*	8
Diagnóstico de HF:	
• Certeza: ≥8 puntos • Probable: 6-7 puntos • Posible: 3-5 puntos • Improbable: <3 puntos	

Figura de World Health Organization (WHO)(140)

Otros criterios de diagnóstico son los basados en *the Simon Broome register* del Reino Unido (229) (Tabla 9) y el Programa MedPed de EE.UU(230). (Tabla 10).

Tabla 9. Criterios diagnósticos del *Simon Broome Familial Hypercholesterolemia Register* para el diagnóstico de hipercolesterolemia familiar

Criterios	Descripción	
A	Colesterol total superior a 7,5 mmol/litro en adultos o superior a 6,7 mmol/litro en niños de edad superior a 16 años. O LDL colesterol superior a 4,9 mmol/litro en adultos o de 4,0 mmol/litro en niños mayores de 16 años.	
B	Xantomas tendinosos en el paciente o en familiares de primer grado.	
C	Diagnóstico genético de mutación en el gen del LDLr o en el gen de la ApoB.	
D	Historia familiar de infarto de miocardio antes de los 50 años en un familiar de segundo grado o antes de los 60 años en uno de primer grado.	
E	Historia familiar de concentraciones de colesterol superiores a 7,5 mmol/litro en familiares de primero o segundo grado.	
Diagnóstico	**Definitivo**	Criterio ***a*** y criterio ***b*** o ***c***
	Probable	Criterio ***a*** y criterio ***d*** o criterio ***a*** y ***e***

Tabla 10. Puntos de corte de CT total en el diagnóstico de HF de la escala MedPed.

	Límites de colesterol total (mmol/litro)			
Edad	Familiares de 1º grado con HF	Familiares de 2º grado con HF	Familiares de 3º grado con HF	Población general
< 20	5,7	5,9	6,2	7,0
20-29	6,2	6,5	6,7	7,5
30-39	7,0	7,2	7,5	8,8
≥ 40	7,5	7,8	8,0	9,3
Diagnóstico	Si los niveles de colesterol total exceden estos límites			

Los criterios que se usan más frecuentemente en Europa, y recomendados por la SEA, son los criterios holandeses. En la actualidad, el diagnóstico de la HF mediante el estudio genético se ha facilitado gracias a las técnicas de secuenciación masiva (NGS, next generation sequencing), este método ha permitido el cribado en cascada familiar y además es muy coste-efectivo. La secuenciación cada vez es más completa y hoy en día, podemos estudiarla en los genes de *LDLR, APOB, PCSK9, LDLRAP1, APOE y STAP1* (231). Es recomendable en aquellos casos con puntuación > 6, de lo contrario la especificidad disminuiría y por ello el fracaso en el diagnóstico. En general, las mutaciones con un diagnóstico definitivo o probable se presentan entre el 60-80% de los casos (140).

4.5.1 Cribado familiar

La EAS recomienda el cribado en cascada, para la identificación de familiares HF, basándose en el criterio del *National Institute for Health and Care Excellence (NICE)*, que considera un diagnóstico más eficaz cuando se valoran tanto pruebas genéticas como concentraciones de cLDL. Es recomendable hacer una valoración temprana a los 2 años de edad, en aquellas familias con sospecha de HF con unos antecedentes precoces de enfermedad CV: en familiares de primer grado o segundo, en hombres <55 años; en mujeres <65 años; si además el niño cuenta con factores de riesgo cardíaco; o en algún familiar de primer grado o segundo grado el CT es >240 mg/dL. Para evitar confusiones entre los 9 y 11 años, el cribado permite el conocimiento de la situación del adolescente, antes de que

comience con los cambios propios de la pubertad. El registro de *Cascade Screening for Awareness and Detection, CASCADE* la eficacia del diagnóstico precoz en la prevención de eventos (232).

En el *Consensus Statement of the European Atherosclerosis Society* de 2013, Nordestgaard et al. reflejaron que la mayoría de los países en el mundo, necesitan reforzar sus estrategias para el diagnóstico de esta enfermedad, estableciendo unidades clínicas específicas para el manejo de esta patología (67).

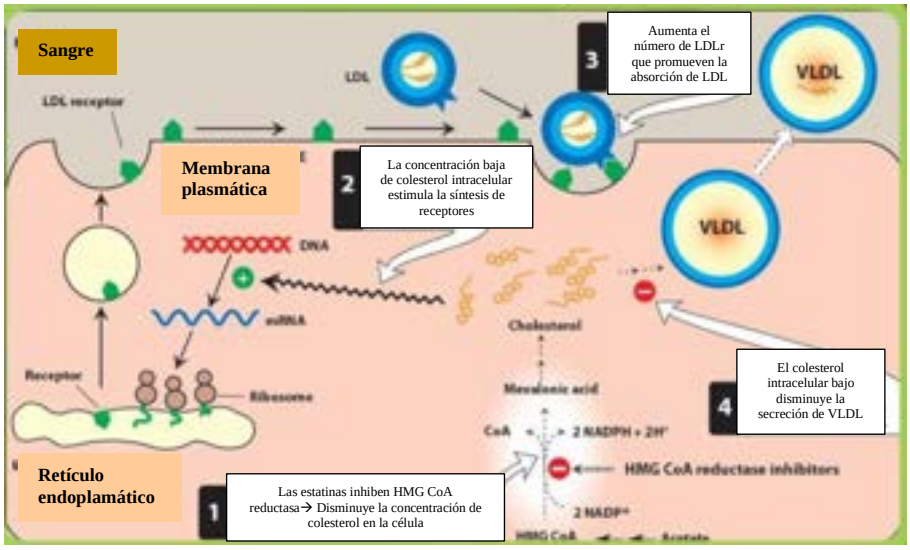
4.6 Tratamiento

4.6.1 Estatinas

En 1960 la American Heart Association (AHA) estableció la importancia de disminuir las concentraciones de colesterol en la sangre asociado al riesgo CV. Hoy en día, son los fármacos más utilizados para la prevención de enfermedad CV tanto en prevención primaria como secundaria. La evidencia de numerosos ensayos clínicos ha establecido que por cada disminución de 38,7 mg/dL de cLDL tras 5 años tratamiento con este hipolipemiante, la mortalidad disminuye un 10%(217).

El mecanismo de acción de las estatinas se establece mediante la inhibición de la enzima HMG-CoA reductasa. La síntesis de colesterol se ve reducida por dicha inhibición. En los hepatocitos se activa una estimulación de los LDLr que conllevan a un aclaramiento de cLDL equilibrando el déficit intracelular del mismo (233,234) (Figura 18).

Figura 18. Mecanismo de acción de estatinas



Adaptado de Harvey RA(235). Mecanismo de acción de estatinas. *Abreviaturas: LDL: lipoproteínas de baja densidad; LDLR: Receptor de lipoproteínas de baja densidad; VDLR: lipoproteínas de muy baja densidad.*

Existen diferentes tipos de estatinas, y dependiendo de su biodisponibilidad, capacidad de atravesar las membranas celulares o afinidad a los lípidos, tendrán mayor o menor actividad. Las estatinas liposolubles, como simvastatina y lovastatina, se unen a las proteínas plasmáticas, presentan una biodisponibilidad baja de <5% por el efecto del citocromo P450. Las estatinas hidrosolubles, como pravastatina, rosuvastatina y pitavastatina, no se metabolizan por el mismo mecanismo, si no que necesitan del anión orgánico transportador de polipéptidos (OATP1B1). Cuentan con una variación de la biodisponibilidad de 12% de la atorvastatina hasta 51% de la Pitavastatina (236).

- Las de alta intensidad, de media disminuyen el cLDL ≥50%.
- Las de moderada intensidad disminuyen el cLDL entre 30-50%.
- Las de baja intensidad disminuyen el cLDL entre 20-30% (141).

Para los pacientes con HF el tratamiento de elección, recomendado por la EAS, es la estatinas de máxima potencia, que generalmente necesita estar asociado a otros tratamientos para lograr los objetivos esperados. En la siguiente tabla se muestran los diferentes tipos e intensidades de estatinas (Tabla 11).

Tabla 11. Clasificación de las estatinas

Alta intensidad (reducción de cLDL ≥50%)	Moderada intensidad (reducción de cLDL 30-50%)	Baja intensidad (reducción de cLDL<30%)
Atorvastatina 40-80 mg Rosuvastatina 20-40 mg	Atorvastatina 10-20 mg Rosuvastatina 5-10 mg Simvastatina 20-40 mg Pravastatina 40-80 mg Lovastatina 40 mg Fluvastatina 40-80 mg Pitavastatina 2-4 mg	Simvastatina 10 mg Pravastatina 10-20 mg Lovastatina 20 mg Fluvastatina 20-40 mg Pitavastatina 1 mg

Adaptada de Stone NJ et al (237)

4.6.2 Ezetimiba

Es un fármaco que inhibe la absorción intestinal de colesterol. Es un inhibidor selectivo del transportador de colesterol intestinal *Niemann-Pick C1-Like1 (NPC1L1).* Las reducciones de cLDL son en torno a 15-25%, pero generalmente en pacientes con HF se asocia a estatinas esperando un efecto sinérgico. Es un fármaco muy bien tolerado, con pocos efectos secundarios, y que ha demostrado que la reducción de cLDL coadministrado es superior a la que se aplica al doblar la dosis de estatina (238–240).

4.6.3 Resinas secuestrantes de ácidos biliares

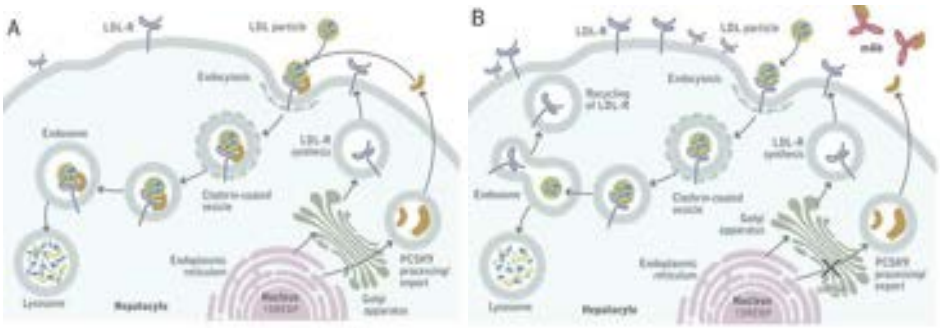
Las resinas quelantes reducen el colesterol al inhibir el ciclo enterohepático de las sales biliares, actuando sin tener efecto a nivel sistémico. Son fármacos utilizados preferentemente en niños por haber demostrado seguridad y eficacia a largo plazo. Consiguen una reducción del cLDL de 20-25%. Dosis más altas de colestipol 30 g y resincolestiramina 24 g alcanzan hasta un 30-35%. Generalmente, son una opción para pacientes intolerantes a

estatinas, además dado que no producen toxicidad sistémica, también pueden utilizarse en embarazadas. La adherencia al tratamiento, sin embargo, es baja, ya que condiciona efectos secundarios en el intestino, como pesadez, estreñimiento, flatulencias. El mejor tolerado es el colesevelam (241,242).

4.6.4 Inhibidores de PCSK9

Los inhibidores de PCSK9 actuales son anticuerpos monoclonales que inhiben la proteína PCSK9 y que han conseguido reducir el cLDL hasta un 60%. PCSK9 se sintetiza en el hepatocito y se secreta en el plasma. Una vez ahí se une con LDLr formando un complejo e internalizándose por endocitosis y degradado en el interior de lisosomas. Cuando la unión al complejo es inhibida por un anticuerpo monoclonal, el cLDL se introduce en el interior de la célula, pero el LDLr vuelve a la superficie del hepatocito con la consecuente unión a una nueva partícula de cLDL (243,244). (Figura 19).

Figura 19. Inhibidores PCSK9



Robert Stoekenbroek (243). *American Society for Biochemistry and Molecular Biology. A) Degradación del receptor de lipoproteínas de baja densidad (LDLR) mediada por PCSK9.B) Inhibición PCSK9.*

En 2015 fueron aprobados por *US Food and Drug Administration y la European Medicines Agency (EMA),* dos anticuerpos monoclonales, Evolocumab y Alirocumab, enfocándose principalmente en pacientes con HF y con enfermedad CV. En 2017, se desarrolló un ensayo clínico, conocido como FOURIER (*Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk),* donde se estudiaron 1242 centros de 49 países, con un total de 27.564 pacientes. Con una diferencia de 1,5 % de eventos

CV que fueron recogidos entre pacientes tratados con evolocumab y con placebo, siendo un 9,8% en el caso de los pacientes que recibieron el tratamiento y un 11,3% los que inyectaron placebo (245,246). Más tarde fue publicado el ensayo *ODYSSEY OUTCOMES* 18.924 pacientes que habían presentado síndrome coronario agudo en los 12 meses previos, con cifras de cLDL >70 mg/dL con tratamiento convencional con dosis máximas de estatinas. Se obtuvieron resultados similares con recurrencias de eventos en un 9,5 % con alirocumab y 11.1% con placebo (75). Ambos estudios disminuyeron el cLDL por debajo de 70 mg/dL, alcanzando los objetivos planteados previamente y sin efectos secundarios relevantes. Dichos tratamientos en la actualidad son administrados en una selección de pacientes que incluyen pacientes que presentan HFHe o HFHo y pacientes con factores de riesgo de recurrencia de evento CV (246).

4.7 Recomendaciones en la dieta

Se ha revisado ampliamente la relación de sufrir un evento CV con los patrones dietéticos, demostrando una asociación causal entre la dieta y el riesgo CV. Los estudios randomizados conocidos como "*metabolic ward studies*", estudios de cohortes y randomizados, muestran que la ingesta de grasas saturadas conlleva un aumento de cLDL y además un alto nivel en la concentración de estas partículas a largo plazo puede ocasionar eventos CV (247–249). El estudio *Prevención con Dieta Mediterránea (PREDIMED)* donde los participantes siguieron una dieta rica en aceite de oliva virgen extra o bien nueces, comparado con una dieta baja en estos alimentos mostraron una incidencia de un 30% más baja en eventos CV (250). Sin embargo, son necesarios más ensayos randomizados a largo y corto plazo con una intervención que tenga en cuenta los factores de riesgo como resultado.

Los suplementos de fitoesteroles como sitosterol, campesterol, y estigmasterol presentes en aceites vegetales y en cantidades limitadas en frutas y verduras, legumbres y granos han demostrado reducir un 7-10% los niveles de cLDL y CT, con un consumo medio de 2 g/día (251). Sin embargo, actualmente, no hay estudios que evidencien el efecto a nivel CV.

La monacolina y levadura de arroz rojo, son inhibidores de la coenzima A (HMG-CoA) reductasa, el efecto clínico presenta un 20% de reducción con una dosis de 2,5 -10 mg /día. Estos productos pueden presentar contaminantes en sus preparaciones y problemas de seguridad no estudiados por el momento (252).

Otros suplementos como la fibra, la soja, berberina, y los ácidos grasos omega 3 han demostrado también tener un limitado efecto en los niveles lipídicos, pero todavía son necesarios más estudios que puedan evidenciar su efecto a largo plazo en los eventos CV(140).

Tabla 12. Objetivos para la prevención de enfermedades cardiovasculares

Tabaco	Evitar la exposición del tabaco
Dieta	Basada en frutas, verduras, granos, pescados, y baja en grasas saturadas
Actividad física	3,5-7 h/semana de actividad moderada e intensa (30-60 min/día)
Peso	BMI 20–25 kg/m^2, cintura <94 cm (hombres) y <80 cm (mujeres).
Tensión arterial	<140/90 mmHg
cLDL	**Muy alto riesgo en prevención primaria o secundaria:** reducción del cLDL ≥50% con respecto al valor inicial y un objetivo de cLDL de <1,4 mmol / L (<55 mg / dL) **Riesgo alto:** un régimen terapéutico que logra una reducción de cLDL ≥50% con respecto al valor inicial y un objetivo de cLDL de <1,8 mmol / L (<70 mg / dL). **Riesgo moderado:** Un objetivo de <2,6 mmol / L (<100 mg / dL). **Riesgo bajo:** Un objetivo de <3,0 mmol / L (<116 mg / dL).
Non-cHDL	<85 mg/dL, 100 mg/dL y 130 mg/dL muy alto riesgo, alto riesgo, moderado riesgo respectivamente.
Apo B	<65 mg/dL, 80 mg/dL y 100 mg/dL, muy alto riesgo, alto riesgo, moderado riesgo respectivamente.
Triglicéridos	Recomendación de <1.7 mmol/L (<150 mg/dL)
Diabetes	HbA1c: <7% (<53 mmol/mol)

Adaptado de 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk (140). *Apo: apolipoproteína; IMC: índice de masa corporal; HbA1c: hemoglobina glicosilada; cHDL: colesterol unido a lipoproteínas de alta densidad; cLDL: colesterol unido a lipoproteínas de baja densidad.*

A continuación, se resume en la figura un resumen de las estrategias de diagnóstico y tratamiento para el manejo de HFHe. (Figura 20).

Figura 20. Resumen de las estrategias de diagnóstico y tratamiento.

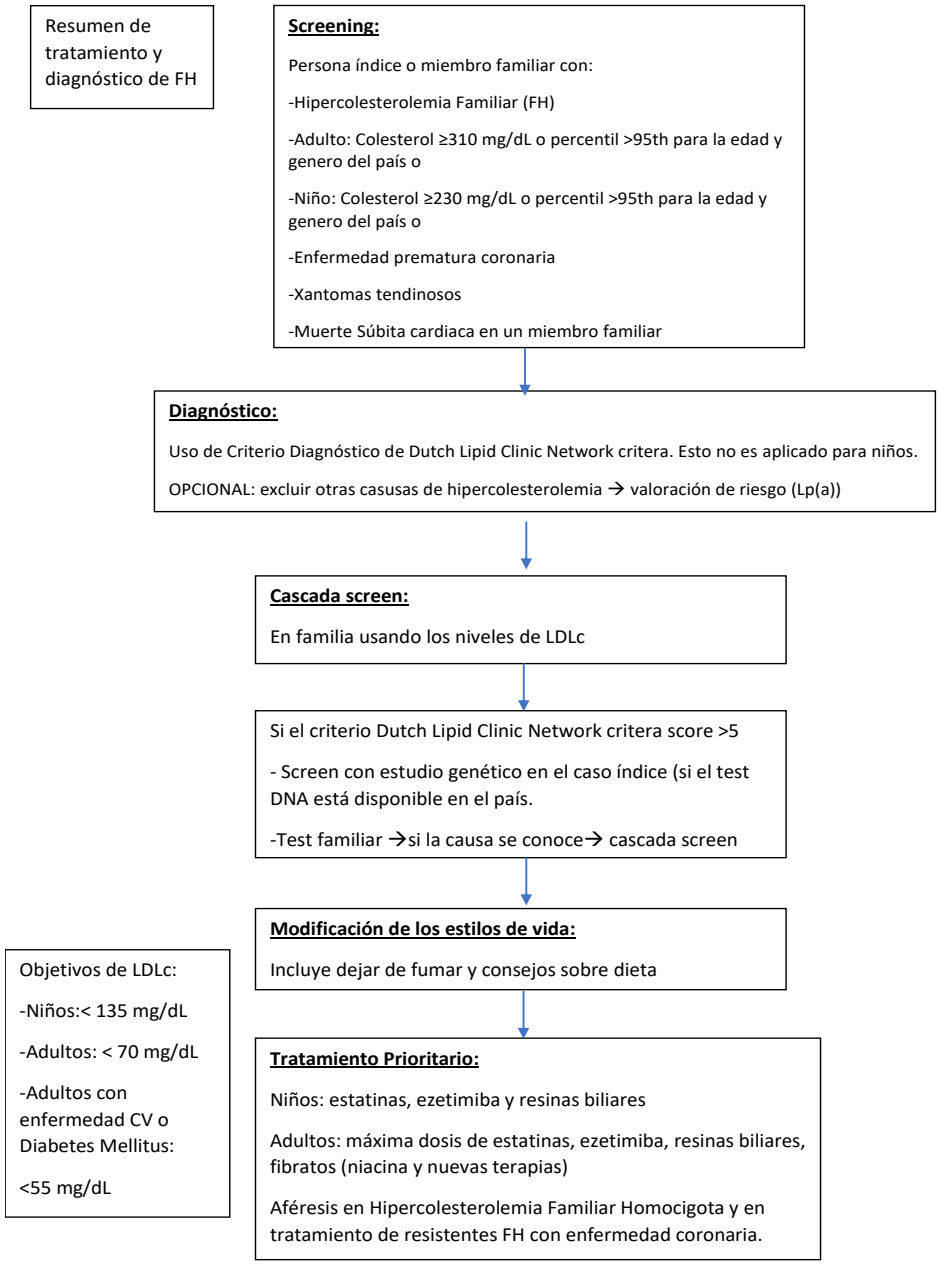


Figura Adaptada de Nordestgaard et al. (106)

JUSTIFICACIÓN

Justificación

La hipercolesterolemia familiar heterocigota (HFHe) es la enfermedad monogénica más frecuente en el adulto, afecta entre 1/200 y 1/250 sujetos, lo que supone entre 14 y 34 millones de personas en el mundo (253,254). Las mutaciones responsables estudiadas hasta la actualidad son aquellas que afectan a los genes que incluyen *LDLR, APOB, PCSK9* y *APOE* (253). Las características clínicas que presentan estos pacientes se deben a un aumento de las concentraciones de las partículas LDL. Por ello presentan depósitos superficiales de colesterol en diferentes tejidos, como arco corneal, xantelasmas y xantomas tendinosos, y una elevada incidencia de enfermedad coronaria prematura personal y familiar. Además, la trasmisión es autosómica codominante y con una alta penetrancia y expresividad (67).

Los estudios realizados a lo largo de estos años han analizado que sin tratamiento un 50% de los varones y un 33% de las mujeres desarrollará enfermedad CV antes de los 55 años y 60 años respectivamente. Los registros internacionales de HFHe como el Simon Broome británico evidenció un riesgo hasta 100 superior de enfermedad coronaria en los varones menores de 40 años afectos de HFHe respecto a la población general en la era pre-estatinas (67,140,229).

En los años 80 con la aparición de las estatinas, se empezó a disponer de tratamiento hipolipemiante efectivo, el cual no se tenía hasta ese momento. Actualmente disponemos de fármacos potentes y seguros para reducir el cLDL, donde se incluyen los inhibidores de la HMGCoA reductasa o estatinas, los inhibidores de NPC1L1, como la ezetimiba, los iPCSK9, como alirocumab y evolocumab, y los inhibidores de angiopoyetina- like proteína 3 (ANGPLTL3) (140,255).

Por otro lado, el análisis de los genes cada vez es más preciso, ya no solamente es analizado clínicamente mediante los criterios diagnósticos de *Dutch Lipid Clinics Network*. Además, a lo largo de los últimos años contamos con un diagnóstico basado en test genéticos definidos por las recomendaciones del *American College of Medical Genetics ACMG*(67). El estudio genético se aplica no solamente a aquellos sujetos de alto riesgo de enfermedad coronaria, sino también a aquellos pacientes analizados en base a criterios

lipídicos, es decir, un subgrupo de personas con posible HF que podría haberse infravalorado cuando el diagnóstico estaba muy centrado en la enfermedad CV.

Son varios los estudios que vienen demostrando estos últimos años la evolución en el fenotipo de estos pacientes, mostrando una enfermedad que pudiera ser más benigna en los años venideros. Dos potentes ensayos clínicos como *FOURIER* y *ODYSSEY OUTCOMES* mostraron una clara disminución de 1,5 % de eventos CV que fueron recogidos entre pacientes tratados con iPCSK9 y con placebo a lo largo de tres años. Ambos estudios disminuyeron el cLDL por debajo de 70 mg/dL, alcanzando los objetivos planteados y sin mostrar efectos secundarios (75,76). Otros estudios analizados en nuestro grupo demostraron que tras tres años de tratamiento mediante iPCSK9 junto con estatinas y ezetimiba los xantomas tendinosos observados en una serie de pacientes, habían disminuido progresivamente (256). En el caso de los xantelasmas depositados de pacientes con HFHe, tratados con dicho fármaco, y continuado en el tiempo durante 26 meses, demostró una regresión de las lesiones lipídicas que podían revertirse con un gran descenso de las concentraciones de cLDL (208).

El fenotipo clásico de la HFHe, muy probablemente, se ha modificado sustancialmente, en especial por una mayor esperanza de vida libre de enfermedad cardiovascular en estos pacientes, por otro lado, un diagnóstico más preciso y un tratamiento más eficaz. Es por ello, que previsiblemente el fenotipo de la enfermedad sea mucho más heterogéneo del que se mostraba en sus descripciones iniciales. Además, se considera la hipótesis de que los fármacos hipolipemiantes utilizados durante mucho tiempo en estos pacientes puedan haber influido en la presentación de otras comorbilidades, favoreciéndolas como la diabetes o mostrando un efecto protector. Se plantea por tanto el estudio de enfermedades potencialmente asociadas con la HFHe diferentes a la enfermedad coronaria clásica, así como de potenciales morbilidades asociadas con el tratamiento hipolipemiante prolongado. Para lograr estos objetivos hemos hecho dos aproximaciones diferentes. En primer lugar, hemos analizado patologías que aparecen en sujetos homocigotos que expresan un fenotipo mucho más grave pero que no estaban descritas en los sujetos heterocigotos. Este ha sido el caso de la enfermedad valvular aórtica que es una complicación de los sujetos homocigotos pero que es desconocida en los heterocigotos. En

segundo caso es la hiperlipoproteinemia(a). La Lp(a) está aumentada en los homocigotos pero su concentración en sangre en heterocigotos no está bien determinada, ni el potencial efecto que la mutación responsable de la hipercolesterolemia pudiera jugar. En el análisis del fenotipo actual de los sujetos HFHe un tema no analizado es la potencial diferencia entre la herencia materna o paterna de las enfermedades asociadas con HF. Este efecto es conocido en otras enfermedades genéticas pero no en la HF. La segunda aproximación ha sido analizar patologías que se han asociado de forma no consistente con el tratamiento hipolipemiante, una de ellas las cataratas en las que había descripciones aisladas en cohortes seguidas hasta 5 años pero sin datos definitivos al respecto. Por último, y para tener una visión holística de enfermedades que pudieran no haberse descrito previamente, hemos analizado un grupo numeroso de familias con HFHe y un grupo de familias control, analizando la mortalidad de los dos grupos en todos los familiares de primer grado de los sujetos probando HFHe. De este modo podríamos por lado actualizar a fecha actual la enfermedad cardiovascular en la HFHe y por otro identificar potenciales patologías que hubiesen pasado desapercibidas hasta la actualidad. Todo ello para conocer en profundidad la repercusión de todo el conjunto de factores que han influido en los sujetos HFHe los últimos años y mejorar en lo posible su tratamiento futuro.

COMPENDIO DE PUBLICACIONES
ESTUDIOS PRINCIPALES

Compendio de publicaciones: estudios principales

Estudio 1 Enfermedad valvular aórtica en ancianos con Hipercolesterolemia Familiar Heterocigótica. Impacto de la terapia hipolipemiante.

1.1 Antecedentes

La Hipercolesterolemia Familiar es una enfermedad común autosómica codominante causada principalmente por mutaciones del gen *LDLR*. La prevalencia de la HFHe es de aproximadamente 1/200-500 personas en la mayoría de los países [1]. Los pacientes con HFHe muestran concentraciones plasmáticas muy elevadas de colesterol unido a lipoproteínas de baja densidad (cLDL) [1,2]. Sin un tratamiento hipolipemiante, aproximadamente el 50% de los hombres y el 30% de las mujeres desarrollarán una enfermedad coronaria antes de los 50 años [1,3,4]. El tratamiento hipolipemiante ha disminuido la enfermedad coronaria en la HFHe y muchos pacientes están alcanzando una esperanza de vida equiparable al resto de la población [4]. Esta mayor tasa de supervivencia puede derivar en otras enfermedades relacionadas con la hipercolesterolemia y/o con los defectos en el LDLr, que no se revierten con las estatinas o que podían estar pasando desapercibidas por disponer de una esperanza de vida más corta. El aumento de nuevos fenotipos ya se ha descrito en la HFHo. Los pacientes pediátricos con HFHo, morían de aterosclerosis coronaria extremadamente prematura, pero dado que el riesgo de enfermedad coronaria se ha reducido sustancialmente gracias a la aféresis de LDL desde la infancia [5-7], a medida que envejecen, muestran un anillo aórtico calcificado, calcificación ascendente de la aorta y un mayor riesgo de estenosis aórtica (EA) grave [6]. La EA es un proceso inflamatorio y degenerativo causado por el daño endotelial consecuencia de la infiltración lipídica, fibrosis progresiva y calcificación, que acaba causando el estrechamiento de la zona valvular aórtica [8]. Curiosamente, la EA ha aumentado progresivamente en los últimos años en la mayoría de los países, incluido en España [9]. Los factores de riesgo para el desarrollo de EA incluyen la edad, la hipercolesterolemia, la DM y la hipertensión, factores de riesgo tradicionales también implicados en el desarrollo de arteriosclerosis [10]. Desafortunadamente, el tratamiento hipolipemiante no ha

demostrado reducir la progresión de la EA a largo plazo, por ello los mecanismos del desarrollo de EA son desconocidos hasta el momento [11]. Existen varios factores que pueden predisponer a esta enfermedad en pacientes con HFHe. En general, las concentraciones cLDL son muy elevadas desde la infancia; además el tratamiento crónico con estatinas parece favorecer la calcificación vascular, que se asocia con la reducción de los componentes lipídicos e inflamatorios en las placas de ateroma [12]. Por otro lado, muchos pacientes con HFHe tienen una concentración elevada de Lp(a), un factor de riesgo independiente de calcificación vascular y de la válvula aórtica (AoVC) [13,14]. Casi la mitad de la población general de edad avanzada (> 75 años) tiene indicios de AoVC y una fracción de ellos padece EA [13]. En consecuencia, la alta mortalidad por cardiopatía coronaria precoz por HFHe [15] podrían estar ocultando la AoVC y la EA que aparecerían en edades avanzadas de la vida. Las mutaciones de *LDLR* predicen fuertemente la AoVC [16], pero aún se desconoce si los pacientes de edad avanzada con HFHe tienen un mayor riesgo de EA.

1.2 Objetivos

Planteamos la hipótesis de que muchos sujetos con HFHe en tratamiento crónico con estatinas, no solo tienen AoVC, sino también parámetros hemodinámicos alterados de la función de la válvula aórtica, incluso cumpliendo con los criterios diagnósticos catalogados de Estenosis Aórtica (EA). Nuestro objetivo fue estudiar estas diferencias funcionales comparando pacientes con HFHe con controles. Además, este estudio evaluó la prevalencia actual de EA en sujetos con HFHe ≥65 años en tratamiento crónico con estatinas. Finalmente, fueron analizados los factores de riesgo potenciales para el desarrollo de EA en HFHe.

1.3 Material y métodos

Este es un estudio observacional, multicéntrico, de casos y controles. En el estudio participaron cinco unidades de lípidos de toda España. Se reclutaron casos de HFHe con los siguientes criterios: edad ≥65 años; una mutación patogénica en un gen candidato para HF (*LDLR, APOB o PCSK9*) en el sujeto o en un familiar de primer grado; niveles

históricos de cLDL ≥220 mg/dL sin tratamiento hipolipemiante; y tratamiento con estatinas ≥5 años. Los controles se seleccionaron de familiares de pacientes con HFHe de las cinco unidades de lípidos, que incluían: ausencia de hipercolesterolemia (cLDL <190 mg/dL sin tratamiento hipolipemiante); edad ≥55 años; y convivientes de HFHe >25 años de convivencia o hermanos HFHe sin diagnóstico positivo. Se reclutaron, además, casos adicionales de HFHe de familiares de casos cuando cumplían con los criterios de inclusión. Los participantes fueron excluidos si tenían antecedentes personales de cardiopatía reumática.

El componente clave fue la valoración de un ecocardiograma transtorácico. Los datos de laboratorio se obtuvieron de los registros en las unidades de lípidos con fechas <1 año a la ecografía cardíaca, si cumplían con el criterio de tratamiento hipolipemiante estable. Cuando estos datos no estuvieron disponibles, se extrajo una muestra de sangre durante la visita. Todos los procedimientos se realizaron localmente, en cada unidad de lípidos.

Todos los sujetos dieron su consentimiento informado por escrito al protocolo, que fue aprobado por un comité ético central (Comité Ético de Investigación Clínica de Aragón, CEICA).

Ecocardiografía transtorácica

Los ecocardiogramas transtorácicos convencionales fueron realizados por cardiólogos certificados a nivel nacional en ecocardiografía, según el protocolo para este estudio. Los estudios de ecocardiograma se enfocaron en la válvula aórtica con la misma posición para todos los pacientes.

Se realizaron las siguientes mediciones:

- gradiente medio de presión de la válvula aórtica;
- velocidad aórtica máxima (Vmax);
- área de la válvula aórtica;
- área de la válvula aórtica indexada al área de superficie corporal;
-válvula de aorta bicúspide o tricúspide;
- engrosamiento valvular> 3 mm;
- y calcificación de las valvas de la válvula aórtica.

El cardiólogo que realizaba los ecocardiogramas era ciego al estudio, no conocía el diagnóstico de HFHe. El grado de calcificación de la válvula aórtica se puntuó de la siguiente manera: 1, sin calcificación; 2, levemente calcificado (pequeñas manchas aisladas); 3, moderadamente calcificado (múltiples manchas grandes); y 4, muy calcificado (engrosamiento extenso y calcificación de todas las cúspides). La EA se diagnosticó según las guías de *American College of Cardiology/American Heart Association Task Force on Practice Guidelines*. Se considera EA con cualquiera de los siguientes hallazgos: gradiente medio de presión de la válvula aórtica ≥20 mm; Vmax ≥2 m/s; y área de la válvula aórtica ≤1 cm^2. Los estadios de EA fueron leves (Vmax 2-2,9 m/s y gradiente de presión de la válvula <20 mm y área de la válvula aórtica>1 cm^2); moderada (Vmax 3-3,9 m/s o gradiente de presión de la válvula 20-39 mm y área de la válvula aórtica >1 cm^2) y severa (gradiente de presión de la válvula ≥40 mm; o Vmax ≥4 m/s; o área de la válvula aórtica ≤1 cm^2). La esclerosis de la válvula aórtica (ASc), una afección aórtica más leve, se definió en presencia de engrosamiento (>3 mm) y/o calcificación de la válvula aórtica sin obstrucción significativa del flujo (Vmax <2 m/s) o criterios de EA.

Entrevista clínica

Sobre la información de los datos clínicos, se recogió edad, sexo, nivel de estudios, antecedentes de tabaquismo, hipertensión, diabetes, antecedentes personales de enfermedad cardiovascular y antecedentes familiares de enfermedad cardiovascular en familiares de primer grado, edad a la que ocurrieron los eventos cardiovasculares, valores de lípidos sin tratamiento y antecedentes de tratamiento hipolipemiante. El nivel de educación se clasificó en primaria, secundaria y educación superior. El tabaquismo actual se definió por fumar en el presente o haber fumado en el último año. Los exfumadores se definieron como sujetos que habían fumado al menos 50 cigarrillos en su vida, pero que no habían fumado en el último año. El consumo de tabaco se registró como el número de paquetes diarios fumados multiplicado por el número de años fumados.

Referente al tratamiento hipolipemiante, registramos la edad a la que comenzó el tratamiento con estatinas, la estatina prescrita con mayor frecuencia, la dosis de estatina, el uso de ezetimiba, la edad a la que comenzó el tratamiento con ezetimiba y estatina y la dosis que se prescribía como tratamiento actual.

En los participantes con enfermedad cardiovascular previa, se registró la edad del primer evento y el tipo de evento: infarto de miocardio; síndrome coronario agudo con hospitalización; accidente cerebrovascular isquémico; revascularización coronaria, carotídea o periférica; la muerte súbita; o aneurisma aórtico.

Examen físico

En el examen físico se registró talla, peso, presión arterial sistólica y diastólica y presencia de xantomas tendinosos. El índice de masa corporal (IMC) se calculó como el peso en kilogramos dividido por el cuadrado de la altura en metros.

Pruebas de laboratorio

Cuando los valores de lípidos actuales (<1 año) no estaban disponibles, se obtuvo una muestra de sangre para determinar, CT, TG, cHDL, Apo B, Lp(a), glucosa y HbA1c. En cada centro se realizaron mediciones de laboratorio y conservación de muestras.

Definiciones

La hipertensión arterial se definió como presión arterial sistólica ≥140 mmHg, presión arterial diastólica ≥90 mmHg o uso actual de medicación antihipertensiva. La diabetes se definió como glucosa plasmática en ayunas ≥126 mg/dL, HbA1c ≥6,5% o uso actual de medicación antidiabética.

Análisis estadístico

Los datos se expresan como media (desviación estándar) o porcentaje. Para las comparaciones entre casos y controles, las variables ecográficas de la válvula aórtica y la presencia de niveles de afectación de la válvula aórtica se analizaron mediante regresiones lineales y logísticas basadas en ecuaciones de estimación generalizada (GEE) con diferentes niveles de ajuste: no ajustada, ajustada por sexo y edad y además se ajustó para la concentración de cLDL sin tratamiento. Los análisis estratificados para casos y controles y los restringidos a casos se basaron en modelos lineales generalizados, e incluyeron, para estimar su influencia, la variable concentración de cLDL sin tratamiento o años de vida con fármacos hipolipemiantes. La influencia del cLDL y el tratamiento hipolipemiante se

estudiaron por separado en los estratos de casos y controles. Todos los análisis se realizaron con el software estadístico R versión 3.4.4. y el paquete "gee" versión 4.13.19.

1.4 Resultados

El equipo de investigación reclutó 205 sujetos, 112 casos y 93 controles. La edad media fue de 71,8 años y 70,0 años en los grupos de casos y controles, respectivamente. Además del CT y cLDL sin tratamiento hipolipemiante, la edad, la prevalencia de enfermedad cardiovascular previa y los antecedentes familiares de prevalencia de enfermedad cardiovascular prematura, también fueron más altos en el grupo de HFHe que en el grupo de control. El IMC fue similar en ambos grupos. No hubo diferencias en tabaquismo, hipertensión y DM tipo 2 (tabla 1). Todos los casos estaban en tratamiento hipolipemiante con un tiempo medio de tratamiento de 22,5 (8,7) años.

Caracterización de la válvula aórtica

El gradiente medio de presión de la válvula aórtica fue mayor en los casos (7,4 mmHg) que en los controles (5,0 mmHg), después de ajustar por edad y sexo (P = 0,003). Los pacientes con HFHe, en comparación con los controles, tenían mayor Vmax (1,7 m/s y 1,5 m/s, respectivamente, p = 0,011), y menor área de la válvula aórtica (2,0 cm^2 y 2,4 cm^2, respectivamente, p <0,001)). Entre los pacientes con HFHe, la puntuación media de calcificación valvular de las valvas de la válvula aórtica fue mayor y el engrosamiento valvular fue más prevalente (P = 0,004). La fracción de eyección del ventrículo izquierdo tendió a ser menor en los casos (65,7% vs 67,2%, P = 0,056). Todas las válvulas estudiadas fueron tricúspides. La EA con criterios moderados o graves y la esclerosis aórtica, fueron más prevalentes entre los HFHe (7% vs 1%, OR ajustado por edad y sexo 8,33, IC 95% 1,22, 57,10, P = 0,031; y 55% vs 32%, OR ajustado por edad y sexo 1,90; IC del 95%: 1,04; 3,47; P = 0.061 respectivamente) (Tabla 2) y aumentó con la edad (Figura 1). Además, las comparaciones de las mediciones aórticas ajustadas y la prevalencia de estenosis para las concentraciones de cLDL sin tratamiento, no mostraron ninguna diferencia significativa. Por lo tanto, cLDL podría estar justificando en parte, las diferencias de válvulas entre HFHe y controles, pero como el cLDL es parte de la definición de caso y control, para aclarar el problema, se realizaron regresiones estratificadas.

Mostraron que la edad, pero no el cLDL, se asoció significativamente con todas las variables de la válvula aórtica entre los controles. Además la Vmax y la puntuación de calcificación de la válvula aórtica también se asociaron con la concentración de cLDL sin tratamiento entre los casos de HFHe. El gradiente medio de la válvula aórtica aumentó 4,1 (2,1, 6,1) mmHg/10 años entre los casos, mientras que solo aumentó 0,8 (0,0, 1,6) mmHg/10 años entre los controles de diferentes grupos de edad.

Todos los datos clínicos y de laboratorio fueron similares en todos los estadios de la válvula aórtica, excepto por la presencia de xantomas de tendón.

Factores de riesgo de enfermedad valvular

Para evaluar cómo el tratamiento con estatinas entre los pacientes con HFHe podría modificar los parámetros de la válvula aórtica entre estos pacientes, utilizamos modelos que incluían el sexo, la edad y la duración del tratamiento con estatinas. El área de la válvula aórtica disminuyó y la puntuación de calcificación de la válvula aórtica aumentó significativamente con la edad ($P < 0,001$) e independientemente de las estatinas. La fracción de eyección fue independiente de la edad, pero disminuyó con la duración del tratamiento con estatinas ($P = 0,005$). El gradiente medio de la válvula aórtica aumentó con la edad en los casos y controles, pero con una tasa de incremento más alta en la HFHe (Figura 2).

Estudio 2. La hipercolesterolemia de herencia materna no modifica el fenotipo cardiovascular en la Hipercolesterolemia Familiar

2.1 Antecedentes

La Hipercolesterolemia Familiar (HF) es una enfermedad autosómica codominante caracterizada por concentraciones muy elevadas de colesterol unido a cLDL, depósitos superficiales de colesterol en forma de arcos corneales y xantomas tendinosos, y alto riesgo de enfermedad CV prematura en ausencia de un tratamiento hipolipemiante adecuado (1, 2). Las concentraciones de cLDL de HFHe tienden a ser aproximadamente el doble que las de los sujetos de la población general y su riesgo de enfermedad CV en las primeras décadas de vida, especialmente enfermedad coronaria, es hasta 100 veces mayor (3). Sin embargo, una característica de la HFHe es la gran variabilidad en la presentación clínica, incluidas las concentraciones de cLDL, y la presencia de xantomas tendinosos o enfermedad de las arterias coronarias (4). Esta variabilidad es multifactorial y se ha asociado con: el gen responsable de la HF, con un fenotipo más severo en portadores de mutaciones de *LDLR* que en aquellos con mutación en *APOB, PCSK9 o APOE* (5, 6); el tipo de mutación causal, con peor fenotipo en los portadores de alelos nulos que en los portadores de alelos defectuosos (7); la interacción con otros genes, como *ABCA1 o PSCK9* (8, 9); y la presencia de factores de riesgo de enfermedad CV comunes a la población general, como tabaquismo, diabetes, colesterol unido a cHDL y niveles elevados de Lp(a) (10). A pesar de todo esto, el origen de gran parte de esta variación clínica en HFHe sigue siendo desconocido (1, 4).

Uno de los factores potenciales asociados con la variación clínica de los sujetos con HFHe es el origen parental del defecto genético. Algunos de los fenómenos relativamente frecuentes en la naturaleza que podrían explicar diferencias en el fenotipo de enfermedades monogénicas son la denominada *impronta genómica,* que consiste en que el nivel de expresión de los alelos de un gen depende de su origen parental (11); y un efecto materno, donde el fenotipo de la descendencia está determinado no solo por el ambiente y genotipo postnatal sino también por el ambiente durante la gestación (12). Estos fenómenos epigenéticos se producen por modificaciones de la cromatina, principalmente por

metilación del ADN, acetilación de histonas o la interacción de ARN no codificantes con el ADN. La inducción de la metilación del ADN está muy influenciada por el entorno materno (13). Los genes de *impronta genómica* no se han asociado con HF hasta el momento (11). Sin embargo, se ha atribuido un efecto maternal en la HFHe debido a un posible efecto de la hipercolesterolemia materna durante el embarazo que condicionaría una memoria metabólica durante la edad adulta (14). Se ha observado que los sujetos con HFHe de origen materno pueden tener niveles más altos de cLDL (15) y una mayor mortalidad por enfermedad CV que los sujetos con HFHe de origen paterno (16). Sería similar a lo que ocurre con el tabaquismo de la madre, con una dieta rica en grasas saturadas durante el embarazo (17), o con el bajo peso al nacer, asociados con riesgo de diabetes (18), hipertensión arterial o enfermedad cardiovascular ateromatosa en la edad adulta (19).

El efecto de la hipercolesterolemia durante el embarazo se ha estudiado en diferentes modelos animales, observando que dicha enfermedad favorece el desarrollo temprano de lesiones arterioscleróticas en recién nacidos y un mayor riesgo de diabetes e hipertensión arterial en la edad adulta (20, 21). Sin embargo, se desconoce si este efecto sucede en los humanos. El tratamiento hipolipemiante está contraindicado durante el embarazo y, dado que los niveles de colesterol aumentan fisiológicamente durante el segundo y tercer trimestre del embarazo, el aumento del colesterol es sustancial en las mujeres embarazadas con HFHe (22). Por tanto, la HF es un buen modelo para identificar si la hipercolesterolemia severa durante el embarazo en mujeres con HFHe condiciona el fenotipo en la descendencia y explica, al menos en parte, las diferencias que encontramos entre sujetos adultos con HFHe.

2.2 Objetivos

El objetivo de este análisis fue identificar las diferencias potenciales en la antropometría, los depósitos de lípidos superficiales, las comorbilidades y las concentraciones de lípidos entre sujetos de origen maternal o paternal de hipercolesterolemia, dentro de un gran grupo de HFHe.

2.3 Material y métodos

Este estudio observacional, transversal, multicéntrico desarrollado a nivel nacional en España, se diseñó para identificar diferencias en la HFHe según el origen parental de la hipercolesterolemia. Los datos de los pacientes con HFHe se obtuvieron del Registro de Dislipidemias de la Sociedad Española de Aterosclerosis (SEA). El Registro de Dislipidemias de la SEA es un registro activo online, donde 65 unidades de lípidos certificadas, de diferentes regiones españolas, informan de casos con varios tipos de hiperlipidemias primarias (23). Los criterios de inclusión y la recopilación de datos se estandarizaron entre las unidades en 5 sesiones antes del reclutamiento de los casos. Se obtuvo el consentimiento informado por escrito de cada paciente incluido en el estudio; el protocolo del estudio se ajusta a las directrices éticas de la Declaración de Helsinki de 1975; el protocolo de estudio ha sido previamente aprobado por el Comité Ético de Investigación Clínica de Aragón.

Los sujetos con HFHe eran elegibles para su inclusión en este análisis si tenían un diagnóstico clínico o genético de HFHe. El diagnóstico clínico se basó en los criterios diagnósticos propuestos por los Criterios *Dutch Lipid Clinic Network*: 6-8 puntos (probable) y >8 puntos (definitivo). El diagnóstico genético se seleccionó en base a la mutación patogénica que portaba el probando para HF. La definición de patogenicidad de mutaciones siguió las recomendaciones del ACMG del *American College of Medical Genetics* (1). Los HFHo fueron excluidos de este estudio. Los pacientes en los que la herencia parental de HF era desconocida, no se incluyeron en el análisis final.

Variables de estudio

- *Entrevista clínica*

En el registro se incluyen datos de HFHe, como los antecedentes de salud personal y familiar, de antropometría, exploración física, datos de laboratorio, presencia de enfermedad CV, edad en la que se inició el tratamiento con estatinas, antecedentes de tratamiento hipolipemiante y datos genéticos sobre mutaciones en *LDLR, APOB o PCSK9* (positivo, negativo o desconocido).

- *Historial de salud familiar*

La información sobre la transmisión de la hipercolesterolemia derivada de los padres fue transmitida por el probando y confirmada a partir de las historias clínicas del paciente. La enfermedad CV se definió como: coronaria (infarto de miocardio, procedimiento de revascularización coronaria, muerte súbita); cerebral (accidente cerebrovascular con déficit neurológico > 24 h sin sangrado en las pruebas de imagen cerebral); enfermedad vascular periférica (claudicación intermitente con índice tobillo-brazo <0,9 o revascularización arterial de miembros inferiores) o aneurisma aórtico abdominal sintomático o asintomático.

- *Pruebas de laboratorio*

Los niveles de lípidos y lipoproteínas se analizaron en ayunas sin tratamiento hipolipemiante durante al menos 6 semanas.

Definiciones

La hipertensión arterial se definió como presión arterial sistólica ≥140 mmHg o presión arterial diastólica ≥90 mmHg o consumo de medicación antihipertensiva. El IMC se calculó mediante el peso en kilogramos dividido por el cuadrado de la altura en metros. La DM se definió según el consumo de medicamentos antidiabéticos. Fumador actual se definió como fumador actual o fumador en el último año. Exfumador se definió como un sujeto que había fumado al menos 50 cigarrillos en su vida, pero que no había fumado en el último año.

Realizamos este estudio de acuerdo con la Declaración de Helsinki para la protección de los derechos y el bienestar de las personas que participan en la investigación biomédica.

Análisis estadístico

Las variables se expresaron como media (desviación estándar) o porcentaje. Las diferencias no ajustadas entre los grupos de padres se analizaron con la prueba t de Student o la prueba de chi-cuadrado, según correspondía. Se utilizaron modelos de regresión lineal y logística ajustados por edad y sexo para definir las características clínicas observadas y para comparar las diferencias entre el origen parental. Las diferencias en la prevalencia de comorbilidades se estudiaron con modelos de regresión logística ajustados por edad, sexo

e IMC. Se realizó un análisis de sensibilidad restringiendo el conjunto de datos a aquellos sujetos con mutación genética confirmada. Todos los análisis de datos se realizaron con SPSS versión 22 y R versión 3.6.0. Se realizó un cálculo de potencia post-hoc para analizar la diferencia de prevalencia de enfermedad cardiovascular según el origen parental de la HF, P <0,05 se consideró estadísticamente significativo.

2.4 Resultados

Características clínicas

Los pacientes con HFHe se agruparon en 1231 HFHe-madre-descendientes y 1174 HFHe-padre-descendientes, de 45,7 (16,3) años y 44,8 (16,7) años, respectivamente. En 884 sujetos el origen parental de la enfermedad fue desconocido. Las principales características de los tres grupos se presentan en la Tabla 1 y en la Tabla Suplementaria 1. Los sujetos con origen hipercolesterolémico parental desconocido eran más mayores que los otros dos grupos. Sin embargo, no se encontraron otras diferencias en el resto de las variables estudiadas entre los tres grupos que diferían en el origen parental, incluyendo CT, TG, cLDL, cHDL y Lp(a), puntuaciones de DLCN o intensidad o duración del tratamiento hipolipemiante. No hubo diferencias en ninguna de estas variables ajustadas por edad y sexo entre los HFHe de origen materno o paterno (Tabla 2), considerándose solo aquellos sujetos HFHe con confirmación genética (Tabla 3). Todas las variables se analizaron estratificadas según el sexo, sin observar diferencias estadísticas entre hombres y mujeres. (Tablas complementarias 4-7).

Prevalencia de enfermedades cardiovasculares, obesidad, diabetes e hipertensión.

La prevalencia de estas morbilidades se presenta en las Tablas 4 y 5. No diferían entre grupos, incluso después de ajustar por edad, sexo e IMC, cuando éste aplicaba (no fue ajustado por IMC en los resultados antropométricos). Como era de esperar, la prevalencia de DM fue baja en ambos grupos y respecto a las enfermedades estudiadas no hubo ninguna tendencia a mostrar diferencias entre los tres grupos. Estimamos que nuestra muestra tiene un 80% de poder estadístico para detectar un riesgo relativo de 1,367 entre

dos grupos de 1202 personas cuando la prevalencia global es de 12 casos por 100 con un umbral alfa de 0,05.

Prevalencia de enfermedades cardiovasculares, obesidad, diabetes e hipertensión por grupos de edad.

Para identificar en profundidad las posibles diferencias en la prevalencia de la morbilidad y las diferencias en su evolución según la edad, estudiamos todas las variables y morbilidades según las décadas de edad. Ninguna de las variables estudiadas mostró diferencias entre grupos de origen parental. La prevalencia de DM, enfermedad CV, concentración de cLDL y presión arterial aumentaron progresivamente y de forma similar, a medida que aumentaba la edad.

Estudio 3. Lipoproteína (a) en hipercolesterolemia hereditaria. Influencia de la causa genética, gen defectuoso y tipo de mutación.

3.1 Antecedentes

La Lp(a) es una variante de las lipoproteínas de baja densidad (LDL) (1) causada por una glicoproteína adicional, llamada apolipoproteína(a) (Apo(a)), unida covalentemente a la apolipoproteína B100 (apoB) (2, 3). Apo(a) está codificada por el gen *LPA*, que deriva de la evolución del gen del plasminógeno. Apo(a) contiene 10 subtipos diferentes de plasminógeno kringle IV (KIV), 1 copia de kringle V y un dominio de proteasa inactivo (4). El tamaño de la isoforma Apo(a) varía, dependiendo del número de copias de KIV tipo 2 (de 1 a 40) y está inversamente relacionado con la concentración plasmática de Lp(a) (5). Alrededor del 25% de los sujetos tienen una concentración de Lp(a)>50 mg/dL, una cifra considerada clínicamente relevante, que aumenta el riesgo cardiovascular (5, 6). El gen *LPA* determina más del 90% la variabilidad en los niveles plasmáticos de Lp(a), con una discreta influencia de factores ambientales, incluida la dieta (7).

La concentración alta de Lp(a) es un factor de riesgo independiente de enfermedad CV (6, 8). Los estudios epidemiológicos, de aleatorización mendeliana, de asociación de todo el genoma y, muy recientemente, de intervención con iPCSK9 han mostrado una relación lineal y positiva entre la concentración de Lp(a) y el riesgo de infarto de miocardio e ictus isquémico (2, 9-11). Además, la estenosis de la válvula aórtica aumenta en sujetos con concentraciones elevadas de Lp(a) (10, 12). Sin embargo, el mecanismo responsable del efecto proaterogénico de Lp(a) es mayormente desconocido (2, 13). De hecho, muchos otros aspectos de la fisiopatología de Lp(a) son poco conocidos (1, 2, 14). El LDLr es el principal receptor responsable del catabolismo de LDL, pero su participación en el catabolismo de Lp(a) es controvertida. Los pacientes homocigotos con HF que portan dos alelos de *LDLR* nulos tienen una concentración de Lp(a) dos veces mayor que los miembros de la familia que no son HF, con un claro efecto de dosificación genética (15), mientras que los estudios en sujetos con HFHe han demostrado resultados no concluyentes. Sin embargo, otros estudios in vitro e in vivo han descartado el LDLr como una vía

significativa para el catabolismo de Lp(a) (16). Los estudios clínicos no son del todo consistentes y se acompañan de importantes sesgos de limitación, principalmente la heterogeneidad en los criterios utilizados para el diagnóstico de HF, la población estudiada, los genes responsables de la enfermedad o el dominio proteico afectado por el defecto genético.

3.2 Objetivos

El objetivo del presente estudio es comparar la concentración de Lp(a) en controles (extraídos de una población sana) con sujetos con diferentes hipercolesterolemias genéticas, para explorar si la concentración de cLDL, el gen defectuoso involucrado y el dominio de la proteína donde ocurre el defecto, están asociados con la concentración de Lp(a).

3.3 Material y métodos

Sujetos con hipercolesterolemia familiar

Los pacientes fueron remitidos por médicos de atención primaria a la Unidad de Lípidos del Hospital Universitario Miguel Servet de Zaragoza para el estudio de su hipercolesterolemia. Se incluyeron todos los pacientes remitidos desde enero de 2006 a marzo de 2020, ≥18 años, con diagnóstico clínico de hipercolesterolemia primaria, con sospecha de HFHe y con estudio genético completo de los genes responsables de HF. El protocolo de derivación de los pacientes a nuestra Unidad, se basa en criterios previamente acordados e incluye concentraciones de CT>300 mg/dL (una vez excluidas las causas secundarias).

Se definió hipercolesterolemia primaria cuando cLDL ≥190mg/dL o no-cHDL ≥220 mg/dL o apoB ≥120 mg/dL y TG <400 mg/dL en ausencia de causas secundarias de hipercolesterolemia: índice de masa corporal >35 kg/m^2, hormona estimulante del tiroides >6 mU/L, creatinina >2,0 mg/dL, diabetes mal controlada (HbA1c> 7,5%), colestasis (bilirrubina directa >1 mg/dL) o uso de fármacos que favorezcan trastornos del metabolismo lipídico. En aquellos pacientes con sospecha de hipercolesterolemia genética, se incluyeron a todos los sujetos con hipercolesterolemia primaria grave (cLDL ≥220

mg/dL si la edad era <40 años o ≥230 mg/ dL si la edad era ≥40 años), transmisión vertical de hipercolesterolemia en la familia y cLDL> en el percentil 95 en al menos un familiar de primer grado (Figura 1). Los resultados del análisis genético (detallados a continuación) permitieron clasificar a los pacientes en HFHe (aquellos con mutaciones "patogénicas" y "probablemente patogénicas" en genes canónicos de HF (N = 511), pacientes con una mutación de significado incierto (N = 69) y pacientes con mutación negativa (aquellos sin mutación funcional en genes conocidos en la HF) (N = 886) (Figura 1).

Controles

Los controles se seleccionaron del estudio *Aragon Workers Health Study* (AWHS). El AWHS es un estudio de cohorte longitudinal de factores de riesgo cardiovascular y aterosclerosis subclínica que se viene estudiando desde 2009 (17). La Lp(a) se determinó al inicio del estudio en un subconjunto aleatorio de participantes. Todos los individuos ≥18 años de AWHS, con determinación de Lp(a) se incluyeron como controles (N = 1221). Todos los participantes firmaron un consentimiento informado, aprobado por el Comité Ético de Investigación Clínica de Aragón antes de ser incluidos en el estudio.

- *Datos clínicos*

Se recogieron antecedentes personales de diabetes, hipertensión, tabaquismo, enfermedades cardiovasculares y tratamiento farmacológico, antecedentes familiares de hipercolesterolemia y enfermedades cardiovasculares en casos y controles. Durante la misma visita se realizó una exploración física que incluyó talla, peso, perímetro de cintura, presencia de arco corneal y xantomas tendinosos.

- *Medidas de laboratorio*

Se obtuvo una muestra de sangre después de 10 horas de ayuno y sin tratamiento hipolipemiante durante al menos 5 semanas, excepto en aquellos sujetos con antecedentes personales de enfermedad cardiovascular o riesgo de enfermedad CV muy alto. Ningún paciente estaba siendo tratado con iPCSK9, un tratamiento que podría disminuir la concentración de Lp(a). En los casos y controles se determinaron los niveles de CT, TG, cHDL, Lp(a), Apo A1 y Apo B, glucosa, ácido úrico, creatinina y enzimas hepáticas y

musculares. Los valores de cLDL se calcularon mediante la ecuación de Friedewald y el cLDL corregido por Lp(a) se calculó restando 1/3 de la concentración de Lp(a) al valor de cLDL (18). Todas las mediciones bioquímicas se realizaron en un laboratorio central como se describió anteriormente (19). Los valores de lípidos se ajustaron en aquellos participantes que tomaban fármacos hipolipemiantes según la terapia con la que estaban tratados (20). La Lp(a) se determinó mediante nefelometría, en un sistema de inmunoquímica IMMAGE 800® (Beckman Coulter, EE. UU.). Los resultados de Lp(a) por debajo del umbral de detección se imputaron a 0,5 mg/dL, a la mitad de ese umbral. Las muestras de los sujetos incluidos en este estudio fueron cedidas por el Biobanco del Sistema de Salud de Aragón (PT17 / 0015/0039) con la correspondiente aprobación de los Comités Ético y Científico.

- *Estudio genético*

En todos los sujetos con sospecha clínica de HF se estudiaron los genes *LDLR* (NM_000527.4), *APOB* (NM_000384.2) y *PCSK9* (NM_174936.3) con las plataformas Progenika Biopharma Grifols (Derio, España) (21) o GENinCode (Terrassa-Barcelona, España) (22) como se describió anteriormente. Estas plataformas incluyen mutaciones puntuales, grandes reordenamientos y variaciones en el número de copias. Además, todos los sujetos fueron sometidos a secuenciación del exón 4 de *APOE* (NM_000041.4),), ya que se ha descrito como causa de HF (23).

El polimorfismo genético *LPA* (NM_005577.4) responsable de la variabilidad del tamaño de Lp(a) se analizó mediante una metodología basada en PCR en tiempo real (24).

- *Funcionalidad de las mutaciones*

Las variantes genéticas en los genes *LDLR, APOB yCK9* se clasificaron como "patogénicas", "probablemente patogénicas", "significado incierto", "probablemente benignas" y "benignas" según las directrices del *American College of Medical Genetics and Genomics* (ACMG) (25).

- *Dominios defectuosos del receptor de LDL*

Las mutaciones se clasificaron según el dominio afectado y la ubicación del gen (exón, intrón o UTR). Las mutaciones en *LDLR* se dividieron en exónicas, intrónicas y UTR y, además, las mutaciones exónicas también se clasificaron en sus dominios proteicos correspondientes: péptido señal, dominio de unión a ligando (LBD), dominio homólogo al precursor de factor de crecimiento epidérmico A (EGF -A), dominio homólogo al precursor de EGF-B, hélice β, dominio homólogo al precursor de EGF-C, dominio de unión a azúcares por O, dominio transmembrana y dominio citosólico. Las mutaciones en *APOB* se dividieron en categorías exónicas, intrónicas y UTR. Las mutaciones de *PCSK9* se dividieron en dominios proteicos: péptido señal, prodominio, dominio catalítico y dominio C-terminal.

- *Puntuación poligénica*

El score poligénico (SP) se basó en 12 variaciones comunes de un solo nucleótido (SNV) (Tabla complementaria 1) identificadas como aumento de cLDL a partir de estudios de consorcio de asociación de genoma de poblaciones europeas-caucásicas (26)

- *Cohorte de validación*

Todos los sujetos no emparentados del Registro de Dislipidemias de la Sociedad Española de Aterosclerosis (SEA), y excluidos los sujetos del Hospital Universitario Miguel Servet, con los mismos criterios de inclusión y exclusión que el grupo principal de estudio fueron analizados como cohorte de validación. El Registro de Dislipidemias de la SEA es un registro online activo, en el que las unidades de lípidos certificadas de todas las regiones de España notifican casos de varios tipos de hiperlipidemias primarias (27). Los sujetos se definieron con los mismos criterios. Se obtuvo el consentimiento informado por escrito de cada paciente incluido en el registro.

-<u>Análisis estadístico</u>

Las variables se expresaron como media (desviación estándar), mediana (rango intercuartílico) o porcentaje. Las diferencias no ajustadas entre los grupos se analizaron con *Kruskal-Wallis Rank Sum Test* or *Chi-squared Test*, según fuera apropiado. Se

utilizaron modelos de regresión lineal ajustados por edad, sexo e IMC para estudiar los lípidos plasmáticos. La Lp(a), las repeticiones de kringle y los TG fueron logarítmicamente transformados para las regresiones. Estos análisis se limitaron a los sujetos que disponían de las variables completas para las variables de ajuste. Para estudiar la bondad del ajuste, se realizó con pruebas de razón de verosimilitud (test de heterogeneidad entre grupos). Para la Lp(a) donde los test fueron altamente significativos, se realizaron test post-hoc por pares de acuerdo a los coeficientes de regresión. Se creó un modelo de regresión adicional para estudiar la interacción de cLDL y el grupo (controles vs HFHe) para Lp(a).

3.4 Resultados

Características clínicas de los pacientes y controles hipercolesterolémicos

El estudio incluyó a 1466 sujetos con hipercolesterolemia hereditaria remitidos para estudio genético a la clínica de lípidos y a 1221 sujetos de control del estudio AWHS. Según el estudio genético, los sujetos con hipercolesterolemia se clasificaron en tres grupos: HFHe con mutación patogénica (n = 511); sujetos con una mutación, pero con implicaciones funcionales de significado incierto (n = 69); y sujetos sin mutación identificada en ninguno de los genes HF (n = 886) (Figura 1). Las principales características de los diferentes grupos se presentan en la Tabla Complementaria 2. Como era de esperar, los grupos de hipercolesterolemia tenían concentraciones más altas de CT, cLDL, Apo B y cHDL, mientras que el IMC fue significativamente menor que el del grupo control, según los criterios de inclusión de hipercolesterolemia primaria. Las diferencias entre las variables de lípidos fueron estadísticamente significativas después de ajustar por edad, sexo e IMC (Tabla 1, grupos restringidos a casos de variables completas, ver Tabla complementaria 3). Con respecto a la concentración de Lp(a), los sujetos con mutaciones negativas tenían una concentración significativamente más alta que la HFHe, y ambas más altas que el grupo de control (Figura 2A). El porcentaje de sujetos con concentración de Lp(a) ≥50 mg/dL en HFHe con una mutación patogénica, sujetos con una mutación, pero con implicaciones funcionales de significado incierto, y en sujetos con mutación negativa fue del 31,1%, 43,5% y 52,3% respectivamente. Estos porcentajes fueron todos

significativamente más altos que en los controles (23,1%), (p <0,01) (Tabla complementaria 4).

Concentración de Lp(a) en HF según el gen responsable

La etiología de la HFHe 511, según el gen responsable fue: *LDLR* 443 sujetos, *APOB* 27 sujetos, portadores de la mutación p.(Leu167del) en *APOE* 37 y *PCSK9* 4 sujetos. La lista completa de mutaciones se presenta en la Tabla complementaria 5. Los lípidos diferían entre los grupos de genes después de ajustar por edad, sexo e IMC (Tabla 2, grupos restringidos a casos de variables completas). La Lp(a) difirió entre los sujetos con mutación *LDLR, APOB y APOE* (p <0,001). La concentración media geométrica ajustada por edad, sexo e IMC de la Lp(a) fue mayor en HF dependiente de *APOB*, 36,5 mg/dL (IC 95% 22,0, 60,8), intermedia en HF dependiente de *LDLR*, 21,7 mg/dL (IC del 95% 17,9, 26,4) y el más bajo en portadores de la mutación p.(Leu167del) en *APOE*, 7,99 mg/dL (IC del 95% 4,9, 12,7). Todas las diferencias de Lp(a) entre grupos con un número razonable de casos (excepto mutaciones en el gen *PCSK9*, N = 4), fueron estadísticamente significativas por pares (Figura 2B). La media geométrica de las repeticiones de *LPA* KIV-2 no difirió entre los subgrupos de genes HF y las estimaciones y las diferencias para la Lp(a) permanecieron sin cambios después del ajuste por el número de repeticiones de KIV-2.

Efecto de las mutaciones de *LDLR* sobre la concentración de Lp(a) según el dominio proteico afectado en el LDLr

La ubicación de las mutaciones de *LDLR* en los 443 sujetos se muestra en la Tabla complementaria 5. No hubo diferencias en la concentración de Lp(a) entre los grupos, ni después de agrupar las mutaciones entre los 4 grupos principales: alelos nulos, dominio de unión al ligando, dominio homólogo al precursor EGF y de ayuste (*splicing)*; ni entre los grupos: alelos defectuosos vs defectos nulos.

Asociación de la concentración de Lp(a) con cLDL

Hubo una asociación positiva entre cLDL y la concentración de Lp(a) (Figura 2C). Sin embargo, esta asociación fue más intensa en el grupo control (AWHS) que en el grupo de HFHe, con una diferencia muy significativa en las pendientes entre los grupos (p =

0,006). El colesterol calculado en Lp(a) contribuyó en mayor medida a cLDL en los controles que en HFHe, especialmente si el cLDL estaba por encima de 200 mg/dL. A misma concentración de cLDL (por ejemplo, 200 mg/dL), la Lp(a) estimada fue significativamente menor en HFHe que en los sujetos de control (p = 0,025). La Lp(a) fue independiente del colesterol LDL-noLp(a) (mg/dL) en HFHe en contraste con la población control, en la que hubo una intensa asociación negativa (Figura 2D).

Efecto del score poligénico

El score poligénico (SP) se realizó en un subconjunto de 216 sujetos correspondientes a todos los sujetos consecutivos estudiados desde enero de 2018: 137 en el grupo de mutación negativa, 32 con una mutación del gen HF de significado incierto y 47 sujetos con HFHe. Las características clínicas y bioquímicas fueron similares a las del grupo principal (Tabla complementaria 6). Las concentraciones en Lp(a) se mantuvieron alrededor de 15 mg/dL más altas en el grupo con mutación negativa en comparación con HFHe, 50,4 mg/dL (IQR 19,0, 119,0) vs 36,5 mg/dL (IQR 7,94, 58,4), respectivamente (p = 0,071). El SP fue significativamente mayor en sujetos con mutaciones negativas que en sujetos con HFHe (Tabla complementaria 6). Cuando el grupo de mutaciones negativas se dividió según terciles de SP, los sujetos en el tercil más alto tenían una tendencia no significativa a concentraciones más altas de cLDL y apoB. Sin embargo, la concentración de Lp(a) fue elevada homogéneamente en los tres grupos, sin diferencias entre ellos (Tabla complementaria 7).

Cohorte de validación

El grupo hipercolesterolémico del Registro de Dislipidemias de la SEA estuvo compuesto por 707 HFHe con mutación patogénica; 74 pacientes con una mutación de significado incierto; y 398 sujetos con mutaciones negativas (Tabla 3, grupos restringidos a casos de variables completas). De manera similar a lo que ocurrió en el grupo de estudio principal, los sujetos con mutaciones negativas tenían una concentración de Lp(a) significativamente más alta que la HFHe, y ambas más altas que el grupo de control (Tabla 3 y Figura 1A complementaria). En este grupo de validación había 671 HFHe con una mutación patogénica en *LDLR* y 36 HFHe con mutación en *APOB*, todos con la mutación p.(Arg3527Gln), generalmente denominada *APOB-3500*. Las medias geométricas

ajustadas de Lp(a) fueron 22,2 mg/dL (IC del 95%: 19,2; 25,6) y 34,9 mg/dL (IC del 95%: 22,2; 55,0), respectivamente (p = 0,045) (Tabla complementaria 8 y Figura complementaria 1B). La asociación entre cLDL y cLDL-noLp(a) con Lp(a) mostró un patrón similar al de la cohorte principal (Figuras suplementarias 1C y 1D). En este grupo, no se mostraron mutaciones patogénicas *APOE o PCSK9*.

Estudio 4 Cirugía de cataratas en ancianos con Hipercolesterolemia Familiar Heterocigótica en tratamiento prolongado con estatinas.

4.1 Antecedentes

Las cataratas causan un tercio de la ceguera en todo el mundo, junto con los errores de refracción no corregidos y el glaucoma (1) y cada año se realizan entre 20 y 25 millones de intervenciones quirúrgicas de cataratas en todo el mundo (2). Las cataratas se definen como una degradación de la calidad óptica del ojo debido a la opacidad del cristalino. Varias propiedades del cristalino disminuyen gradualmente con la edad y, en consecuencia, la vejez es el factor de riesgo más importante en la formación de cataratas. Otros factores de riesgo habituales son la DM; uso prolongado de corticosteroides tópicos, sistémicos, intravítreos, inhalados u orales; cirugía intraocular previa; trauma; de fumar; y exposición a luz ultravioleta B (3, 4).

Las estatinas son inhibidores de la enzima HMGCoA reductasa comúnmente utilizada como fármacos hipolipemiantes (5). Se utilizan para reducir el colesterol en sangre, lo que ha demostrado ser una estrategia muy eficaz para prevenir enfermedades cardiovasculares en sujetos de alto riesgo. Desde que se asoció una pérdida de visión por cataratas irreversibles por triparanol, que fue el primer fármaco sintético reductor del colesterol (6), algunos informes han asociado el uso de estatinas con el desarrollo de cataratas, aunque con resultados contradictorios. Un metaanálisis reciente que incluye estudios observacionales ha concluido que las estatinas aumentan ligeramente el riesgo de cataratas (5, 7), mientras que en los ensayos clínicos aleatorizados las estatinas no aumentan el riesgo de cataratas (8). Este tema se ha revisado recientemente por varios consejos de la *American Heart Association* y su conclusión ha sido que las estatinas no aumentan el riesgo de cataratas (9). Sin embargo, estos estudios observacionales y ensayos clínicos se han realizado en poblaciones en las que la prevalencia de cataratas no es muy prevalente, ya que se excluyeron a pacientes ≥ 75 años, y en las que el uso de estatinas se limita a solo unos pocos años, habitualmente menos de 5 años.

La HF es una enfermedad monogénica caracterizada por un aumento anormal de los niveles de colesterol unido a cLDL desde el nacimiento y un consecuente riesgo elevado de enfermedad coronaria. Solo los pacientes con HFHe alcanzan mayor edad. Además, los sujetos con HFHe son un grupo de pacientes en los que se ha utilizado una alta dosis de estatinas potentes durante décadas por esta mayor esperanza de vida respecto a los HFHo. Por lo tanto, los sujetos de edad avanzada con HFHe sometidos a una potente terapia hipolipemiante durante décadas pueden ser un modelo de población atractivo para explorar efectos secundarios inesperados (10, 11). Por el momento, no se ha estudiado el desarrollo de cataratas en HFHe.

4.2 Objetivos

Nuestro objetivo fue estudiar la asociación del uso de cataratas y estatinas en un grupo de ancianos con HFHe bajo tratamiento prolongado con estatinas y compararlos con un grupo control.

4.3 Material y métodos

Características del estudio

Este es un estudio observacional, multicéntrico, de casos y controles. Estudiamos HFHe casos y controles de cinco Unidades de lípidos de España. El protocolo había sido publicado previamente (12) y fue diseñado para explorar morbilidades no cardiovasculares en ancianos con HFHe. Los criterios de inclusión de los sujetos reclutados fueron los siguientes: pacientes con edades ≥65 años; con una mutación patogénica en un gen candidato para HF (*LDLR, APOB o PCSK9*) en el sujeto o en un pariente de primer grado; niveles de cLDL ≥ 220 mg/dL sin tratamiento hipolipemiante; y tratamiento con estatinas ≥5 años. Los controles se seleccionaron de familiares de pacientes con HFHe en ausencia de hipercolesterolemia (cLDL <190 mg/dL sin tratamiento hipolipemiante). Todos los sujetos dieron su consentimiento informado para su inclusión antes de participar en el estudio. El estudio se realizó de acuerdo con la Declaración de Helsinki y el protocolo fue aprobado por el Comité de Ética de C.I. PI19 / 440.

Evaluaciones:

La entrevista clínica recogió datos de edad, sexo, hábito tabáquico y antecedentes personales de hipertensión, DM, cirugía de cataratas y cardiopatía cardiovascular. La cirugía de cataratas se confirmó mediante la revisión de las historias médicas. Además, se recogieron los tratamientos hipolipemiantes que estaban consumiendo, como estatinas, ezetimiba e iPCSK9. Se incluyeron el tipo de fármaco y la dosis prescrita como tratamiento actual, el tratamiento más común utilizado de por vida, la dosis y el momento en que se inició el tratamiento hipolipemiante. El IMC se calculó como el peso en kilogramos dividido por el cuadrado de la altura en metros.

Análisis estadístico

Analizamos la asociación de cataratas con HFHe mediante GEE, utilizando modelos logísticos con varios niveles de ajuste: no ajustado, ajustado por sexo y edad, y además ajustado por concentración de cLDL sin tratamiento. Los datos para los casos y controles se muestran como media (desviación estándar) o porcentaje. El estudio de la influencia del cLDL y el tratamiento con estatinas se analizó dentro de los estratos (casos y controles por separado) con modelos lineales generalizados. Todos los análisis se realizaron con el software estadístico R versión 3.4.4. y el paquete “gee” versión 4.13.19.

4.4 Resultados

Se recopilaron datos de 205 sujetos (112 HFHe y 93 controles) de 71,8 (6,5) años y 70,0 (7,3) años, respectivamente. No hubo diferencias en las características clínicas entre casos y controles, a excepción de los datos sobre antecedentes de enfermedades cardiovasculares, que fueron más frecuentes en los familiares de los sujetos cosanguineos HFHe (el 45,0 de HFHe frente al 25,8% familiares no-HFHe) y en entre casos y controles (el 27,7 frente al 16,1%); $p < 0,05$ en ambos casos. Asimismo, la concentración de cLDL fue mayor en los casos: 314 mg/dL vs 138 mg/dL ($p < 0,01$). La duración media del uso del tratamiento con estatinas en HFHe fue de 22,5 (8,7) años. Noventa y nueve de los 112 (88,4%) tomaban estatinas de alta potencia (atorvastatina 40-80 mg y rosuvastatina 20-40 mg). No observamos diferencias en tabaquismo, hipertensión y DM entre casos y controles

(12). El antecedente de cirugía de cataratas estuvo presente en el 25,2% de los casos y en el 16,1% de los controles. Esta diferencia no fue estadísticamente diferente sin ajustar, ni después de ajustar por edad y sexo, ni adicionalmente por cLDL (Tabla 1). Cuando se clasificaron los casos de acuerdo con la presencia o ausencia de cirugía de cataratas no hubo diferencias en las variables clínicas, excepto la edad que fue mayor en el HFHe con cirugía de cataratas (Tabla Suplementaria). También analizamos la asociación de edad, años en tratamiento con estatinas y cLDL sin tratamiento hipolipemiante con cirugía de cataratas. La edad se asoció fuertemente con un riesgo relativo de 2,06 (IC 1,09, 4,02) por 10 años entre los casos y 2,57 (IC 1,13, 6,28) entre los controles. El número de años en tratamiento con estatinas (estudiado entre los casos) y cLDL sin tratamiento hipolipemiante no mostró asociación con la cirugía de cataratas (tabla 2).

Estudio 5. Causas actuales de muerte en hipercolesterolemia familiar

5.1 Antecedentes

La Hipercolesterolemia Familiar (HF) es un trastorno autosómico codominante y la enfermedad metabólica monogénica más común en la población. La prevalencia de HFHe es de aproximadamente 1 / 200-500 personas en la mayoría de los países (1,2). La HF está causada por mutaciones en los genes que controlan la captación celular del colesterol plasmático y que incluyen el *LDLR, APOB, PCSK9 y APOE* (1). Los pacientes con HFHe muestran una concentración plasmática muy alta de cLDL, aproximadamente el doble de los sujetos sin HF de la población general, a menudo oscilando entre 250-400 mg/dL, depósitos de colesterol en tejidos superficiales como el arco corneal y xantomas de tendones y alto riesgo de enfermedad CV prematura en ausencia de un tratamiento hipolipemiante adecuado (3,4). El riesgo de desarrollar enfermedad CV prematura aumenta 10 veces en estos pacientes con respecto a la población general, especialmente la enfermedad coronaria (EC) en pacientes jóvenes (4,5). Los registros internacionales de HFHe como el británico Simon Broome muestran un riesgo hasta 100 más alto de enfermedad CV en hombres HFHe menores de 40 años con HFHe en la era anterior a las estatinas, tratamiento que no estuvo disponible hasta finales de la década de 1980 (6). La esperanza de vida de los sujetos HFHe se había calculado entre 10-30 años menor para mujeres y hombres, respectivamente, en relación con la población no HF (7). En los últimos años se ha producido una disminución de la enfermedad CV en la HFHe, como hemos podido comprobar recientemente en nuestro medio (4,8) probablemente debido a un diagnóstico precoz y un tratamiento hipolipemiante intensivo.

Dos hechos importantes han ocurrido en el análisis de morbilidad y mortalidad de la HFHe en las últimas décadas. En primer lugar, se han estudiado en profundidad las bases genéticas de la HFHe y el estudio genético proporciona un diagnóstico de certeza que obvia el sesgo diagnóstico basado en el riesgo de enfermedad CV como uno de los principales criterios para el diagnóstico de FHHe (9); y los fármacos hipolipemiantes de la segunda corriente, que incluyen estatinas, ezetimiba e iPCSK9, han modificado sustancialmente el

fenotipo lipídico y, en consecuencia, el espectro clínico de la enfermedad (5,10). De esta forma, si el tratamiento está bien establecido durante las primeras décadas de vida, la HFHe es una enfermedad mucho menos agresiva que antes. La complejidad del trasfondo genético de la HF, el uso de múltiples fármacos durante décadas, una mayor esperanza de vida asociada al tratamiento y cambios en los factores ambientales y sociales podrían conducir a un fenotipo mucho más heterogéneo que el descrito en el siglo pasado (5). Además, otras comorbilidades podrían estar asociadas al fenotipo HFHe que estaban ocultas por la enfermedad CV, o asociadas al tratamiento hipolipemiante, como la diabetes favorecida por las estatinas (11). Conocer el efecto de los diferentes tipos genéticos de HFHe a largo plazo y el impacto de un tratamiento hipolipemiante prolongado son fundamentales para un manejo adecuado de esta enfermedad en los próximos años.

5.2 Objetivos

El objetivo de este análisis fue estudiar las causas actuales de muerte cardiovascular y no cardiovascular de la HFHe y las posibles diferencias con una población control.

5.3 Material y métodos

Este es un estudio observacional de casos y controles diseñado para describir la morbilidad y mortalidad actual en sujetos con HFHe. Los casos de HFHe fueron reclutados en la Unidad de Lípidos del Hospital Universitario Miguel Servet, Zaragoza, España, y sus parejas no consanguíneas fueron reclutadas como controles. Los datos sobre los familiares de primer grado de los casos y controles, incluidos los familiares fallecidos, se recopilaron a partir de un cuestionario de participantes y la revisión de sus registros médicos. Se obtuvo el consentimiento informado por escrito de cada caso y control incluidos en el estudio; el protocolo del estudio se ajusta a las directrices éticas de la Declaración de Helsinki de 1975; y el protocolo de estudio fue previamente aprobado por el Comité Ético de Investigación Clínica de Aragón de la Institución.

Los criterios de inclusión para los casos consistieron en los siguientes requisitos: edad ≥ 30 y ≤ 60 años en el momento de la inscripción en el estudio; HFHe genéticamente

diagnosticado; e historia personal de hipercolesterolemia con niveles de cLDL> 220 mg/dL sin tratamiento hipolipemiante. Los controles se seleccionaron entre familiares no consanguíneos de similar edad (± 5 años) de pacientes con HFHe (compañeros del caso que convivían con ellos); edad ≥ 30 y ≤ 60 años en el momento de la inclusión en el estudio; y cumpliendo que ni ellos ni ningún familiar de primer grado tenían diagnóstico clínico de HFHe y que tenían cLDL <190 mg/dL sin fármacos hipolipemiantes.

Entrevista clínica

Los participantes fueron entrevistados para recopilar información personal sobre antecedentes de enfermedad cardiovascular, factores de riesgo CV, comorbilidades, uso de medicamentos, valores de lípidos y hospitalizaciones, y además, una historia familiar detallada incluyendo estos datos de todos los familiares de primer grado (padres, hermanos y descendencia), y también la edad y la causa de muerte de los fallecidos. La información sobre los antecedentes de hipercolesterolemia, el uso de fármacos hipolipemiantes y la edad y la causa de la muerte se confirmaron a partir de los registros médicos del paciente. Si un familiar de primer grado de un caso presentaba cLDL> 220 mg/dL en al menos una ocasión y/o cLDL>160 mg/dL bajo tratamiento con estatinas, se les etiquetaba como pertenecientes al grupo HFHe. Así, los grupos de análisis se denominaron “familiares HFHe”, “familiares no HFHe” y “familiares control”. En este informe solo se presentan datos sobre muertes de miembros de la familia mayores de 18 años.

Estudio genético.

Todos los casos de HFHe entrevistados en este estudio tenían un diagnóstico genético de HFHe y eran portadores de una variante "patogénica" o "probablemente patogénica" según las directrices del *American College of Medical Genetics and Genomics* (ACMG) (12) en *LDLR* (NM_000527 .4), genes *APOB* (NM_000384.2) o *PCSK9* (NM_174936.3). El análisis del gen HF se estudió con las plataformas *Progenika Biopharma Grifols* (Derio, España) (13) o *GEN inCode* (Terrassa-Barcelona, España) (14).

Análisis estadístico

Los datos se expresan como desviación estándar media para las variables numéricas con distribución normal y se analizaron con la prueba t de Student, mientras que las que no tienen distribución normal se expresan como mediana [rango intercuartílico] y se analizan con la prueba U de Mann-Whitney. Las variables cualitativas se expresan en porcentaje y se analizaron mediante la prueba X2. Para la comparación de variables de categoría no dicotómicas se utilizaron las pruebas ANOVA y Kruskal-Wallis. Las tasas de mortalidad se calcularon utilizando la estimación de Kaplan-Meier basada en la edad, y los grupos se compararon mediante la prueba de rango logarítmico. La asociación entre la HFHe y la mortalidad CV y no CVD se calculó mediante la regresión de Cox multivariante. Se generó un modelo que incluyó la covariable de edad, y se calculó con técnicas apropiadas para analizar muestras complejas para tener en cuenta que los datos estaban agrupados en familias.

5.4 Resultados

Características clínicas de casos y controles

Reclutamos 166 sujetos, 83 casos de HFHe y 83 controles. Las edades medias fueron 54,3 años y 54,5 años, respectivamente, sin diferencias de edad y sexo entre los grupos. El IMC, la presión arterial sistólica y la presión arterial diastólica fueron similares en ambos grupos. La prevalencia de hipertensión arterial y DM2 tampoco mostró diferencias. La enfermedad CV tendió a ser más prevalente en los casos de HFHe que en los controles 8,4% y 2,4% respectivamente (P = 0,08). El cLDL y el total no tratado fueron más altos en los casos que en los controles. El tratamiento con estatinas estuvo presente en todos los casos y en el 22,9% de los controles. El inicio del tratamiento hipolipemiante fue de 32,8 años en los HFHe y de 51,3 años en los controles (tabla 1).

Estudio familiar

Analizamos 813 familiares de primer grado de casos y controles dentro de familias de casos 211 miembros eran HFHe y 219 no HFHe. Descartamos 11 familiares de primer grado de casos con fenotipo HFHe ambiguo (Figura 1). El grupo familiar de control estuvo compuesto por 372 sujetos.

Mortalidad de Enfermedad CV y no enfermedad CV entre casos y familiares de primer grado de control

Identificamos 62 fallecidos entre familiares HFHe, 53 en no HFHe y 100 en controles (Figura 1). El porcentaje de muertos y la edad media de fallecimiento fueron similares en los tres grupos, siendo ligeramente superior en los familiares HFHe, 29,4% frente al 24,2% en los familiares no HFHe y 26,9% en los familiares control. La edad promedio de muerte fue de aproximadamente 4 años menos en el grupo de HFHe. La proporción de muertes por enfermedad CV fue superior en el grupo de HFHe (59,7% en HFHe frente a 37,7% en no HFHe y 37,4% en controles, P = 0,012). Otras causas de muerte, incluida la muerte por cáncer, no mostraron diferencias significativas entre los tres grupos (Tabla 2). Además, estudiamos las diferencias de mortalidad entre hombres y mujeres. El porcentaje de sujetos fallecidos no mostró diferencias entre los grupos, sin embargo, los sujetos FHHe murieron aproximadamente 4 años antes que los no HFHe y los controles, aunque la diferencia no fue estadísticamente significativa. La causa de muerte según la enfermedad CV en los hombres fue del 69% en los HFHe, frente al 38,5% de los no HFHe y el 37,0% de los controles, respectivamente (P = 0,01). Se observó el mismo patrón en las mujeres, aunque la edad de muerte fue de aproximadamente 7 años más tarde entre las mujeres que entre los hombres, y de manera similar en los 3 grupos. (Tabla 3 y Tabla 4). La razón de riesgo de muerte por enfermedad CV fue 2,85 veces superior (IC del 95%, 1.73-4.69) en HFHe con respecto a los otros dos grupos, y sin diferencias entre no HFHe y controles. Esta razón de riesgo fue de 2,95 en hombres (IC del 95%, 1,52-5,75) y de 3,44 en las mujeres en HFHe (IC del 95%, 1,66-7,10) (Tabla 5). La separación de las curvas comenzó a los 50 años en hombres, aumentando progresivamente con la edad (Figura 2). En hombres HFHe, el mayor riesgo apareció aproximadamente 5 años antes que en las mujeres HFHe (Figura suplementaria 1).

Mortalidad de CV o no CV entre los casos de los padres y los miembros de la familia controles

Dado que la mayoría de las muertes correspondieron a los padres de los casos y controles, analizamos la mortalidad en este grupo de sujetos. Hubo 116 muertes entre los padres: 24 (72,7%) HFHe, 35 (72,9%) no HFHe y 57 (70,4%) controles; y 77 defunciones

entre madres: 33 (66,0%) HFHe, 13 (39,4%) no HFHe y 31 (37,8%) controles. Estas diferencias se magnificaron en los padres. El porcentaje de muertes por enfermedad CV fue superior entre HFHe respecto a los otros dos grupos, aunque la diferencia fue significativa solo en hombres; y respecto a la edad de muerte por enfermedad CV, fueron más jóvenes tanto los hombres como las mujeres HFHe. No encontramos diferencias estadísticamente significativas entre las muertes por causas no CV (Figura 3), pero las madres de los sujetos control, tenían una mayor tendencia a morir de cáncer, en comparación con las madres de los sujetos HFHe (P = 0,092) (Tablas complementarias 1 y 2).

DISCUSIÓN y CONCLUSIONES

Discusiones y conclusiones estudiadas en el compendio de publicaciones

Estudio 1 Enfermedad valvular aórtica en ancianos con Hipercolesterolemia Familiar Heterocigótica. Impacto de la terapia hipolipemiante.

1.5 Discusión

En el presente estudio describimos la prevalencia de enfermedad aórtica en pacientes con HFHe ≥ 65 años tratados con fármacos hipolipemiantes de forma prolongada. La afectación de la válvula aórtica en la HFHe ha sido previamente explorada, pero de acuerdo a la bibliografía analizada, este es el primer trabajo enfocado en ancianos, la población de mayor relevancia clínica debido a la estrecha relación de la EA con la edad, y el primero en describir la prevalencia de EA y evaluar el papel potencial del tratamiento con estatinas en el desarrollo de enfermedad aórtica en HFHe.

Prevalencia de EA en HFHe

En nuestro estudio, la prevalencia de EA fue de más de tres veces superior que la reportada hasta ahora, para este grupo de edad en la población general, 1.5-3%, y también fue mayor que en nuestro grupo control [19-22]. En vista de esta prevalencia sustancialmente elevada, la ecocardiografía transtorácica sistemática probablemente esté justificada para el cribado de EA en ancianos con HFHe mayores de 65 años.

Varios estudios han analizado previamente el engrosamiento o calcificación de la válvula aórtica en la HFHe. Ten Kake y col. compararon una cohorte de los Países Bajos de 59 sujetos con HFHe con controles y demostraron, que los pacientes con HFHe, especialmente aquellos con mutaciones patogénicas para *LDLR*, mostraban una mayor prevalencia de AoVC (41% versus 21%, respectivamente, P <0,001) [16]. Esta relación es similar a la que observamos en nuestro estudio. Sin embargo, los autores probablemente no informaron de la EA porque la muestra era más pequeña que la nuestra e incluían pacientes más jóvenes. En el *Cardiovascular Health Study*, la HF clínicamente definida, se asoció con AoVC y esclerosis aórtica, pero no se pudo demostrar una asociación con EA [23,24]. En nuestro estudio, además de incluir criterios clínicos lipídicos, todos los casos

se definieron por tener una mutación genética en un gen causante de hipercolesterolemia familiar, analizada directamente en el caso o en un pariente consanguíneo. Está bien establecido que la definición de HF basada exclusivamente en criterios clínicos incluye otras formas de hipercolesterolemia genética [25,26], y los sujetos hipercolesterolémicos con una mutación genética, incluso con una concentración similar de cLDL en el momento del diagnóstico, tienen fenotipos cardiovasculares más graves. [27].

Factores de riesgo de enfermedad valvular aórtica

Nuestros resultados apoyan que los factores de riesgo de aterosclerosis, incluido el cLDL elevado a lo largo de la vida, son factores de riesgo importantes para la EA [28]. La EA probablemente se produce por una combinación de estrés mecánico y daño endotelial de las válvulas aórticas, lo que conduce a la posterior inflamación de la válvula, fibrosis, calcificación y estrechamiento progresivo de la válvula [8]. El estrés mecánico afecta la función endotelial y facilita la infiltración de lípidos y células inflamatorias (células T) en la válvula. Todos estos mecanismos están implicados en la actividad inflamatoria [29,30]. Como resultado, los fibroblastos se diferencian en miofibroblastos que, bajo la influencia de la angiotensina, promueven el engrosamiento de la válvula [8]. El tratamiento de la hipercolesterolemia no previene de la progresión de EA moderada a grave, una vez que la EA ya está establecida [31], esto sugiere que la hipercolesterolemia juega un papel en las fases iniciales, pero con poco efecto una vez que la enfermedad está ya avanzada [32]. Nuestro estudio respaldaría las directrices actuales que recomiendan el tratamiento temprano e intensivo de la HFHe para prevenir en un futuro, no solo la enfermedad coronaria, sino también la enfermedad valvular [4].

Estatinas y valvulopatías

El tratamiento hipolipemiante se asocia con un aumento de la calcificación vascular [33] debido, en parte, a la formación de hueso en las células óseas potenciada por las estatinas, derivado de un aumento en la expresión del gen BMP-2 [34]. En consecuencia, se ha especulado que el tratamiento hipolipemiante, especialmente el tratamiento prolongado con estatinas, puede favorecer el desarrollo de AoVC y esclerosis aórtica [35]. Sin embargo, otros estudios como Al Kindi et al. demostraron que la calcificación vascular

asociada a las estatinas no se relaciona con la calcificación valvular [36]. Actualmente, los pacientes con HFHe son los pacientes a los que se prescriben fármacos hipolipemiantes de forma más temprana, intensa y prolongada. Por lo tanto, nuestra muestra de ancianos con HFHe, expuestos a estatinas durante una media de 22,5 años, es excelente para estudiar esta asociación potencial. Encontramos que el número de años en tratamiento con estatinas no es un factor de riesgo de EA, lo que indica que, si existe algún riesgo de EA con estatinas, se ve altamente compensado por el efecto beneficioso sobre el cLDL, como se sugirió previamente [19].

Limitaciones del estudio

Se trata de un proyecto multicéntrico en el que se han realizado estudios ecocardiográficos por parte de diferentes investigadores y, por tanto, puede existir cierto grado de variabilidad en las medidas. Sin embargo, todas las ecografías fueron realizadas por cardiólogos expertos en ecocardiografía, con alta experiencia en hospitales del Sistema Nacional de Salud español, y todas ellas certificadas a nivel nacional con criterios homogéneos y estrictos. Además, los ecocardiografistas estaban cegados al diagnóstico de HFHe para evitar sesgos. Las mediciones de la funcionalidad de la válvula aórtica pueden variar en presencia de una función sistólica anormal [37]. Aunque no fue un criterio de exclusión, ninguno de nuestros casos y controles presentó fracción de eyección del ventrículo izquierdo (FEVI) <40% o miocardiopatía hipertrófica, por lo que esta limitación no juega un papel importante en nuestro estudio y todas las medidas de la superficie valvular se realizaron mediante ecuación de continuidad, el mejor procedimiento estandarizado. Por último, los participantes fueron quienes informaron de los datos sobre su historia personal de uso de fármacos hipolipemiantes y sobre los antecedentes familiares de enfermedad cardiovascular, que podrían haber sido sesgados por falta de exactitud en el recuerdo. Sin embargo, el resultado principal y los criterios de inclusión y exclusión para los grupos de casos y controles se basaron en datos objetivos, realizados o verificados por el equipo de investigación. Habría sido importante tener la exposición al cLDL a lo largo de la vida para estudiar el efecto del cLDL acumulativo en la EA. Teniendo en cuenta que estamos tratando con pacientes ancianos, esto no ha sido factible. Sin embargo, hemos

considerado el cLDL en el momento del diagnóstico y el número de años que estuvieron bajo tratamiento con estatinas, considerando que ambos datos son buenos subrogados de la exposición al cLDL de por vida.

1.6 Conclusiones

- Los sujetos ≥65 años con HFHe en tratamiento prolongado con estatinas durante un período medio de más de 22 años muestran más enfermedad valvular aórtica y mayor frecuencia de EA que los controles.

- Los factores de riesgo independientes para la enfermedad de la válvula aórtica en HFHe fueron la edad y el cLDL antes del tratamiento.

- La duración del tratamiento con estatinas no se asoció con ninguna medición de la válvula aórtica. Por lo tanto, la exposición prolongada de cLDL en lugar del tiempo de exposición a las estatinas explicaría este riesgo más alto.

- Estos resultados sugieren que los sujetos HFHe de edad avanzada debieran ser explorados para detectar la presencia de enfermedad aórtica y enfatizan la importancia del tratamiento hipolipemiante temprano en la población con HFHe para prevenir no solo la enfermedad coronaria sino también la enfermedad valvular aórtica.

- Además, nuestro estudio proporciona apoyo adicional a los estudios sobre EA y sobre el papel de la hipercolesterolemia en la EA.

Estudio 2. La hipercolesterolemia de herencia materna no modifica el fenotipo cardiovascular en la hipercolesterolemia familiar

2.5 Discusión

Varios estudios han sugerido que la hipercolesterolemia materna podría aumentar la enfermedad CV del adulto en la descendencia (20). Por ello, estudiamos este tema en un grupo de HFHe y observamos que no existen diferencias significativas en el fenotipo incluyendo la enfermedad CV, la DM, la hipertensión o los niveles plasmáticos de lípidos según el origen parental del defecto genético. La HF es un buen modelo para estudiar el efecto de la hipercolesterolemia en la descendencia como consecuencia del gran aumento de los niveles lipídicos que tienen las mujeres con HF durante el embarazo, normalmente alcanzan hasta el doble de concentración de CT que las de las madres sin HF y superiores a 400 mg/dL. Nuestros hallazgos no apoyan que la hipercolesterolemia materna tenga un efecto deletéreo en la descendencia.

Estos resultados están en línea con otros estudios que no encontraron diferencias en los niveles de lípidos y lipoproteínas entre los HFHe que habían heredado la HF de la madre o del padre (24). Además, Tonstad et al., no observaron ninguna diferencia en el grosor de la íntima-media carotídea y la prevalencia de placa entre los niños con HFHe a pesar del origen de los padres (25). Sin embargo, Van der Graf et al. habían observado previamente que los descendientes de madres con hipercolesterolemia, presentan un ligero aumento de los niveles de CT, cLDL y Apo B en fases avanzadas de la vida (15); y además, la HF heredada de las madres portadoras de la mutación V408M en el gen *LDLR*, se asoció con un exceso de mortalidad significativamente mayor que la HF transmitida por los padres (riesgo relativo 2,2; p = 0,048) (16). Probablemente los diferentes criterios de inclusión entre estudios o el número de sujetos estudiados podrían explicar las diferencias encontradas.

Nuestro estudio también aporta información relevante sobre el papel de la hipercolesterolemia durante el embarazo, y en el desarrollo posterior de complicaciones cardiovasculares, independientemente de su causa. En estudios previos se sugería que la

hipercolesterolemia materna aceleraba el desarrollo de arteriosclerosis en la descendencia tanto en conejos (26) como en ratones (27), independientemente de si la hipercolesterolemia en la madre era inducida por manipulación genética, dieta o ambas, e independientemente del cLDL posnatal (19). El efecto de la hipercolesterolemia durante el embarazo en humanos apenas se ha estudiado. En un estudio post mortem, que analizaba el arco aórtico y la aorta abdominal de 156 niños normocolesterolémicos de 1 a 13 años, que fallecieron por traumatismo y otras causas (*Fate of Early Lesions in Children Study*), mostró una asociación entre el colesterol materno y la presencia de lesiones iniciales de arteriosclerosis en los niños (20). Sin embargo, esto no se ha confirmado posteriormente.

Durante el embarazo, se produce un aumento fisiológico de los niveles de colesterol materno. Es un mecanismo adaptativo que responde a las mayores demandas de colesterol durante el embarazo y se conoce como "hipercolesterolemia fisiológica materna" (28). Además, algunas mujeres presentan una alteración, denominada "hipercolesterolemia suprafisiológica materna", que se asocia con modificaciones vasculares fetoplacentarias. Sin embargo, la hipercolesterolemia materna no afecta los niveles de lípidos neonatales (29, 30) porque la concentración plasmática de colesterol en el feto es un proceso altamente regulado, mayormente independiente del colesterol plasmático materno. El colesterol en el feto proviene de la síntesis "*de novo*" o del transporte placentario. El colesterol se transporta en la placenta humana de la madre al feto a través de la captación de las lipoproteínas maternas de colesterol por la placenta, atravesando el trofoblasto y el endotelio y dando salida a los aceptores en el feto. En el lado apical del sincitiotrofoblasto (STB), la captación de colesterol de la circulación materna proviene de las partículas LDL y HDL, a través de los LDLr y SR-BI respectivamente y se secretan en el lado basal hacía el estroma velloso (12, 31, 32). Los mecanismos por los que el colesterol es transportado a las células endoteliales para finalmente llegar a la circulación fetal son en su mayoría desconocidos (33, 34) pero dos proteínas altamente reguladas, ABCA1 y ABCG1 son responsables de transportar el colesterol de la placenta a las Apo A1 libres de lípidos y partículas HDL (35) sin la participación del LDLr que se expresa pobremente en el lado basal del sincitiotrofoblasto (30).

La principal implicación clínica de nuestros resultados es que el manejo clínico de los sujetos con HFHe no debe ser diferente, dependiendo de si la herencia es materna o paterna, ya que el fenotipo lipídico y las complicaciones a largo plazo son similares en ambos grupos. Existe una tendencia en la práctica clínica a infrautilizar medicamentos cardíacos efectivos entre las mujeres respecto a los hombres (36-38) y esto podría acentuarse en la HF, ya que los antecedentes familiares de enfermedad cardiovascular prematura son un factor potenciador del riesgo en la población general (39), y la enfermedad cardiovascular prematura es menos frecuente en las mujeres con HFHe (10). Por tanto, el riesgo de tener antecedentes de enfermedad prematura es mayor si la herencia es paterna.

Este sesgo potencial no se observa en nuestro estudio ya que el manejo clínico es muy similar entre sujetos con herencia paterna o materna. Ni los años de estatinas ni el porcentaje de sujetos con tratamiento hipolipemiante de alta intensidad fueron diferentes según la herencia paterna. Lo probable es que se deba a que los pacientes de nuestro estudio, proceden de unidades especializadas (23) donde las recomendaciones terapéuticas se basan mayoritariamente en factores de riesgo individuales según las guías vigentes (40). Creemos que nuestros datos son sólidos respecto a la ausencia de un efecto clínico relevante en la hipercolesterolemia de origen monogénico. Sin embargo, se debe explorar si otras formas de hipercolesterolemia durante el embarazo juegan un papel relevante en el futuro.

Algunas limitaciones podrían surgir en nuestro estudio. La asignación de los padres ha sido “autoinformada”, aunque para evitar sesgos, esta información se comprobó en el historial médico. Por este motivo, el 27% de los sujetos fueron excluidos del análisis ya que no se pudo verificar la asignación. En segundo lugar, nos basamos en un diagnóstico de HFHe según criterios clínicos y respecto a el diagnóstico genético de los HFHe de origen materno y paterno no estuvo disponible en el 10,1% y el 11,5%, respectivamente, aunque tampoco encontramos diferencias al considerar solo a aquellos sujetos con diagnóstico genético positivo.

2.6 Conclusiones

- En la HF no existen diferencias en el fenotipo lipídico, en la prevalencia de enfermedad cardiovascular, en la edad de inicio de enfermedad cardiovascular o en las complicaciones cardiometabólicas como DM e hipertensión asociados al origen maternal o paternal de la hipercolesterolemia.

- Estos hallazgos implican que la hipercolesterolemia materna no confiere un riesgo adicional a la descendencia en etapas más avanzadas de la vida y que un posible efecto materno no es relevante en la HF.

Estudio 3. Lipoproteína (a) en hipercolesterolemia hereditaria. Influencia de la causa genética, gen defectuoso y tipo de mutación

3.5 Discusión

En nuestro estudio describimos las concentraciones de Lp(a) en diferentes tipos de hipercolesterolemias hereditarias, incluyendo dos grandes cohortes independientes de pacientes con HFHe monogénica y mutaciones negativas, algunos de ellos diagnosticados de hipercolesterolemia poligénica. También analizamos el efecto del gen responsable de la HF en la concentración de Lp(a) y la posible implicación de los diferentes dominios proteicos afectados por el defecto genético. La concentración más alta de Lp(a) se observó en sujetos con hipercolesterolemia hereditaria pero sin una mutación patogénica en los genes de HF, independientemente del score de la hipercolesterolemia poligénica. Los sujetos con HFHe tenían una concentración de Lp(a) más alta que la población general pero más baja que los sujetos con mutaciones negativas; y este aumento de Lp(a) en HFHe estuvo relacionado con el gen involucrado, pero sus diferencias no se explicaron por el número de kringle IV tipo 2 de *LPA*. La Lp(a) más alta en HFHe no se explica por su concentración más alta de cLDL y, en la HF dependiente de *LDLR*, los niveles de Lp(a) no son diferentes dependiendo del dominio de la proteína del LDLr afectada. Varios estudios han analizado la concentración de Lp(a) en sujetos con HF obteniendo una amplia variación

de resultados: desde presentar valores más altos (28-32) hasta no presentar diferencias respecto a una población control (33-35). Kraft y col. estudiaron la concentración de Lp(a) en HFHo, HFHe y familiares sin HFHe, mostrando un efecto dependiente de la dosis sobre la concentración de Lp(a). La HFHo mostró mayor concentración que los sujetos heterocigotos y éstos, a su vez, mayor concentración que los controles, sin diferencias en el genotipo *LPA* (36). Sjouke et al (31) encontraron el mismo efecto de dosis. Nuestro estudio concuerda con el hallazgo de una mayor concentración de Lp(a) en HFHe. Sin embargo, existen, al menos, dos sesgos potenciales importantes en la asociación previamente descrita de Lp(a) y FH. Primero, Lp(a) es un factor de riesgo independiente de enfermedad CV. En estudios previos, los criterios de selección para el diagnóstico de HF incluían enfermedad coronaria (EC) y cardiopatía isquémica (8, 11), tal y como se recoge en el *Dutch Lipid Clinic Network Score* o en los criterios de *Simon Broome* para HF (28–30), esto implica un sesgo evidente de disponer de una mayor Lp(a), como se ha señalado recientemente (32). En nuestro estudio, hemos utilizado criterios de diagnóstico para HF basados en cLDL, y no en enfermedad CV. En realidad, solo el 8% de nuestros sujetos con HFHe tenían enfermedad CV, un porcentaje mucho más bajo que el publicado anteriormente (37). El segundo potencial sesgo es tomar de referencia el análisis genético para la definición de HFHe, al tiempo que se incluyen variantes genéticas de significado incierto (34, 38), mientras que otros estudios solo se basaron en criterios clínicos (28, 29, 39). El presente estudio incluye en todos los sujetos, las mutaciones en todos los genes responsables reconocidos de HF, además de considerar su funcionalidad. Además, hemos excluido a aquellos sujetos con un diagnóstico genético incierto.

La concentración de Lp(a) juega un papel importante en la determinación de la concentración de cLDL y en aquellos sujetos con Lp(a) muy alta, se podrían estar clasificando como HFHe (30). Debido al solapamiento entre HF e hipercolesterolemia que es secundaria a niveles altos de Lp(a), es importante precisar el diagnóstico genético de HF para evaluar la concentración de Lp(a) en estos pacientes. Por el mismo motivo, la medición de Lp(a) es muy recomendable en sujetos con diagnóstico clínico de HFHe(6) pero también en sujetos con sospecha de hipercolesterolemia poligénica.

Uno de los resultados más destacables de nuestro estudio es la diferente concentración de Lp(a) en HF según el gen responsable de HF. Los sujetos con mutaciones en *APOB* tienen concentraciones más altas de Lp(a) mientras que los sujetos con mutación en *APOE* tienen concentraciones más bajas en comparación con los portadores de mutaciones en *LDLR*, sin mostrar diferencias según el número de repeticiones de KIV tipo 2. Sjouke y col. estudiaron las concentraciones de Lp(a) en 13 heterocigotos y 2 homocigotos para mutaciones *APOB*, obteniendo valores medios de 50,3 mg/dL (IQR 18,7; 120,9) y 205,5 mg/dL, respectivamente, ambos valores superiores a los de los sujetos control (31). En la misma línea, Van der Kooek et al. demostraron que el defecto de FDB (defecto familiar de *APOB*) aumenta la concentración y la variabilidad de Lp(a), y sugirió que *APOB* puede ser un gen que afecte a la variabilidad de los niveles de Lp(a) en plasma (40). Se desconoce el mecanismo que explica la elevación de Lp(a) en la HFHe dependiente de *APOB*. Knight y col. compararon la composición de Apo B de las partículas de LDL y Lp(a) en sujetos heterocigotos FDB. Demostraron que las proporciones de *APOB* mutante y de tipo salvaje eran similares en las partículas de Lp(a), lo que indica que, en HFHe causada por una mutación en *APOB*, el mecanismo probablemente implicado en el aumento de Lp(a), sería un aumento de la síntesis, y no de una disminución del catabolismo (41).

Otro hallazgo importante fue que los portadores de la mutación *APOE* p.(Leu167del) tenían una Lp(a) más baja que otras formas de HF. La variación genética en *APOE* está involucrada en la concentración de Lp(a): Moriarty et al. analizaron las isoformas comunes de *APOE* y demostraron un aumento del 65% en Lp(a) en los sujetos con ε4/ε4 en comparación con los sujetos con ε2/ε2 (18). Recientemente, Croyal et al. han demostrado la estrecha asociación entre las partículas de VLDL-apo E y la producción de Lp(a) (42). Nuestro grupo ha descrito recientemente la mayor captación hepática de partículas de VLDL que contienen p.(Leu167del) en *APOE* (23), por lo que especulamos que los mecanismos probables que influyen en la reducción de la concentración de Lp(a) asociados con una disminución de la apo E normal que contiene VLDL, podrían ser una disminución de producción y /o formación de Lp(a).

Los resultados de nuestro estudio refuerzan el hecho de que el LDLr apenas juega ningún papel en el catabolismo de Lp(a). Varios estudios en humanos, ratones y cultivos

celulares *in vitro* han descartado un papel significativo del LDLr en el catabolismo de Lp(a) (1). Demostramos que la concentración de Lp(a) en HFHe es independiente del tipo de defecto del LDLr, lo que respalda que el receptor no está involucrado en el catabolismo de Lp(a). Se han demostrado varios receptores de partículas de Lp(a), distintos del LDLr, que utilizan Apo B, Apo(a) o fosfolípidos oxidados (OxPL) como ligandos (1), por ello tampoco se puede descartar si pudieran estar implicados en el aumento de la concentración de Lp(a) en HFHe.

Nuestro artículo tiene la relevancia de que todos los sujetos han sido analizados genéticamente, solo se han incluido sujetos con mutaciones claramente patogénicas, se analiza el efecto sobre la concentración de Lp(a) de acuerdo al gen responsable y de *LDLR* en el caso del dominio de la proteína afectada y finalmente la selección de la HF no se realizó en base a la enfermedad cardiovascular.

Limitaciones del estudio

Se trata de un estudio observacional, retrospectivo, realizado en un solo centro, con un número limitado de pacientes con mutaciones distintas al *LDLR*, especialmente en *APOE*. La población de control fue principalmente masculina, y el genotipado de *LPA* se limitó al número de KIV tipo 2 y no estuvo disponible en todos los sujetos, pero éstos fueron seleccionados al azar. No todos los sujetos del estudio se sometieron al análisis del SP; sin embargo, se obtuvo en un subgrupo representativo sin llegar a mostrar relación entre la concentración de Lp(a) y la Hipercolesterolemia Poligénica. Dos tercios de los sujetos con mutaciones negativas tenían un SP superior a 0,93, lo que se ha utilizado como criterio para el diagnóstico de Hipercolesterolemia Poligénica, en ausencia de mutaciones en los genes FH (43). La forma de seleccionar a los pacientes en función de un cLDL elevado puede enriquecer a los sujetos con un Lp(a) elevado, lo que contribuyó al cLDL calculado (33). No podemos excluir completamente este sesgo de selección, aunque parece poco probable debido a la pequeña contribución del colesterol transportado en Lp(a) al cLDL total en HFHe en nuestro estudio. Finalmente, nuestros resultados son representativos de la población española de origen genético caucásico.

Afortunadamente, nuestro trabajo también tiene varios puntos fuertes. Los sujetos fueron estudiados genéticamente, cubriendo todos los genes canónicos para FH (44) y excluyendo aquellas mutaciones sin mutación funcional, y todos los resultados se comprobaron en una cohorte independiente.

3.6 Conclusiones

- La Lp(a) está elevada en las hipercolesterolemias genéticas incluyendo, la HP y la HFHe.
- La concentración de Lp(a) en HFHe es variable dependiendo del gen responsable de HF; y que sus diferencias no se explican por el número de kringle IV tipo 2 de LPA o por el Score Poligénico.
- La Lp(a) más alta en HFHe no se explica por su cLDL más alto, y en la HF dependiente de *LDLR*, los niveles de Lp(a) no son diferentes según el dominio proteico afectado.

Estudio 4 Cirugía de cataratas en ancianos con Hipercolesterolemia Familiar Heterocigótica en tratamiento prolongado con estatinas.

4.5 Discusión

Nuestro estudio muestra que la prevalencia de la cirugía de cataratas no aumenta en personas mayores con HFHe sometidas a tratamiento hipolipemiante durante más de 20 años. Esto sugiere que ni la hipercolesterolemia grave, ni el uso prolongado de estatinas son factores de riesgo relevantes en el desarrollo de cataratas. Hasta donde sabemos, este es el primer estudio que explora la presencia de cataratas en esta población, un subgrupo paradigmático de pacientes en los que el tratamiento con altas dosis de estatinas potentes es la primera línea de tratamiento.

El presente estudio tiene la fortaleza de ser realizado en un grupo de pacientes con tres principales características diferenciales de estudios previos: concentraciones muy elevadas de cLDL desde el nacimiento, uso de estatinas a dosis elevadas durante > 20 años, y vejez, es decir, grupo con alta incidencia de cataratas. Nuestro estudio coincide en que la edad es el principal factor asociado al desarrollo de cataratas. Es importante destacar que nuestros datos confirman los resultados de un análisis previo sobre la seguridad de las estatinas de uso prolongado con respecto al desarrollo de cataratas y plantean dudas sobre la supuesta asociación de la concentración de colesterol con las cataratas. Los estudios preclínicos mostraron que el colesterol tiene un papel importante en la integridad de la membrana y se suponía que la inhibición de la síntesis de colesterol causaba el desarrollo de cataratas (7). Además, se debe tener en cuenta que las estatinas ejercen sus beneficios en un amplio espectro de afecciones oftálmicas a través de sus efectos hipocolesterolémicos y pleiotrópicos, que pueden contribuir a hacerlas seguras con respecto a las cataratas (5).

4.6 Conclusiones

- La prevalencia de la cirugía de cataratas no fue significativamente diferente entre HFHe y controles.

- La presencia de cataratas no se asoció ni con el cLDL ni con la duración del tratamiento con estatinas.

- En el presente estudio, la HFHe no fue un factor de riesgo de cataratas y el tratamiento prolongado con estatinas no favoreció el desarrollo de cataratas.

Estudio 5. Causas actuales de muerte en hipercolesterolemia familiar

5.5 Discusión

En el presente trabajo analizamos las posibles diferencias en la mortalidad en un grupo de familias HFHe de una Unidad de Lípidos comparando familiares HFHe, no HFHe y no consanguíneos con el objetivo de actualizar la muerte por enfermedad CV y no enfermedad CV en HFHe en la era del tratamiento hipolipemiante. HFHe es un buen modelo para estudiar el efecto sobre la mortalidad de la hipercolesterolemia y su relación con los eventos de enfermedad CV (15). Además, los pacientes con HFHe suelen estar sometidos a un tratamiento crónico hipolipemiante intensivo y nuestros resultados están en consonancia con el beneficio que se obtiene en la enfermedad CV con la reducción de cLDL en la población general (16,17). Presumimos que la prevalencia de muerte por enfermedad CV ha disminuido durante los últimos años en estos pacientes y nuestros resultados parecen apoyarlo, debido a que se observó que la mortalidad por enfermedad CV en este grupo de familias fue menor y apareció más tarde que las cohortes de HFHe reportadas en las últimas décadas del siglo pasado (7,17). Sin embargo, todavía encontramos un aumento en la muerte por enfermedad CV con respecto a los pacientes no HFHe, especialmente en los hombres HFHe, que fallecieron 6,8 años más jóvenes en comparación con los otros grupos familiares. Tradicionalmente, se ha considerado que en pacientes con HFHe y sin tratamiento hipolipemiante, aproximadamente el 50% de los hombres y el 30% de las mujeres desarrollarán enfermedad CV antes de los 50 años (18,19) con una esperanza de vida estimada entre 20 y 30 años menor (7). Sin embargo, la muerte por enfermedad CV podría haber estado sesgada en esos estudios: históricamente, ya que la HFHe se ha diagnosticado clínicamente en función de las elevaciones de cLDL, enfermedad CV prematura personal y familiar y la presencia de xantomas tendinosos o arco corneal. Los criterios más comunes para el diagnóstico, incluidos los de *Dutch Lipid Clinic Network* (20) y el *registro Simon Broome* (21) incluyen enfermedad CV o factores de riesgo de enfermedad CV, como los xantomas tendinosos (18,22,23). De esta manera, los sujetos con HFHe o sus familiares en los que predominó la enfermedad cardíaca,

tuvieron más posibilidades de ser diagnosticados clínicamente de HF. La caracterización genética de la HF en los últimos años ha demostrado que el fenotipo HFHe es más heterogéneo de lo que se creía anteriormente, incluida la presencia de enfermedad CV. En una publicación reciente de los Países Bajos, la enfermedad cardíaca estaba presente en el 7,4% de 25.137 HFHe diagnosticados genéticamente, a pesar de que la edad media era de 38 años y el 71,1% no tomaba fármacos hipolipemiantes (24). En consecuencia, una proporción significativa de HFHe podría haber pasado desapercibida al aplicar los criterios diagnósticos tradicionales.

Nuestra cohorte se basa en niveles muy altos de cLDL (> 220 mg/dL sin tratamiento hipolipemiante) y un diagnóstico genético positivo para una mutación causal en un gen canónico para HF. Además, los pacientes fueron remitidos a la clínica por sus médicos de atención primaria, debido niveles altos de cLDL (25). Por ello, creemos que nuestra cohorte ha superado el sesgo anterior. Existe evidencia sólida, mediante estudios observacionales de HFHe, que ha demostrado una reducción de los episodios de enfermedad CV importantes en pacientes que están tomando un tratamiento hipolipemiante, cuando se inicia en etapas tempranas de la vida y se mantiene durante años (26, 27). En consecuencia, la supervivencia sin enfermedad CV, con un inicio temprano de estatinas en estos sujetos, podría ser bastante similar a la del resto de la población (28). En nuestro estudio, mostramos a un grupo grande de HFHe que estaban en tratamiento hipolipemiante durante una medida de 25 años. Además, la mayoría de sus familiares HFHe han estado con tratamiento con estatinas en algún momento de sus vidas. La prevalencia de ECV estimada en este estudio, 7% en HFHe, está en línea con otros estudios de HFHe genéticamente definida (24,29).

También hemos analizado la mortalidad no CV en estas familias de HFHe genéticamente definida, con un gran historial de tratamiento hipolipemiante con dos propósitos: primero, comprobar si la terapia hipolipemiante podría desempeñar un papel en otras comorbilidades, y segundo, explorar si la propia mutación causante de la HF podría estar asociada a otras morbilidades distintas de la enfermedad CV una vez que los sujetos con HFHe vivan lo suficiente sin enfermedad CV, algo que, hasta ahora, se habría ocultado dada la mortalidad precoz. En este estudio, la mortalidad no CV no mostró diferencias significativas entre HFHe y no HF ni entre ninguno de los grupos separados por sexo. Hubo

una tendencia en las mujeres HFHe a morir más tarde por causas no CV que las mujeres sin HF, aunque la diferencia no alcanzó significación estadística. Planteamos la hipótesis de que podría deberse a los estilos de vida más saludables en sujetos con HFHe como nuestro grupo había mostrado anteriormente (8).

Nuestro estudio tiene algunas limitaciones. En su diseño retrospectivo solo se seleccionan los HFHe que vivieron el tiempo suficiente para poder estudiarlos, por lo que los sujetos HFHe que fallecieron antes del análisis no se pudieron estudiar. El gran número de sujetos estudiados, contando con la dificultad de encontrar grandes series de pacientes, nos permite identificar diferencias entre la mortalidad de los grandes grupos de enfermedades. Hasta ahora si alguna enfermedad pudiera estar asociada a la mutación o al tratamiento, podría haber pasado desapercibido. Para concluir, tenemos información del momento de inicio del tratamiento hipolipemiante, pero solo se pudo corroborar completamente, en los casos y los controles. Los puntos fuertes de nuestro artículo incluyen que todos los casos de HFHe se han confirmado genéticamente y que el diagnóstico de HFHe es independiente de la enfermedad CV.

5.6 Conclusiones

- La mortalidad actual por enfermedad CV en la HFHe es menor y se produce más tarde que la descrita en el siglo pasado, pero sigue siendo mayor que en los sujetos sin HF. Probablemente, esto se deba a un mejor control de los factores de riesgo de enfermedad CV, especialmente a el tratamiento hipolipemiante prolongado. Este mejor pronóstico en el riesgo de enfermedad CV no se asocia con cambios en la mortalidad no CV.

DISCUSIÓN GLOBAL DEL COMPENDIO DE PUBLICACIONES

El trabajo de esta tesis, describe varias patologías que pudieran estar modificando el fenotipo clásico de la HF. El estudio detallado de las mutaciones patogénicas, muy especialmente en *LDLR* y el análisis del tratamiento hipolipemiante prolongado que de forma intensiva y precozmente llevan estos pacientes son los dos hechos fundamentales estudiados en nuestro trabajo y que se encuentran fuertemente asociados con el cambio en el fenotipo encontrado.

El fenotipo clásico de la HFHe se viene mostrando desde hace algunos años, como mucho más heterogéneo y menos agresivo que en años anteriores cuando no se disponía de un tratamiento hipolipemiante eficaz para estos sujetos y mostraban un riesgo de sufrir enfermedad cardiovascular antes de los 55-60 años en más del 50% de los varones y en un 33% de las mujeres (67,140,229).

Para comenzar, hemos estudiado en profundidad una enfermedad que hasta ahora estaba relacionada con sujetos HFHo. Los sujetos homocigotos presentan afectación en los parámetros estructurales y hemodinámicos de la válvula aórtica, con calcificación progresiva y un alto riesgo de estenosis aórtica grave (257). En este sentido, una mayor esperanza actual de los pacientes HFHe, que se calculada antes de la era de las estatinas entre 10-30 años menor para hombres y mujeres respectivamente (258,259), junto con la concentración elevada de Lp(a) que muestran muchos pacientes con HFHe parecen explicar el aumento de EA encontrado en nuestro trabajo. El aumento de Lp(a) es un factor de riesgo independiente de calcificación tanto vascular como de la válvula aórtica en la población general (226,260) que parece estar influyendo en la prevalencia actual de la EA en la HFHe. Los estudios previos sobre esta enfermedad han sido orientados en población general de edad avanzada (>75 años), que son aquellos quienes muestran mayor prevalencia de esta afectación valvular(260). Sin embargo, se desconocía si los pacientes HFHe con una mutación patogénica en *LDLR* y con altas concentraciones de cLDL a lo largo de la vida, tenían una mayor prevalencia de AoVC (227). Nuestro estudio demuestra que la HFHe debe ser incluido como un factor de riesgo relevante para la EA junto con la edad (261). Para evitar sesgos, basados en un diagnóstico meramente clínico de HFHe,

nuestro estudio tiene la potencia de que todos los sujetos fueron reclutados y seleccionados no solamente por criterios lipídicos, sino también definidos por una mutación patogénica en un gen candidato, es decir evitando sesgos de selección basados en los fenotipos cardiovasculares más graves (262).

La EA es un proceso que acaba desarrollándose por el daño endotelial crónico que sufre la zona de la válvula aórtica por la infiltración lipídica, inflamación y su consecuente calcificación y estrechamiento valvular. Entre los factores de riesgo de EA, y que se reflejan en nuestro estudio, se incluyen la edad y la hipercolesterolemia. En este sentido, las diferencias entre las válvulas de HFHe y controles, mostraron una clara relación con la edad. Específicamente, entre los casos de HFHe, se asoció una mayor velocidad aórtica máxima y mayor calcificación de las valvas de la válvula aórtica, con la concentración de cLDL sin tratamiento hipolipemiante, sugiriendo que el cLDL elevado a lo largo de la vida, jugaría un importante papel, considerado como factor de riesgo de arteriosclerosis.

En segundo lugar, hemos profundizado en el estudio de las potenciales diferencias en el fenotipo que pudieran plantear el origen maternal o paternal de la mutación responsable de la HFHe. Se había sugerido que la hipercolesterolemia materna podría explicar algunas diferencias entre sujetos, de la misma manera que se explican en otras enfermedades monogénicas mediante la *impronta genómica* (263) o bien por los factores ambientales que sufre la madre durante la gestación, como el tabaco y la dieta rica en grasas saturadas (264). Todavía no se había establecido si el aumento del colesterol durante el embarazo pudiera repercutir en la descendencia, un efecto que en el caso de las madres HFHe, muestran cifras de colesterol hasta dos veces superior que las madres sin enfermedad. Se había sugerido en algunos estudios un aumento del desarrollo de arteriosclerosis asociado a la hipercolesterolemia de origen materno (265,266). Sin embargo, nuestro estudio no mostró diferencias clínicas ni antropométricas dependiendo del origen parental de la hipercolesterolemia, ni el desarrollo posterior de comorbilidades como la enfermedad CV o DM. De esta manera, se hipotetiza que la concentración plasmática de colesterol en el feto, se regula mediante un proceso bien establecido e independiente del LDLr materno (267). El LDLr quedaría limitado al lado basal del sincitiotrofoblasto, de manera que apenas se expresaría en el feto. Probablemente si se ha

observado alguna asociación entre la concentración de colesterol y la memoria metabólica del feto, dependería más bien de los mecanismos asociados a la sobreproducción de hipercolesterolemia, como son el síndrome metabólico, la obesidad o hiperglucemia. Los factores de riesgo asociados a la madre afectarán de manera indirecta al feto, pero no a consecuencia de las altas concentraciones de cLDL en la madre HFHe. Por ello, y de acuerdo con lo que hemos observado, el manejo clínico de los sujetos con HFHe no debe ser diferente dependiendo de la herencia parental porque su riesgo de comorbilidades es independiente del origen.

Por otro lado, hemos descrito las concentraciones de Lp(a) en las distintas hipercolesterolemias hereditarias, desde fenotipos más heterogéneos como se describen en mutaciones negativas, así como un fenotipo más severo, en el caso de las mutaciones patogénicas en *LDLR, APOB o APOE*. Además, en este último, analizamos el impacto del gen responsable de la HFHe en la concentración de Lp(a). Demostramos que el gen involucrado en HFHe, está relacionado con este aumento, aunque no se explica por el número de kringles IV tipo 2. Hasta la fecha se había observado un concentración mayor de Lp(a) en HFHo(268), pero no se había establecido en HFHe, ni tampoco dependiendo del gen responsable de la HF. Nuestro artículo en línea con otros estudios(269), demuestra que el LDLr apenas juega ningún papel en el catabolismo de Lp(a), ya que se muestra independiente del tipo del defecto del LDLr y que los sujetos con HFHe, analizados genéticamente, tienen una concentración de Lp(a) más alta que la población en general y en mayor medida, aquellos con mutación en el gen de *APOB*.

Nuestro estudio también sugirió que la hipercolesterolemia grave, con altas concentraciones de cLDL muy elevadas desde el nacimiento, en pacientes ≥65 años con HFHe, no se asoció como un factor de riesgo principal al desarrollo de cataratas. Sin embargo, coincidimos con estudios previos sobre cataratas, en que la edad es el principal factor asociado al desarrollo de cataratas (270). El presente estudio tiene la fortaleza de ser realizado en un gran grupo de pacientes que componían las tres características diferenciales previamente no estudiadas, es decir, un subgrupo paradigmático de pacientes ancianos, en los que el tratamiento con altas dosis de estatinas potentes es la primera línea de tratamiento.

La segunda aproximación ha sido analizar patologías que se han asociado de forma no consistente con el tratamiento hipolipemiante.

En este sentido, en el análisis de los factores de riesgo asociados a la enfermedad valvular aórtica, el tratamiento con estatinas no demostró diferencias significativas con respecto al resto de HFHe. En aquellos pacientes en los que la enfermedad valvular ya se había establecido en sus fases iniciales, el tratamiento apenas previno la EA en fases más avanzadas de la enfermedad.

Tampoco el tratamiento hipolipemiante con estatinas a altas dosis, fue asociado al desarrollo de cataratas, en estos sujetos con HFHe, sugiriendo de nuevo el factor de edad, como factor de riesgo relevante para el desarrollo de cataratas.

Por último, y para tener una visión holística de enfermedades que pudieran no haberse descrito previamente, estudiamos un gran grupo numeroso de familias con HFHe y un grupo de familias control, para demostrar las posibles diferencias en la mortalidad de los dos grupos entre todos los familiares de primer grado. El objetivo de este trabajo fue mostrar una actualización de las causas de muerte por enfermedad CV y no CV en HFHe en la era del tratamiento hipolipemiante. Por un lado, identificar si nuestra hipótesis apoyaba la idea de una menor prevalencia de muerte por enfermedad CV en sujetos HFHe. En este sentido, nuestro trabajo sugiere que la prevalencia de muerte por enfermedad CV ha disminuido durante estos años, que se había estimado anteriormente muy elevada con una esperanza de vida estimada entre 20 y 30 años menor que el resto de la población (259). Sin embargo, a pesar de una evidente mejoría, todavía nos encontramos que los varones HFHe fallecieron 6,8 años más jóvenes en comparación con los otros grupos familiares sin HF. Para evitar sesgos de selección, nuevamente seleccionamos a los sujetos mediante un diagnóstico genético positivo. Tradicionalmente los criterios para el diagnóstico de HFHe se basaban en criterios clínicos incluidos en los registros internacionales como el de *Simmon Bromme,* de esta manera los pacientes HFHe o sus familiares con enfermedad CV, eran más fácilmente diagnosticados de HFHe que aquellos en los que la enfermedad CV no estuvo presente. Consecuentemente, un gran grupo de HFHe podrían haber pasado desapercibidos al aplicar criterios meramente clínicos.

Nuestra cohorte tiene la potencia suficiente y apoya estudios previos observacionales de HFHe, que sugerían una reducción de los episodios CV en pacientes que comenzaron con tratamiento hipolipemiante en edades tempranas de la vida y se mantuvieron de manera crónica con el tiempo (148,271). Nuestro estudio trata de un grupo de personas HFHe con un consumo medio de 25 años de tratamiento con estatinas. De acuerdo con la prevalencia estimada hasta la fecha, un 7% en HFHe mostraron enfermedad CV en aquellos definidos genéticamente.

De manera adicional analizamos la mortalidad no CV no estudiada hasta el momento, con el propósito por un lado de comprobar si el tratamiento hipolipemiante podía influir en otras comorbilidades asociadas, y, por otro lado, explorar si la propia mutación responsable de la HFHe se relacionaba con otras causas de enfermedad, en el momento en que una mayor esperanza de vida de estos pacientes pudiera permitir aflorar patologías que podrían haber estado ocultas en presencia de una mortalidad cardiovascular precoz. Nuestro estudio muestra que la mortalidad no CV no mostró diferencias entre los sujetos con y sin HF, sin encontrar diferencias entre sexos.

Nuestro estudio presenta algunas limitaciones. Al tratarse de un diseño retrospectivo solo se seleccionan los HeHF que vivieron un tiempo suficiente para poder estudiarlos, por lo que los sujetos que presentaron una mortalidad precoz no pudieron ser representados. En el caso del tratamiento hipolipemiante, y sobre los antecedentes familiares de enfermedad CV, la asignación podría haber sido sesgada por la inexactitud en el recuerdo, aunque de manera minuciosa se comprobaron en los registros médicos.

Para el estudio de los diferentes fenotipos, hubiera sido de interés disponer de la exposición al cLDL de por vida para estudiar el efecto acumulativo en las distintas patologías, sin embargo, al tratarse en algunos casos de una población anciana, esto no ha sido factible. Consideramos de esta manera el cLDL en el momento del diagnóstico y el número de años que mantuvieron el tratamiento hipolipemiante, como datos subrogados de la exposición al cLDL de por vida.

Finalmente, nuestros resultados son representativos de un fenotipo de la población española basado concretamente en el origen genético caucásico.

El conjunto de patologías estudiadas y el amplio grupo de pacientes HFHe presentado, ha sido necesario para conocer en profundidad la repercusión de todo el conjunto de factores que han influido en los sujetos HFHe en los últimos años y que nos van a orientar en la medida de lo posible hacía una mejora terapéutica y una supuesta mejora en la calidad de vida, en vistas de una enfermedad menos agresiva en el futuro.

CONCLUSIONES
Conclusiones finales estudiadas en el compendio de publicaciones

Conclusiones

- El fenotipo actual de la HFHe caracterizada genéticamente es substancialmente diferente al descrito en el siglo pasado.

- Las diferencias fundamentales radican en una menor prevalencia de enfermedad cardiovascular y una menor mortalidad cardiovascular.

- Otros fenotipos no previamente asociados con HFHe aparecen como relevantes, y hemos demostrado que la enfermedad valvular aórtica es uno de ellos, en personas con HFHe con supervivencia ≥65 años.

- El aumento de riesgo de EA está relacionado con la hipercolesterolemia pero no con el tratamiento con estatinas.

- Una de las nuevas descripciones que aporta el presente trabajo es el aumento de la concentración de Lp(a) en HFHe.

- El aumento de Lp(a) no se relaciona con las mutaciones en el gen *LDLR*.

- La HFhe dependiente de mutaciones en *APOB* tienen concentraciones de Lp(a) más elevadas que las HFHe dependiente de mutaciones en el *LDLR*.

-El fenotipo clínico actual de la HFHe en relación con la enfermedad cardiovascular, DM y HTA no depende del origen parental de la mutación responsable de la HFHe.

- En nuestro estudio no hemos evidenciado en sujetos HFHe con mutación en *LDLR* y larga historia de tratamiento con estatinas un riesgo aumentado de cataratas.

Lista de referencias utilizadas en la introducción

1. Repa JJ, Mangelsdorf DJ. The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. Annu Rev Cell Dev Biol. 2000;16:459-81.

2. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science. 4 de abril de 1986;232(4746):34-47.

3. De Leon H, Boue S, Szostak J, Peitsch MC, Hoeng J. Systems Biology Research into Cardiovascular Disease: Contributions of Lipidomics-based Approaches to Biomarker Discovery. Curr Drug Discov Technol. 2015;12(3):129-54.

4. Iqbal J, Hussain MM. Intestinal lipid absorption. Am J Physiol Endocrinol Metab. junio de 2009;296(6):E1183-1194.

5. Hegele RA. Plasma lipoproteins: genetic influences and clinical implications. Nat Rev Genet. febrero de 2009;10(2):109-21.

6. Dominiczak MH, Caslake MJ. Apolipoproteins: metabolic role and clinical biochemistry applications. Ann Clin Biochem. noviembre de 2011;48(Pt 6):498-515.

7. Ye SQ, Kwiterovich PO. Influence of genetic polymorphisms on responsiveness to dietary fat and cholesterol. Am J Clin Nutr. noviembre de 2000;72(5 Suppl):1275S-1284S.

8. Mahley RW, Innerarity TL, Rall SC, Weisgraber KH. Plasma lipoproteins: apolipoprotein structure and function. J Lipid Res. 1 de diciembre de 1984;25(12):1277-94.

9. Ridker PM. LDL cholesterol: controversies and future therapeutic directions. Lancet. 16 de agosto de 2014;384(9943):607-17.

10. Feingold KR. Introduction to Lipids and Lipoproteins. En: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., editores. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000 [citado 20 de agosto de 2021]. Disponible en: http://www.ncbi.nlm.nih.gov/books/NBK305896/

11. Ros E. Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. Atherosclerosis. agosto de 2000;151(2):357-79.

12. Carey MC, Small DM, Bliss CM. Lipid digestion and absorption. Annu Rev Physiol. 1983;45:651-77.

13. Friedman HI, Nylund B. Intestinal fat digestion, absorption, and transport. A review. Am J Clin Nutr. mayo de 1980;33(5):1108-39.

14. Phan CT, Tso P. Intestinal lipid absorption and transport. Front Biosci. 1 de marzo de 2001;6:D299-319.

15. Green PH, Riley JW. Lipid absorption and intestinal lipoprotein formation. Aust N Z J Med. febrero de 1981;11(1):84-90.

16. Li P-S, Fu Z-Y, Zhang Y-Y, Zhang J-H, Xu C-Q, Ma Y-T, et al. The clathrin adaptor Numb regulates intestinal cholesterol absorption through dynamic interaction with NPC1L1. Nat Med. enero de 2014;20(1):80-6.

17. Bietrix F, Yan D, Nauze M, Rolland C, Bertrand-Michel J, Coméra C, et al. Accelerated lipid absorption in mice overexpressing intestinal SR-BI. J Biol Chem. 17 de marzo de 2006;281(11):7214-9.

18. Hussain MM. Intestinal Lipid Absorption and Lipoprotein Formation. Curr Opin Lipidol. junio de 2014;25(3):200-6.

19. Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J. Editors. En: Harrison's Principles of Internal Medicine [Internet]. 20.ª ed. New York, NY: McGraw-Hill Education; 2018 [citado 7 de marzo de 2022]. Disponible en: accessmedicine.mhmedical.com/content.aspx?aid=1181483305

20. Errico TL, Chen X, Martin Campos JM, Julve J, Escolà-Gil JC, Blanco-Vaca F. [Basic mechanisms: structure, function and metabolism of plasma lipoproteins]. Clin Investig Arterioscler. junio de 2013;25(2):98-103.

21. Chen SH, Habib G, Yang CY, Gu ZW, Lee BR, Weng SA, et al. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. Science. 16 de octubre de 1987;238(4825):363-6.

22. Abumrad NA, Davidson NO. ROLE OF THE GUT IN LIPID HOMEOSTASIS. Physiol Rev. julio de 2012;92(3):1061-85.

23. Kindel T, Lee DM, Tso P. The mechanism of the formation and secretion of chylomicrons. Atheroscler Suppl. junio de 2010;11(1):11-6.

24. Redgrave TG. Chylomicron metabolism. Biochem Soc Trans. febrero de 2004;32(Pt 1):79-82.

25. Grundy SM, Mok HY. Chylomicron clearance in normal and hyperlipidemic man. Metabolism. noviembre de 1976;25(11):1225-39.

26. Pedro-Botet Montoya J, Masana Marín L, Carmena Rodríguez R. Capítulo 8 - Alteraciones del metabolismo de las lipoproteínas. En: Farreras Valentí P, Rozman C, editores. Farreras-Rozman Medicina Interna Metabolismo y Nutrición Endocrinología (Décimo sétimo Edição) [Internet]. Madrid: Elsevier; 2014 [citado 26 de agosto de 2021]. p. 44-65. Disponible en: https://www.sciencedirect.com/science/article/pii/B9788490225950000089

27. Mason TM. The role of factors that regulate the synthesis and secretion of very-low-density lipoprotein by hepatocytes. Crit Rev Clin Lab Sci. diciembre de 1998;35(6):461-87.

28. Pedro-Botet J, Mostaza JM, Pintó X, Banegas JR, En Nombre del Grupo de Investigadores EDICONDIS-ULISEA. [Achievement of low-density lipoprotein cholesterol therapeutic goal in lipid and vascular risk units of the Spanish Arteriosclerosis Society]. Clin Investig Arterioscler. octubre de 2013;25(4):155-63.

29. Griffin BA, Packard CJ. Metabolism of VLDL and LDL subclasses. Curr Opin Lipidol. junio de 1994;5(3):200-6.

30. Remmerie A, Scott CL. Macrophages and lipid metabolism. Cell Immunol. agosto de 2018;330:27-42.

31. Rader DJ, Mann WA, Cain W, Kraft HG, Usher D, Zech LA, et al. The low density lipoprotein receptor is not required for normal catabolism of Lp(a) in humans. J Clin Invest. marzo de 1995;95(3):1403-8.

32. Sathiyakumar V, Kapoor K, Jones SR, Banach M, Martin SS, Toth PP. Novel Therapeutic Targets for Managing Dyslipidemia. Trends Pharmacol Sci. agosto de 2018;39(8):733-47.

33. Illingworth DR. Lipoprotein metabolism. Am J Kidney Dis. julio de 1993;22(1):90-7.

34. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. Cell. 26 de marzo de 2015;161(1):161-72.

35. Goldstein JL, Brown MS. The LDL receptor. Arterioscler Thromb Vasc Biol. abril de 2009;29(4):431-8.

36. Lindgren V, Luskey KL, Russell DW, Francke U. Human genes involved in cholesterol metabolism: chromosomal mapping of the loci for the low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-coenzyme A reductase with cDNA probes. Proc Natl Acad Sci U S A. diciembre de 1985;82(24):8567-71.

37. Aguilar-Ballester M, Herrero-Cervera A, Vinué Á, Martínez-Hervás S, González-Navarro H. Impact of Cholesterol Metabolism in Immune Cell Function and Atherosclerosis. Nutrients. 7 de julio de 2020;12(7):2021.

38. Brown MS, Radhakrishnan A, Goldstein JL. Retrospective on Cholesterol Homeostasis: The Central Role of Scap. Annu Rev Biochem. 20 de junio de 2018;87:783-807.

39. Sharpe LJ, Cook ECL, Zelcer N, Brown AJ. The UPS and downs of cholesterol homeostasis. Trends Biochem Sci. noviembre de 2014;39(11):527-35.

40. Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. Science. 3 de julio de 2009;325(5936):100-4.

41. Zanoni P, Velagapudi S, Yalcinkaya M, Rohrer L, von Eckardstein A. Endocytosis of lipoproteins. Atherosclerosis. agosto de 2018;275:273-95.

42. Tavori H, Fan D, Blakemore JL, Yancey PG, Ding L, Linton MF, et al. Serum proprotein convertase subtilisin/kexin type 9 and cell surface low-density lipoprotein receptor: evidence for a reciprocal regulation. Circulation. 18 de junio de 2013;127(24):2403-13.

43. Chen WJ, Goldstein JL, Brown MS. NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of the low density lipoprotein receptor. J Biol Chem. 25 de febrero de 1990;265(6):3116-23.

44. Jeon H, Blacklow SC. Structure and physiologic function of the low-density lipoprotein receptor. Annu Rev Biochem. 2005;74:535-62.

45. Marcel YL, Ouimet M, Wang M-D. Regulation of cholesterol efflux from macrophages. Curr Opin Lipidol. octubre de 2008;19(5):455-61.

46. Wang S, Smith JD. ABCA1 and nascent HDL biogenesis. Biofactors. noviembre de 2014;40(6):547-54.

47. Rosenson RS, Brewer HB, Davidson WS, Fayad ZA, Fuster V, Goldstein J, et al. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. Circulation. 17 de abril de 2012;125(15):1905-19.

48. Applebaum-Bowden D. Lipases and lecithin: cholesterol acyltransferase in the control of lipoprotein metabolism. Curr Opin Lipidol. junio de 1995;6(3):130-5.

49. Barter PJ, Brewer HB, Chapman MJ, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. Arterioscler Thromb Vasc Biol. 1 de febrero de 2003;23(2):160-7.

50. Lewis GF, Rader DJ. New insights into the regulation of HDL metabolism and reverse cholesterol transport. Circ Res. 24 de junio de 2005;96(12):1221-32.

51. Brewer HB. Increasing HDL Cholesterol Levels. N Engl J Med. 8 de abril de 2004;350(15):1491-4.

52. Tsimikas S. A Test in Context: Lipoprotein(a): Diagnosis, Prognosis, Controversies, and Emerging Therapies. J Am Coll Cardiol. 14 de febrero de 2017;69(6):692-711.

53. Berg K. A NEW SERUM TYPE SYSTEM IN MAN--THE LP SYSTEM. Acta Pathol Microbiol Scand. 1963;59:369-82.

54. Knight BL. Lp(a) catabolism in hypercholesterolaemic individuals. Chemistry and Physics of Lipids. 1 de enero de 1994;67-68:233-9.

55. Albers JJ, Marcovina SM, Lodge MS. The unique lipoprotein(a): properties and immunochemical measurement. Clin Chem. 1 de diciembre de 1990;36(12):2019-26.

56. Enkhmaa B, Anuurad E, Zhang W, Berglund L. Significant associations between lipoprotein(a) and corrected apolipoprotein B-100 levels in African-Americans. Atherosclerosis. julio de 2014;235(1):223-9.

57. Collaboration ERF, Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, et al. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA. 22 de julio de 2009;302(4):412-23.

58. Paquette M, Bernard S, Thanassoulis G, Baass A. LPA genotype is associated with premature cardiovascular disease in familial hypercholesterolemia. J Clin Lipidol. agosto de 2019;13(4):627-633.e1.

59. Boffa MB, Koschinsky ML. Oxidized phospholipids as a unifying theory for lipoprotein(a) and cardiovascular disease. Nat Rev Cardiol. mayo de 2019;16(5):305-18.

60. Beaumont JL, Carlson LA, Cooper GR, Fejfar Z, Fredrickson DS, Strasser T. Classification of hyperlipidaemias and hyperlipoproteinaemias. Bull World Health Organ. 1970;43(6):891-915.

61. Durrington P. Dyslipidaemia. Lancet. 30 de agosto de 2003;362(9385):717-31.

62. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. Rev Esp Cardiol (Engl Ed). febrero de 2017;70(2):115.

63. Lipidología en la práctica Clínica [Internet]. [citado 13 de septiembre de 2021]. Disponible en: http://lipidologiaclinica.es/

64. Hovingh GK, Davidson MH, Kastelein JJP, O'Connor AM. Diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J. abril de 2013;34(13):962-71.

65. Cenarro A, Etxebarria A, de Castro-Orós I, Stef M, Bea AM, Palacios L, et al. The p.Leu167del Mutation in APOE Gene Causes Autosomal Dominant Hypercholesterolemia by Down-regulation of LDL Receptor Expression in Hepatocytes. J Clin Endocrinol Metab. 2016;101(5):2113-21.

66. de Ferranti SD, Rodday AM, Mendelson MM, Wong JB, Leslie LK, Sheldrick RC. Prevalence of Familial Hypercholesterolemia in the 1999 to 2012 United States National Health and Nutrition Examination Surveys (NHANES). Circulation. 15 de marzo de 2016;133(11):1067-72.

67. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. Eur Heart J. diciembre de 2013;34(45):3478-3490a.

68. Ahangari N, Sahebkar A, Azimi-Nezhad M, Ghazizadeh H, Moohebati M, Ebrahim M, et al. A Novel Splice Site Variant in the LDLRAP1 Gene Causes Familial Hypercholesterolemia. Iran Biomed J. 1 de septiembre de 2021;25(5):374-9.

69. Rader DJ, Cohen J, Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. J Clin Invest. junio de 2003;111(12):1795-803.

70. Seftel HC, Baker SG, Sandler MP, Forman MB, Joffe BI, Mendelsohn D, et al. A host of hypercholesterolaemic homozygotes in South Africa. Br Med J. 6 de septiembre de 1980;281(6241):633-6.

71. Boerwinkle E, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs HH. Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. J Clin Invest. julio de 1992;90(1):52-60.

72. Schmidt K, Noureen A, Kronenberg F, Utermann G. Structure, function, and genetics of lipoprotein (a). J Lipid Res. 1 de agosto de 2016;57(8):1339-59.

73. Alonso R, Andres E, Mata N, Fuentes-Jiménez F, Badimón L, López-Miranda J, et al. Lipoprotein(a) levels in familial hypercholesterolemia: an important predictor of cardiovascular disease independent of the type of LDL receptor mutation. J Am Coll Cardiol. 20 de mayo de 2014;63(19):1982-9.

74. Langsted A, Kamstrup PR, Benn M, Tybjærg-Hansen A, Nordestgaard BG. High lipoprotein(a) as a possible cause of clinical familial hypercholesterolaemia: a prospective cohort study. Lancet Diabetes Endocrinol. 2016;4(7):577-87.

75. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. N Engl J Med. 29 de noviembre de 2018;379(22):2097-107.

76. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. N Engl J Med. 4 de mayo de 2017;376(18):1713-22.

77. Zhou H, Gong Y, Wu Q, Ye X, Yu B, Lu C, et al. Rare Diseases Related with Lipoprotein Metabolism. Adv Exp Med Biol. 2020;1276:171-88.

78. Franceschini G. Epidemiologic evidence for high-density lipoprotein cholesterol as a risk factor for coronary artery disease. Am J Cardiol. 20 de diciembre de 2001;88(12A):9N-13N.

79. Yoo E-G. Sitosterolemia: a review and update of pathophysiology, clinical spectrum, diagnosis, and management. Ann Pediatr Endocrinol Metab. marzo de 2016;21(1):7-14.

80. Katayama T, Katayama S, Satoh T, Yagi T, Hirose N, Kurita Y, et al. A 19-year-old man with myocardial infarction and sitosterolemia. Intern Med. julio de 2003;42(7):591-4.

81. Farzam K, Morgan RT. Hereditary Sitosterolemia. En: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 [citado 23 de septiembre de 2021]. Disponible en: http://www.ncbi.nlm.nih.gov/books/NBK572142/

82. Chiang JYL, Ferrell JM. Up to date on cholesterol 7 alpha-hydroxylase (CYP7A1) in bile acid synthesis. Liver Res. junio de 2020;4(2):47-63.

83. Brouwers MCGJ, van Greevenbroek MMJ, Stehouwer CDA, de Graaf J, Stalenhoef AFH. The genetics of familial combined hyperlipidaemia. Nat Rev Endocrinol. 14 de febrero de 2012;8(6):352-62.

84. Mata P, Alonso R, Ruíz-Garcia A, Díaz-Díaz JL, González N, Gijón-Conde T, et al. [Familial combined hyperlipidemia: consensus document]. Aten Primaria. octubre de 2014;46(8):440-6.

85. Taghizadeh E, Esfehani RJ, Sahebkar A, Parizadeh SM, Rostami D, Mirinezhad M, et al. Familial combined hyperlipidemia: An overview of the underlying molecular mechanisms and therapeutic strategies. IUBMB Life. septiembre de 2019;71(9):1221-9.

86. Koopal C, Marais AD, Visseren FLJ. Familial dysbetalipoproteinemia: an underdiagnosed lipid disorder. Curr Opin Endocrinol Diabetes Obes. abril de 2017;24(2):133-9.

87. McGINLEY J, Jones H, Gofman J. Lipoproteins and xanthomatous diseases. J Invest Dermatol. julio de 1952;19(1):71-82.

88. Fredrickson DS, Levy RI, Lees RS. Fat transport in lipoproteins--an integrated approach to mechanisms and disorders. N Engl J Med. 2 de febrero de 1967;276(5):273-281 concl.

89. Utermann G, Jaeschke M, Menzel J. Familial hyperlipoproteinemia type III: deficiency of a specific apolipoprotein (apo E-III) in the very-low-density lipoproteins. FEBS Lett. 15 de agosto de 1975;56(2):352-5.

90. Fredrickson DS, Morganroth J, Levy RI. Type III hyperlipoproteinemia: an analysis of two contemporary definitions. Ann Intern Med. febrero de 1975;82(2):150-7.

91. Blum CB. Type III Hyperlipoproteinemia: Still Worth Considering? Prog Cardiovasc Dis. octubre de 2016;59(2):119-24.

92. Connelly PW, Hegele RA. Hepatic lipase deficiency. Crit Rev Clin Lab Sci. diciembre de 1998;35(6):547-72.

93. Dugi KA, Brandauer K, Schmidt N, Nau B, Schneider JG, Mentz S, et al. Low hepatic lipase activity is a novel risk factor for coronary artery disease. Circulation. 18 de diciembre de 2001;104(25):3057-62.

94. Santamarina-Fojo S, González-Navarro H, Freeman L, Wagner E, Nong Z. Hepatic lipase, lipoprotein metabolism, and atherogenesis. Arterioscler Thromb Vasc Biol. octubre de 2004;24(10):1750-4.

95. Chora JR, Alves AC, Medeiros AM, Mariano C, Lobarinhas G, Guerra A, et al. Lysosomal acid lipase deficiency: A hidden disease among cohorts of familial hypercholesterolemia? J Clin Lipidol. abril de 2017;11(2):477-484.e2.

96. Scott SA, Liu B, Nazarenko I, Martis S, Kozlitina J, Yang Y, et al. Frequency of the Cholesteryl Ester Storage Disease Common LIPA E8SJM Mutation (c.894G>A) in Various Racial and Ethnic Groups. Hepatology. septiembre de 2013;58(3):958-65.

97. Abramov A, Schorr S, Wolman M. Generalized xanthomatosis with calcified adrenals. AMA J Dis Child. marzo de 1956;91(3):282-6.

98. Bernstein DL, Hülkova H, Bialer MG, Desnick RJ. Cholesteryl ester storage disease: Review of the findings in 135 reported patients with an underdiagnosed disease. Journal of Hepatology. 1 de junio de 2013;58(6):1230-43.

99. Jones SA, Valayannopoulos V, Schneider E, Eckert S, Banikazemi M, Bialer M, et al. Rapid progression and mortality of lysosomal acid lipase deficiency presenting in infants. Genet Med. mayo de 2016;18(5):452-8.

100. Burton BK, Deegan PB, Enns GM, Guardamagna O, Horslen S, Hovingh GK, et al. Clinical Features of Lysosomal Acid Lipase Deficiency. J Pediatr Gastroenterol Nutr. diciembre de 2015;61(6):619-25.

101. Berglund L, Brunzell JD, Goldberg AC, Goldberg IJ, Sacks F, Murad MH, et al. Evaluation and Treatment of Hypertriglyceridemia: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. septiembre de 2012;97(9):2969-89.

102. Dron JS, Hegele RA. Genetics of Triglycerides and the Risk of Atherosclerosis. Curr Atheroscler Rep. 2017;19(7):31.

103. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, et al. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. Circulation. 20 de junio de 2000;101(24):2777-82.

104. Brunzell JD, Schrott HG. The interaction of familial and secondary causes of hypertriglyceridemia: role in pancreatitis. J Clin Lipidol. octubre de 2012;6(5):409-12.

105. Ayyobi AF, McGladdery SH, McNeely MJ, Austin MA, Motulsky AG, Brunzell JD. Small, dense LDL and elevated apolipoprotein B are the common characteristics for the three major lipid phenotypes of familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol. 1 de julio de 2003;23(7):1289-94.

106. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. Lancet. 16 de agosto de 2014;384(9943):626-35.

107. G. Parhofer K, Laufs U. The Diagnosis and Treatment of Hypertriglyceridemia. Dtsch Arztebl Int. diciembre de 2019;116(49):825-32.

108. Okorodudu DE, Crowley MJ, Sebastian S, Rowell JV, Guyton JR. Inherited lipemic splenomegaly and the spectrum of apolipoprotein E p.Leu167del mutation phenotypic variation. J Clin Lipidol. diciembre de 2013;7(6):566-72.

109. Chait A, Eckel RH. The Chylomicronemia Syndrome Is Most Often Multifactorial: A Narrative Review of Causes and Treatment. Ann Intern Med. 7 de mayo de 2019;170(9):626-34.

110. Pallazola VA, Sajja A, Derenbecker R, Ogunmoroti O, Park J, Sathiyakumar V, et al. Prevalence of familial chylomicronemia syndrome in a quaternary care center. Eur J Prev Cardiol. diciembre de 2020;27(19):2276-8.

111. Baass A, Paquette M, Bernard S, Hegele RA. Familial chylomicronemia syndrome: an under-recognized cause of severe hypertriglyceridaemia. J Intern Med. abril de 2020;287(4):340-8.

112. Williams L, Wilson DP. Editorial commentary: Dietary management of familial chylomicronemia syndrome. J Clin Lipidol. junio de 2016;10(3):462-5.

113. Ewald N, Kloer H-U. Treatment options for severe hypertriglyceridemia (SHTG): the role of apheresis. Clin Res Cardiol Suppl. junio de 2012;7:31-5.

114. Heaney AP, Sharer N, Rameh B, Braganza JM, Durrington PN. Prevention of recurrent pancreatitis in familial lipoprotein lipase deficiency with high-dose antioxidant therapy. J Clin Endocrinol Metab. abril de 1999;84(4):1203-5.

115. Talmud PJ, Shah S, Whittall R, Futema M, Howard P, Cooper JA, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study. Lancet. 13 de abril de 2013;381(9874):1293-301.

116. Futema M, Bourbon M, Williams M, Humphries SE. Clinical utility of the polygenic LDL-C SNP score in familial hypercholesterolemia. Atherosclerosis. octubre de 2018;277:457-63.

117. Sharifi M, Futema M, Nair D, Humphries SE. Polygenic Hypercholesterolemia and Cardiovascular Disease Risk. Curr Cardiol Rep. 2019;21(6):43.

118. Humphries SE, Cooper JA, Seed M, Capps N, Durrington PN, Jones B, et al. Coronary heart disease mortality in treated familial hypercholesterolaemia: Update of the UK Simon Broome FH register. Atherosclerosis. julio de 2018;274:41-6.

119. Ascaso J, Gonzalez Santos P, Hernandez Mijares A, Rojas AM, Masana L, Millan J, et al. Management of Dyslipidemia in the Metabolic Syndrome. Am J Cardiovasc Drugs. 1 de enero de 2007;7(1):39-58.

120. Núñez-Cortés JM, Pedro-Botet J. Atherogenic dyslipemia: the other pandemic, associated with diabesity. Clin Investig Arterioscler. febrero de 2021;33(1):30-2.

121. de la Sierra A, Gorostidi M, Aranda P, Corbella E, Pintó X. Prevalence of Atherogenic Dyslipidemia in Spanish Hypertensive Patients and Its Relationship With Blood Pressure Control and Silent Organ Damage. Rev Esp Cardiol (Engl Ed). julio de 2015;68(7):592-8.

122. Summerhill VI, Grechko AV, Yet S-F, Sobenin IA, Orekhov AN. The Atherogenic Role of Circulating Modified Lipids in Atherosclerosis. Int J Mol Sci. 20 de julio de 2019;20(14):3561.

123. Gofman JW, Delalla O, Glazier F, Freeman NK, Lindgren FT, Nichols AV, et al. The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis and coronary heart disease. J Clin Lipidol. mayo de 2007;1(2):104-41.

124. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. JAMA. 20 de enero de 1984;251(3):351-64.

125. Proctor SD, Mamo JC. Retention of fluorescent-labelled chylomicron remnants within the intima of the arterial wall--evidence that plaque cholesterol may be derived from post-prandial lipoproteins. Eur J Clin Invest. junio de 1998;28(6):497-503.

126. Twickler T, Dallinga-Thie GM, Chapman MJ, Cohn JS. Remnant lipoproteins and atherosclerosis. Curr Atheroscler Rep. marzo de 2005;7(2):140-7.

127. Wilhelm MG, Cooper AD. Induction of atherosclerosis by human chylomicron remnants: a hypothesis. J Atheroscler Thromb. 2003;10(3):132-9.

128. Huang L, Chambliss KL, Gao X, Yuhanna IS, Behling-Kelly E, Bergaya S, et al. SR-B1 Drives Endothelial Cell LDL Transcytosis via DOCK4 to Promote Atherosclerosis. Nature. mayo de 2019;569(7757):565-9.

129. Civeira F, Marco-Benedí V, Cenarro A. Papel de los lípidos en la ateroesclerosis. Rev Esp Cardiol. 1 de diciembre de 2020;20:2-7.

130. Linton MF, Yancey PG, Davies SS, Jerome WG, Linton EF, Song WL, et al. The Role of Lipids and Lipoproteins in Atherosclerosis. En: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., editores. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000 [citado 16 de noviembre de 2021]. Disponible en: http://www.ncbi.nlm.nih.gov/books/NBK343489/

131. Orsó E, Schmitz G. Lipoprotein(a) and its role in inflammation, atherosclerosis and malignancies. Clin Res Cardiol Suppl. 2017;12(Suppl 1):31-7.

132. Ellis KL, Boffa MB, Sahebkar A, Koschinsky ML, Watts GF. The renaissance of lipoprotein(a): Brave new world for preventive cardiology? Prog Lipid Res. octubre de 2017;68:57-82.

133. Tada H, Takamura M, Kawashiri M-A. Lipoprotein(a) as an Old and New Causal Risk Factor of Atherosclerotic Cardiovascular Disease. J Atheroscler Thromb. 1 de julio de 2019;26(7):583-91.

134. Borén J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease:

pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 21 de junio de 2020;41(24):2313-30.

135. Vandivier RW, Henson PM, Douglas IS. Burying the dead: the impact of failed apoptotic cell removal (efferocytosis) on chronic inflammatory lung disease. Chest. junio de 2006;129(6):1673-82.

136. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J. Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. Ann Intern Med. julio de 1961;55:33-50.

137. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J. 1 de agosto de 2016;37(29):2315-81.

138. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, et al. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. JAMA. 20 de noviembre de 2013;310(19):2061-8.

139. Raygor V, Khera A. New Recommendations and Revised Concepts in Recent Guidelines on the Management of Dyslipidemias to Prevent Cardiovascular Disease: the 2018 ACC/AHA and 2019 ESC/EAS Guidelines. Curr Cardiol Rep. 9 de julio de 2020;22(9):87.

140. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 31 de agosto de 2019;

141. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, et al. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. N Engl J Med. 18 de junio de 2015;372(25):2387-97.

142. Denke MA. Weighing in before the fight: low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol versus apolipoprotein B as the best predictor for coronary heart disease and the best measure of therapy. Circulation. 29 de noviembre de 2005;112(22):3368-70.

143. Dobiásová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-

lipoprotein-depleted plasma (FER(HDL)). Clin Biochem. octubre de 2001;34(7):583-8.

144. Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res. enero de 1982;23(1):97-104.

145. Sata T, Havel RJ, Jones AL. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. J Lipid Res. noviembre de 1972;13(6):757-68.

146. Gazi IF, Tsimihodimos V, Tselepis AD, Elisaf M, Mikhailidis DP. Clinical importance and therapeutic modulation of small dense low-density lipoprotein particles. Expert Opin Biol Ther. enero de 2007;7(1):53-72.

147. Ference BA, Majeed F, Penumetcha R, Flack JM, Brook RD. Effect of Naturally Random Allocation to Lower Low-Density Lipoprotein Cholesterol on the Risk of Coronary Heart Disease Mediated by Polymorphisms in NPC1L1, HMGCR, or Both: A 2 × 2 Factorial Mendelian Randomization Study. Journal of the American College of Cardiology. 21 de abril de 2015;65(15):1552-61.

148. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet. 8 de octubre de 2005;366(9493):1267-78.

149. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 21 de agosto de 2017;38(32):2459-72.

150. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk. abril de 1996;3(2):213-9.

151. Ference BA, Kastelein JJP, Ray KK, Ginsberg HN, Chapman MJ, Packard CJ, et al. Association of Triglyceride-Lowering LPL Variants and LDL-C-Lowering LDLR Variants With Risk of Coronary Heart Disease. JAMA. 29 de enero de 2019;321(4):364-73.

152. Berrougui H, Momo CN, Khalil A. Health benefits of high-density lipoproteins in preventing cardiovascular diseases. J Clin Lipidol. diciembre de 2012;6(6):524-33.

153. Lincoff AM, Nicholls SJ, Riesmeyer JS, Barter PJ, Brewer HB, Fox KAA, et al. Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. N Engl J Med. 18 de mayo de 2017;376(20):1933-42.

154. O'Donoghue ML, Fazio S, Giugliano RP, Stroes ESG, Kanevsky E, Gouni-Berthold I, et al. Lipoprotein(a), PCSK9 Inhibition, and Cardiovascular Risk. Circulation. 19 de marzo de 2019;139(12):1483-92.

155. Raal FJ, Giugliano RP, Sabatine MS, Koren MJ, Langslet G, Bays H, et al. Reduction in lipoprotein(a) with PCSK9 monoclonal antibody evolocumab (AMG 145): a pooled analysis of more than 1,300 patients in 4 phase II trials. J Am Coll Cardiol. 8 de abril de 2014;63(13):1278-88.

156. Boekholdt SM, Arsenault BJ, Mora S, Pedersen TR, LaRosa JC, Nestel PJ, et al. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. JAMA. 28 de marzo de 2012;307(12):1302-9.

157. Najam O, Ray KK. Familial Hypercholesterolemia: a Review of the Natural History, Diagnosis, and Management. Cardiol Ther. junio de 2015;4(1):25-38.

158. Nutrition classics. Archives of Internal Medicine, Volume 64, October 1939: Angina pectoris in hereditary xanthomatosis. By Carl Müller. Nutr Rev. abril de 1987;45(4):113-5.

159. Khachadurian AK. THE INHERITANCE OF ESSENTIAL FAMILIAL HYPERCHOLESTEROLEMIA. Am J Med. septiembre de 1964;37:402-7.

160. Goldstein JL, Brown MS. Familial hypercholesterolemia: identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. Proc Natl Acad Sci U S A. octubre de 1973;70(10):2804-8.

161. Moorjani S, Roy M, Gagné C, Davignon J, Brun D, Toussaint M, et al. Homozygous familial hypercholesterolemia among French Canadians in Québec Province. Arteriosclerosis. abril de 1989;9(2):211-6.

162. Abifadel M, Rabès J-P, Jambart S, Halaby G, Gannagé-Yared M-H, Sarkis A, et al. The molecular basis of familial hypercholesterolemia in Lebanon: spectrum of LDLR mutations and role of PCSK9 as a modifier gene. Hum Mutat. julio de 2009;30(7):E682-691.

163. Kotze MJ, De Villiers WJ, Steyn K, Kriek JA, Marais AD, Langenhoven E, et al. Phenotypic variation among familial hypercholesterolemics heterozygous for either one of two Afrikaner founder LDL receptor mutations. Arterioscler Thromb. octubre de 1993;13(10):1460-8.

164. Meiner V, Landsberger D, Berkman N, Reshef A, Segal P, Seftel HC, et al. A common Lithuanian mutation causing familial hypercholesterolemia in Ashkenazi Jews. Am J Hum Genet. agosto de 1991;49(2):443-9.

165. Steyn K, Goldberg YP, Kotze MJ, Steyn M, Swanepoel AS, Fourie JM, et al. Estimation of the prevalence of familial hypercholesterolaemia in a rural Afrikaner community by direct screening for three Afrikaner founder low density lipoprotein receptor gene mutations. Hum Genet. octubre de 1996;98(4):479-84.

166. Austin MA, Hutter CM, Zimmern RL, Humphries SE. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. Am J Epidemiol. 1 de septiembre de 2004;160(5):407-20.

167. Florentin M, Kostapanos MS, Elisaf MS, Liberopoulos EN. Prevalence, Identification, and Scouting for Familial Hypercholesterolaemia Including Registries. Curr Pharm Des. 2018;24(31):3605-15.

168. Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J. 21 de septiembre de 2015;36(36):2425-37.

169. Cuchel M, Bruckert E, Ginsberg HN, Raal FJ, Santos RD, Hegele RA, et al. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. Eur Heart J. 21 de agosto de 2014;35(32):2146-57.

170. Guallar-Castillón P, Gil-Montero M, León-Muñoz LM, Graciani A, Bayán-Bravo A, Taboada JM, et al. Magnitude and management of hypercholesterolemia in the adult population of Spain, 2008-2010: The ENRICA Study. Rev Esp Cardiol (Engl Ed). junio de 2012;65(6):551-8.

171. Awan Z, Choi HY, Stitziel N, Ruel I, Bamimore MA, Husa R, et al. APOE p.Leu167del mutation in familial hypercholesterolemia. Atherosclerosis. diciembre de 2013;231(2):218-22.

172. Fouchier SW, Dallinga-Thie GM, Meijers JCM, Zelcer N, Kastelein JJP, Defesche JC, et al. Mutations in STAP1 are associated with autosomal dominant hypercholesterolemia. Circ Res. 29 de agosto de 2014;115(6):552-5.

173. Berberich AJ, Hegele RA. The complex molecular genetics of familial hypercholesterolaemia. Nat Rev Cardiol. enero de 2019;16(1):9-20.

174. Benn M, Watts GF, Tybjærg-Hansen A, Nordestgaard BG. Mutations causative of familial hypercholesterolaemia: screening of 98 098 individuals from the

Copenhagen General Population Study estimated a prevalence of 1 in 217. Eur Heart J. 1 de mayo de 2016;37(17):1384-94.

175. Schmidt EB, Hedegaard BS, Retterstøl K. Familial hypercholesterolaemia: history, diagnosis, screening, management and challenges. Heart. diciembre de 2020;106(24):1940-6.

176. Chora JR, Medeiros AM, Alves AC, Bourbon M. Analysis of publicly available LDLR, APOB, and PCSK9 variants associated with familial hypercholesterolemia: application of ACMG guidelines and implications for familial hypercholesterolemia diagnosis. Genet Med. junio de 2018;20(6):591-8.

177. Masana L, Civeira F, Pedro-Botet J, de Castro I, Pocoví M, Plana N, et al. [Expert consensus on the detection and clinical management of familial hypercholesterolemia]. Clin Investig Arterioscler. octubre de 2013;25(4):182-93.

178. Ascaso JF, Millán J, Hernández-Mijares A, Blasco M, Brea Á, Díaz Á, et al. Consensus document on the management of the atherogenic dyslipidaemia of the Spanish Society of Arteriosclerosis. Clin Investig Arterioscler. abril de 2017;29(2):86-91.

179. Südhof TC, Goldstein JL, Brown MS, Russell DW. The LDL receptor gene: a mosaic of exons shared with different proteins. Science. 17 de mayo de 1985;228(4701):815-22.

180. Fahed AC, Nemer GM. Familial hypercholesterolemia: the lipids or the genes? Nutr Metab (Lond). 22 de abril de 2011;8(1):23.

181. Horton JD, Cohen JC, Hobbs HH. Molecular biology of PCSK9: its role in LDL metabolism. Trends Biochem Sci. febrero de 2007;32(2):71-7.

182. Francke U, Brown MS, Goldstein JL. Assignment of the human gene for the low density lipoprotein receptor to chromosome 19: synteny of a receptor, a ligand, and a genetic disease. Proc Natl Acad Sci U S A. mayo de 1984;81(9):2826-30.

183. Fokkema IFAC, Taschner PEM, Schaafsma GCP, Celli J, Laros JFJ, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat. mayo de 2011;32(5):557-63.

184. Russell DW, Brown MS, Goldstein JL. Different combinations of cysteine-rich repeats mediate binding of low density lipoprotein receptor to two different proteins. J Biol Chem. 25 de diciembre de 1989;264(36):21682-8.

185. Esser V, Limbird LE, Brown MS, Goldstein JL, Russell DW. Mutational analysis of the ligand binding domain of the low density lipoprotein receptor. J Biol Chem. 15 de septiembre de 1988;263(26):13282-90.

186. Kozarsky K, Kingsley D, Krieger M. Use of a mutant cell line to study the kinetics and function of O-linked glycosylation of low density lipoprotein receptors. Proc Natl Acad Sci U S A. junio de 1988;85(12):4335-9.

187. Schneider WJ. Lipoprotein receptors. En: New Comprehensive Biochemistry [Internet]. Elsevier; 2002 [citado 15 de octubre de 2021]. p. 553-72. (Biochemistry of Lipids, Lipoproteins and Membranes, 4th edition; vol. 36). Disponible en: https://www.sciencedirect.com/science/article/pii/S016773060236023X

188. Yokode M, Pathak RK, Hammer RE, Brown MS, Goldstein JL, Anderson RG. Cytoplasmic sequence required for basolateral targeting of LDL receptor in livers of transgenic mice. J Cell Biol. abril de 1992;117(1):39-46.

189. Al-Allaf FA, Coutelle C, Waddington SN, David AL, Harbottle R, Themis M. LDLR-Gene therapy for familial hypercholesterolaemia: problems, progress, and perspectives. Int Arch Med. 13 de diciembre de 2010;3:36.

190. Hobbs HH, Leitersdorf E, Goldstein JL, Brown MS, Russell DW. Multiple crm- mutations in familial hypercholesterolemia. Evidence for 13 alleles, including four deletions. J Clin Invest. marzo de 1988;81(3):909-17.

191. Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, et al. The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA. Cell. noviembre de 1984;39(1):27-38.

192. Lehrman MA, Schneider WJ, Südhof TC, Brown MS, Goldstein JL, Russell DW. Mutation in LDL receptor: Alu-Alu recombination deletes exons encoding transmembrane and cytoplasmic domains. Science. 11 de enero de 1985;227(4683):140-6.

193. Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat. 1992;1(6):445-66.

194. Innerarity TL, Weisgraber KH, Arnold KS, Mahley RW, Krauss RM, Vega GL, et al. Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding. Proc Natl Acad Sci U S A. octubre de 1987;84(19):6919-23.

195. Horton JD, Cohen JC, Hobbs HH. Molecular biology of PCSK9: its role in LDL metabolism. Trends Biochem Sci. febrero de 2007;32(2):71-7.

196. Abifadel M, Rabès J-P, Devillers M, Munnich A, Erlich D, Junien C, et al. Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (PCSK9) gene in cholesterol metabolism and disease. Hum Mutat. abril de 2009;30(4):520-9.

197. Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet. febrero de 2005;37(2):161-5.

198. Cohen JC, Boerwinkle E, Mosley TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med. 23 de marzo de 2006;354(12):1264-72.

199. Brown MS, Goldstein JL. Biomedicine. Lowering LDL--not only how low, but how long? Science. 24 de marzo de 2006;311(5768):1721-3.

200. Santos RD, Gidding SS, Hegele RA, Cuchel MA, Barter PJ, Watts GF, et al. Defining severe familial hypercholesterolaemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. Lancet Diabetes Endocrinol. octubre de 2016;4(10):850-61.

201. Civeira F, Ros E, Jarauta E, Plana N, Zambon D, Puzo J, et al. Comparison of genetic versus clinical diagnosis in familial hypercholesterolemia. Am J Cardiol. 1 de noviembre de 2008;102(9):1187-93, 1193.e1.

202. Civeira F, International Panel on Management of Familial Hypercholesterolemia. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. Atherosclerosis. marzo de 2004;173(1):55-68.

203. Zak A, Zeman M, Slaby A, Vecka M. Xanthomas: clinical and pathophysiological relations. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. junio de 2014;158(2):181-8.

204. Civeira F, Castillo S, Alonso R, Meriño-Ibarra E, Cenarro A, Artied M, et al. Tendon xanthomas in familial hypercholesterolemia are associated with cardiovascular risk independently of the low-density lipoprotein receptor gene mutation. Arterioscler Thromb Vasc Biol. septiembre de 2005;25(9):1960-5.

205. Reina D, Jericó C, Estrada P, Navarro V, Torrente V, Armario P, et al. Ecografía en el diagnóstico y manejo de los xantomas tendinosos en la hipercolesterolemia familiar. Reumatol Clin. 1 de septiembre de 2019;15(5):305-6.

206. Bergman R. Xanthelasma palpebrarum and risk of atherosclerosis. Int J Dermatol. mayo de 1998;37(5):343-5.

207. Kim J, Kim YJ, Lim H, Lee SI. Bilateral circular xanthelasma palpebrarum. Arch Plast Surg. julio de 2012;39(4):435-7.

208. Civeira F, Perez-Calahorra S, Mateo-Gallego R. Rapid resolution of xanthelasmas after treatment with alirocumab. J Clin Lipidol. octubre de 2016;10(5):1259-61.

209. Fernández A, Sorokin A, Thompson PD. Corneal arcus as coronary artery disease risk factor. Atherosclerosis. agosto de 2007;193(2):235-40.

210. Shanoff HM, Little JA. STUDIES OF MALE SURVIVORS OF MYOCARDIAL INFARCTION DUE TO «ESSENTIAL» ATHEROSCLEROSIS. 3. CORNEAL ARCUS: INCIDENCE AND RELATION TO SERUM LIPIDS AND LIPOPROTEINS. Can Med Assoc J. 17 de octubre de 1964;91:835-9.

211. Rosenman RH, Brand RJ, Sholtz RI, Jenkins CD. Relation of corneal arcus to cardiovascular risk factors and the incidence of coronary disease. N Engl J Med. 19 de diciembre de 1974;291(25):1322-4.

212. Townsend N, Nichols M, Scarborough P, Rayner M. Cardiovascular disease in Europe--epidemiological update 2015. Eur Heart J. 21 de octubre de 2015;36(40):2696-705.

213. Slack J. Risks of ischaemic heart-disease in familial hyperlipoproteinaemic states. Lancet. 27 de diciembre de 1969;2(7635):1380-2.

214. Austin MA, Hutter CM, Zimmern RL, Humphries SE. Familial hypercholesterolemia and coronary heart disease: a HuGE association review. Am J Epidemiol. 1 de septiembre de 2004;160(5):421-9.

215. Hopkins PN, Toth PP, Ballantyne CM, Rader DJ, National Lipid Association Expert Panel on Familial Hypercholesterolemia. Familial hypercholesterolemias: prevalence, genetics, diagnosis and screening recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol. junio de 2011;5(3 Suppl):S9-17.

216. Koivisto UM, Hämäläinen L, Taskinen MR, Kettunen K, Kontula K. Prevalence of familial hypercholesterolemia among young north Karelian patients with coronary heart disease: a study based on diagnosis by polymerase chain reaction. J Lipid Res. febrero de 1993;34(2):269-77.

217. Cholesterol Treatment Trialists' (CTT) Collaborators, Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, et al. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. Lancet. 11 de agosto de 2012;380(9841):581-90.

218. Ford I, Murray H, McCowan C, Packard CJ. Long-Term Safety and Efficacy of Lowering Low-Density Lipoprotein Cholesterol With Statin Therapy. Circulation. 15 de marzo de 2016;133(11):1073-80.

219. Pérez de Isla L, Alonso R, Mata N, Fernández-Pérez C, Muñiz O, Díaz-Díaz JL, et al. Predicting Cardiovascular Events in Familial Hypercholesterolemia: The

SAFEHEART Registry (Spanish Familial Hypercholesterolemia Cohort Study). Circulation. 30 de mayo de 2017;135(22):2133-44.

220. Perez-Calahorra S, Laclaustra M, Marco-Benedí V, Lamiquiz-Moneo I, Pedro-Botet J, Plana N, et al. Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia. Atherosclerosis. mayo de 2019;284:245-52.

221. Mortality in treated heterozygous familial hypercholesterolaemia: implications for clinical management. Scientific Steering Committee on behalf of the Simon Broome Register Group. Atherosclerosis. enero de 1999;142(1):105-12.

222. Mundal L, Igland J, Ose L, Holven KB, Veierød MB, Leren TP, et al. Cardiovascular disease mortality in patients with genetically verified familial hypercholesterolemia in Norway during 1992-2013. Eur J Prev Cardiol. enero de 2017;24(2):137-44.

223. Mönestam E. Long-term outcome of cataract surgery: 20-year results from a population-based prospective study. J Cataract Refract Surg. diciembre de 2019;45(12):1732-7.

224. Besseling J, Hovingh GK, Huijgen R, Kastelein JJP, Hutten BA. Statins in Familial Hypercholesterolemia: Consequences for Coronary Artery Disease and All-Cause Mortality. J Am Coll Cardiol. 19 de julio de 2016;68(3):252-60.

225. Vuorio A, Watts GF, Schneider WJ, Tsimikas S, Kovanen PT. Familial hypercholesterolemia and elevated lipoprotein(a): double heritable risk and new therapeutic opportunities. J Intern Med. enero de 2020;287(1):2-18.

226. Vongpromek R, Bos S, Ten Kate G-JR, Yahya R, Verhoeven AJM, de Feyter PJ, et al. Lipoprotein(a) levels are associated with aortic valve calcification in asymptomatic patients with familial hypercholesterolaemia. J Intern Med. agosto de 2015;278(2):166-73.

227. Ten Kate G-JR, Bos S, Dedic A, Neefjes LA, Kurata A, Langendonk JG, et al. Increased Aortic Valve Calcification in Familial Hypercholesterolemia: Prevalence, Extent, and Associated Risk Factors. J Am Coll Cardiol. 22 de diciembre de 2015;66(24):2687-95.

228. Sprecher DL, Schaefer EJ, Kent KM, Gregg RE, Zech LA, Hoeg JM, et al. Cardiovascular features of homozygous familial hypercholesterolemia: analysis of 16 patients. Am J Cardiol. 1 de julio de 1984;54(1):20-30.

229. Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group. BMJ. 12 de octubre de 1991;303(6807):893-6.

230. Williams RR, Hunt SC, Schumacher MC, Hegele RA, Leppert MF, Ludwig EH, et al. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. Am J Cardiol. 15 de julio de 1993;72(2):171-6.

231. Reeskamp LF, Tromp TR, Defesche JC, Grefhorst A, Stroes ES, Hovingh GK, et al. Next-generation sequencing to confirm clinical familial hypercholesterolemia. Eur J Prev Cardiol. 27 de julio de 2020;2047487320942996.

232. McGowan MP, Hosseini Dehkordi SH, Moriarty PM, Duell PB. Diagnosis and Treatment of Heterozygous Familial Hypercholesterolemia. J Am Heart Assoc. 16 de diciembre de 2019;8(24):e013225.

233. Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F. New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol Ther. diciembre de 1999;84(3):413-28.

234. Bellosta S, Paoletti R, Corsini A. Safety of statins: focus on clinical pharmacokinetics and drug interactions. Circulation. 15 de junio de 2004;109(23 Suppl 1):III50-57.

235. Clark MA, Finkel R Rey, Jose A, Whalen K. Pharmacology. 2012.

236. Sirtori CR, Mombelli G, Triolo M, Laaksonen R. Clinical response to statins: mechanism(s) of variable activity and adverse effects. Ann Med. agosto de 2012;44(5):419-32.

237. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 1 de julio de 2014;63(25 Pt B):2889-934.

238. Strilchuk L, Tocci G, Fogacci F, Cicero AFG. An overview of rosuvastatin/ezetimibe association for the treatment of hypercholesterolemia and mixed dyslipidemia. Expert Opin Pharmacother. abril de 2020;21(5):531-9.

239. Lamb YN. Rosuvastatin/Ezetimibe: A Review in Hypercholesterolemia. Am J Cardiovasc Drugs. agosto de 2020;20(4):381-92.

240. Phan BAP, Dayspring TD, Toth PP. Ezetimibe therapy: mechanism of action and clinical update. Vasc Health Risk Manag. 2012;8:415-27.

241. Out C, Groen AK, Brufau G. Bile acid sequestrants: more than simple resins. Curr Opin Lipidol. febrero de 2012;23(1):43-55.

242. Hou R, Goldberg AC. Lowering low-density lipoprotein cholesterol: statins, ezetimibe, bile acid sequestrants, and combinations: comparative efficacy and safety. Endocrinol Metab Clin North Am. marzo de 2009;38(1):79-97.

243. Stoekenbroek RM, Kastelein JJP, Huijgen R. PCSK9 inhibition: the way forward in the treatment of dyslipidemia. BMC Med. 12 de octubre de 2015;13:258.

244. Handelsman Y, Lepor NE. PCSK9 Inhibitors in Lipid Management of Patients With Diabetes Mellitus and High Cardiovascular Risk: A Review. J Am Heart Assoc. 22 de junio de 2018;7(13):e008953.

245. Giugliano RP, Keech A, Murphy SA, Huber K, Tokgozoglu SL, Lewis BS, et al. Clinical Efficacy and Safety of Evolocumab in High-Risk Patients Receiving a Statin: Secondary Analysis of Patients With Low LDL Cholesterol Levels and in Those Already Receiving a Maximal-Potency Statin in a Randomized Clinical Trial. JAMA Cardiol. 1 de diciembre de 2017;2(12):1385-91.

246. Ascaso JF, Civeira F, Guijarro C, López Miranda J, Masana L, Mostaza JM, et al. Indications of PCSK9 inhibitors in clinical practice. Recommendations of the Spanish Sociey of Arteriosclerosis (SEA), 2019. Clin Investig Arterioscler. junio de 2019;31(3):128-39.

247. Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Arch Intern Med. 13 de abril de 2009;169(7):659-69.

248. Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. Ann Intern Med. 18 de marzo de 2014;160(6):398-406.

249. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. PLoS Med. 23 de marzo de 2010;7(3):e1000252.

250. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Retraction and Republication: Primary Prevention of Cardiovascular Disease with a Mediterranean Diet. N Engl J Med 2013;368:1279-90. N Engl J Med. 21 de junio de 2018;378(25):2441-2.

251. Musa-Veloso K, Poon TH, Elliot JA, Chung C. A comparison of the LDL-cholesterol lowering efficacy of plant stanols and plant sterols over a continuous dose range: results of a meta-analysis of randomized, placebo-controlled trials. Prostaglandins Leukot Essent Fatty Acids. julio de 2011;85(1):9-28.

252. Li Y, Jiang L, Jia Z, Xin W, Yang S, Yang Q, et al. A meta-analysis of red yeast rice: an effective and relatively safe alternative approach for dyslipidemia. PLoS One. 2014;9(6):e98611.

253. Scriver CR, editor. The metabolic & molecular bases of inherited disease. 8th ed. New York: McGraw-Hill; 2001. 4 p.

254. Akioyamen LE, Genest J, Shan SD, Reel RL, Albaum JM, Chu A, et al. Estimating the prevalence of heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. BMJ Open. 1 de septiembre de 2017;7(9):e016461.

255. Gaudet D, Gipe DA, Pordy R, Ahmad Z, Cuchel M, Shah PK, et al. ANGPTL3 Inhibition in Homozygous Familial Hypercholesterolemia. N Engl J Med. 20 de 2017;377(3):296-7.

256. Bea AM, Perez-Calahorra S, Marco-Benedi V, Lamiquiz-Moneo I, Jarauta E, Mateo-Gallego R, et al. Effect of intensive LDL cholesterol lowering with PCSK9 monoclonal antibodies on tendon xanthoma regression in familial hypercholesterolemia. Atherosclerosis. agosto de 2017;263:92-6.

257. Palcoux J-B, Atassi-Dumont M, Lefevre P, Hequet O, Schlienger J-L, Brignon P, et al. Low-density lipoprotein apheresis in children with familial hypercholesterolemia: follow-up to 21 years. Ther Apher Dial. junio de 2008;12(3):195-201.

258. Perak AM, Ning H, de Ferranti SD, Gooding HC, Wilkins JT, Lloyd-Jones DM. Long-Term Risk of Atherosclerotic Cardiovascular Disease in US Adults With the Familial Hypercholesterolemia Phenotype. Circulation. 5 de julio de 2016;134(1):9-19.

259. WHO Human Genetics Programme. Familial hypercholesterolaemia (FH): report of a WHO consultation, Paris, 3 October 1997 [Internet]. World Health Organization; 1998 [citado 23 de noviembre de 2021]. Report No.: WHO/HGN/CONS/FH/98.7. Disponible en: https://apps.who.int/iris/handle/10665/64162

260. Lindroos M, Kupari M, Heikkilä J, Tilvis R. Prevalence of aortic valve abnormalities in the elderly: an echocardiographic study of a random population sample. J Am Coll Cardiol. abril de 1993;21(5):1220-5.

261. Rajamannan NM. Calcific Aortic Stenosis: Lessons Learned from Experimental and Clinical Studies. Arterioscler Thromb Vasc Biol. febrero de 2009;29(2):162-8.

262. Khera AV, Won H-H, Peloso GM, Lawson KS, Bartz TM, Deng X, et al. Diagnostic Yield and Clinical Utility of Sequencing Familial Hypercholesterolemia Genes in Patients With Severe Hypercholesterolemia. J Am Coll Cardiol. 07 de 2016;67(22):2578-89.

263. Reik W. The Wellcome Prize Lecture. Genetic imprinting: the battle of the sexes rages on. Exp Physiol. marzo de 1996;81(2):161-72.

264. Palinski W, Nicolaides E, Liguori A, Napoli C. Influence of maternal dysmetabolic conditions during pregnancy on cardiovascular disease. J Cardiovasc Transl Res. septiembre de 2009;2(3):277-85.

265. Napoli C, Glass CK, Witztum JL, Deutsch R, D'Armiento FP, Palinski W. Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) study. Lancet. 9 de octubre de 1999;354(9186):1234-41.

266. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, et al. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. J Clin Invest. 1 de diciembre de 1997;100(11):2680-90.

267. Brizzi P, Tonolo G, Esposito F, Puddu L, Dessole S, Maioli M, et al. Lipoprotein metabolism during normal pregnancy. Am J Obstet Gynecol. agosto de 1999;181(2):430-4.

268. Kraft HG, Lingenhel A, Raal FJ, Hohenegger M, Utermann G. Lipoprotein(a) in homozygous familial hypercholesterolemia. Arterioscler Thromb Vasc Biol. febrero de 2000;20(2):522-8.

269. McCormick SPA, Schneider WJ. Lipoprotein(a) catabolism: a case of multiple receptors. Pathology. febrero de 2019;51(2):155-64.

270. Olson RJ, Braga-Mele R, Chen SH, Miller KM, Pineda R, Tweeten JP, et al. Cataract in the Adult Eye Preferred Practice Pattern®. Ophthalmology. 1 de febrero de 2017;124(2):P1-119.

271. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J. julio de 2012;33(13):1635-701.

*Las referencias propias de cada trabajo aparecen en sus correspondientes artículos publicados adjuntos en los anexos.

APÉNDICE

Área temática, factor de impacto y aportación del doctorando en el compendio de publicaciones

ESTUDIO 1. Victoria Marco-Benedí, Martín Laclaustra, Juan M. Casado-Dominguez, Rosa Villa-Pobo, Rocío Mateo-Gallego, Rosa M. Sánchez-Hernández, Marta Blanco Nuez, Emilio Ortega-Martínez de Victoria, Marta Sitges, Juan Pedro-Botet, Jose Puzo, Teresa Villarroel, Fernando Civeira. **Aortic Valvular Disease in Elderly Subjects with Heterozygous Familial Hypercholesterolemia: Impact of Lipid-Lowering Therapy.** *J Clin Med. 2019 Dec 14;8(12):2209.*

- *ÁREA TEMÁTICA*: Medicine General and Internal
- *FACTOR DE IMPACTO:*3,3
- *APORTACIÓN DEL DOCTORANDO:* Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito

ESTUDIO 2: Victoria Marco-Benedí, Martín Laclaustra, Ana M. Bea, Manuel Suarez-Tembra, Núria Plana, Xavier Pinto, Ángel Brea, Rosa M. Sánchez-Hernández, Fernando Civeira. **Maternally inherited hypercholesterolemia does not modify the cardiovascular phenotype in familial hypercholesterolemia.** *Atherosclerosis. 2021 Mar;320:47-52.*

- *ÁREA TEMÁTICA*: Peripheral vascular disease
- *FACTOR DE IMPACTO:* 5,16
- *APORTACIÓN DEL DOCTORANDO:* Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito

ESTUDIO 3: Victoria Marco-Benedí, Ana Cenarro, Martín Laclaustra, Asier Larrea-Sebal, Estíbaliz Jarauta, Itziar Lamiquiz-Moneo, Pilar Calmarza, Ana M. Bea, Núria Plana, Xavier Pintó, César Martín, Fernando Civeira. **Lipoprotein(a) in hereditary hypercholesterolemia. Influence of the genetic cause, defective gene and type of mutation.** *Atherosclerosis. 2021 Aug 23:S0021-9150(21)01270-3*

- *ÁREA TEMÁTICA*: Peripheral vascular disease

- *FACTOR DE IMPACTO*: 5,16

- *APORTACIÓN DEL DOCTORANDO:* Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito

ESTUDIO 4: Victoria Marco-Benedí, Martín Laclaustra, Rosa M. Sánchez-Hernández, Emilio Ortega-Martínez de Victoria, Juan Pedro-Botet, Jose Puzo. **Cataract surgery in elderly subjects with heterozygous familial hypercholesterolemia in prolonged treatment with statins.** *J Clin Med. 2021 Aug 8;10(16):3494*

- *ÁREA TEMÁTICA*: Medicine General and Internal

- *FACTOR DE IMPACTO*: 4,2

- *APORTACIÓN DEL DOCTORANDO:* Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito

ESTUDIO 5: Victoria Marco-Benedí, Ana M Bea, Ana Cenarro, Estíbaliz Jarauta, Martín Laclaustra*, Fernando Civeira*. **Causes of death in familial hypercholesterolemia**. Remitido para publicación

- *ÁREA TEMÁTICA*: Biochemistry and Molecular biology

- *FACTOR DE IMPACTO*: 3,56

- *APORTACIÓN DEL DOCTORANDO:* Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito

	Publicación	Área temática	Factor de impacto	Aportación del doctorando
Estudio 1	Victoria Marco-Benedí, Martín Laclaustra, Juan M. Casado-Dominguez, Rosa Villa-Pobo, Rocío Mateo-Gallego, Rosa M. Sánchez-Hernández, Marta Blanco Nuez, Emilio Ortega-Martínez de Victoria, Marta Sitges, Juan Pedro-Botet, Jose Puzo, Teresa Villarroel, Fernando Civeira. Aortic Valvular Disease in Elderly Subjects with Heterozygous Familial Hypercholesterolemia: Impact of Lipid-Lowering Therapy. J Clin Med. 2019 Dec 14;8(12):2209	Medicine General and Internal	3,3	Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito
Estudio 2	Victoria Marco-Benedí, Martín Laclaustra, Ana M. Bea, Manuel Suarez-Tembra, Núria Plana, Xavier Pinto, Ángel Brea, Rosa M. Sanchez-Hernandez, Fernando Civeira. Maternally inherited hypercholesterolemia does not modify the cardiovascular phenotype in familial hypercholesterolemia. Atherosclerosis. 2021 Mar;320:47-52	Peripheral vascular disease	5,16	Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito
Estudio 3	Victoria Marco-Benedí, Ana Cenarro, Martín Laclaustra, Asier Larrea-Sebal, Estíbaliz Jarauta, Itziar Lamiquiz-Moneo, Pilar Calmarza, Ana M. Bea, Núria Plana, Xavier Pintó, César Martín, Fernando Civeira. Lipoprotein(a) in hereditary hypercholesterolemia. Influence of the genetic cause, defective gene and type of mutation. Atherosclerosis. 2021 Aug 23:S0021-9150(21)01270-3	Peripheral vascular disease	5,16	Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito
Estudio 4	Victoria Marco-Benedí, Martín Laclaustra, Rosa M. Sánchez-Hernández, Emilio Ortega-Martínez de Victoria, Juan Pedro-Botet, Jose Puzo. Cataract surgery in elderly subjects with heterozygous familial hypercholesterolemia in prolonged treatment with statins. J Clin Med. 2021 Aug 8;10(16):3494	Medicine General and Internal	4,2	Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito
Estudio 5	Victoria Marco-Benedí, Ana M Bea, Ana Cenarro, Estíbaliz Jarauta, Martín Laclaustra*, Fernando Civeira*. Causes of death in familial hypercholesterolemia. Remitido para publicación en Lipids in Heath and Diseases	Biochemistry and Molecular biology	3,56	Elaboración del diseño del estudio, recogida de datos, inclusión de los sujetos, análisis de resultados y elaboración del manuscrito

COMPENDIO DE PUBLICACIONES:

ESTUDIOS COMPLEMENTARIOS

	Publicación	Área temática	Factor de impacto	Aportación del doctorando
Estudio 6	Victoria Marco-Benedí, Estíbaliz Jarauta, Martín Laclaustra, Fernando Civeira. Nursing workload. Calculation of cardiovascular risk and therapeutic objectives. Clin Investig Arterioscler. 2021 May;33 Suppl 1:10-17. doi: 10.1016/j.arteri.2020.12.006	Peripheral vascular disease	1,3	Recogida de datos, y colaboración en la elaboración del manuscrito
Estudio 7	Elisenda Climent, Victoria Marco-Benedí, David Benaiges, Xavier Pintó, Manuel Suárez-Tembra, Núria Plana, Hannia Lafuente, Emilio Ortega-Martínez de Victoria, Ángel Brea-Hernando, Àlex Vila, Fernando Civeira, Juan Pedro-Botet. Impact of statin therapy on LDL and non-HDL cholesterol levels in subjects with heterozygous familial hypercholesterolaemia. Nutr Metab Cardiovasc Dis. 2021 May 6;31(5):1594-1603.	Nutrition and dietetics	4,2	Recogida de datos, inclusión de los sujetos
Estudio 8	Ana M Bea, Esther Franco-Marín, Victoria Marco-Benedí, Estíbaliz Jarauta, Irene Gracia-Rubio, Ana Cenarro, Fernando Civeira, Itziar Lamiquiz-Moneo. ANGPTL3 gene variants in subjects with familial combined hyperlipidemia. Sci Rep. 2021 Mar 26;11(1):7002.	Genética	4,3	Recogida y extracción de muestras, recogida de datos.
Estudio 9	Victoria Marco-Benedí, Sofía Pérez-Calahorra, Ana M Bea, Itziar Lamiquiz-Moneo, Lucía Baila-Rueda, Ana Cenarro, Fernando Civeira, Rocío Mateo-Gallego. High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early onset diabetic adults with overweight or obesity. Clin Nutr. 2020 May;39(5):1354-1363.	Nutrition	7,3	Colaboración en la elaboración del diseño del estudio, Recogida y extracción de muestras, recogida de datos, inclusión de los sujetos, análisis de resultados y colaboración en la elaboración del manuscrito
Estudio 10	Sofía Perez-Calahorra, Martin Laclaustra, Victoria Marco-Benedí, Iztiar Lamiquiz-Moneo, Juan Pedro-Botet, Núria Plana, Rosa M Sanchez-Hernandez, Antonio J Amor, Fátima Almagro, Francisco Fuentes, Manuel Suarez-Tembra, Fernando Civeira. Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia. Dyslipemia Registry of Spanish Arteriosclerosis Society. Atherosclerosis. 2019 May;284:245-252.	Peripheral vascular disease	3,9	Recogida de datos, inclusión de los sujetos.

	Publicación	Área temática	Factor de impacto	Aportación del doctorando
Estudio 11	Sofía Pérez-Calahorra, Martin Laclaustra, Victoria Marco-Benedí, Xavier Pinto, Rosa M Sanchez-Hernandez, Núria Plana, Emilio Ortega, Francisco Fuentes, Fernando Civeira. Comparative efficacy between atorvastatin and rosuvastatin in the prevention of cardiovascular disease recurrence. Lipids Health Dis. 2019 Dec 11;18(1):216	Biochemistry and Molecular biology	2,97	Recogida de datos, inclusión de los sujetos.
Estudio 12	Victoria Marco-Benedí, Estíbaliz Jarauta, Sofía Pérez-Calahorra, Ana M Bea, Fernando Civeira. Treatment of a high cardiovascular risk patient with McArdle's disease with PCSK9 inhibitors. Clin Investig Arterioscler. 2019 Mar Apr;31(2):89-92.	Peripheral vascular disease	3,0	Colaboración en la elaboración del diseño del estudio, recogida de datos, análisis de resultados y elaboración del manuscrito
Estudio 13	Victoria Marco-Benedí, Itziar Lamiquiz-Moneo, Luis A Álvarez-Sala, Fernando Civeira. Disappearance of recurrent pancreatitis after splenectomy in familial chylomicronemia syndrome. Atherosclerosis 2018 Aug;275:342-345.	Peripheral vascular disease	4,25	Colaboración en la elaboración del diseño del estudio, recogida de datos, análisis de resultados y colaboración en la elaboración del manuscrito

Journal of
Clinical Medicine

MDPI

Article

Aortic Valvular Disease in Elderly Subjects with Heterozygous Familial Hypercholesterolemia: Impact of Lipid-Lowering Therapy

Victoria Marco-Benedí [1], Martin Laclaustra [1,*], Juan M. Casado-Dominguez [2], Rosa Villa-Pobo [1], Rocío Mateo-Gallego [1,3], Rosa M. Sánchez-Hernández [4], Marta Blanco Nuez [5], Emilio Ortega-Martínez de Victoria [6], Marta Sitges [6], Juan Pedro-Botet [7], Jose Puzo [3,8], Teresa Villarroel [8,9] and Fernando Civeira [1,3,*]

1 Lipid Unit, Hospital Universitario Miguel Servet, IIS Aragón, CIBERCV, 50009 Zaragoza, Spain; vmarcob@iisaragon.es (V.M.-B.); rosavillapobo@hotmail.com (R.V.-P.); rmateo@unizar.es (R.M.-G.)
2 Cardiology Department, Hospital Universitario Miguel Servet, 50009 Zaragoza, Spain; jmcasadod@salud.aragon.es
3 Universidad de Zaragoza, 50009 Zaragoza, Spain; jpuzo@unizar.es
4 Endocrinology Department, Hospital Insular de Gran Canaria, 35016 Las Palmas de Gran Canaria, Spain; rosamariasanher@gmail.com
5 Cardiology Department, Hospital Universitario Dr. Negrín, 35012 Las Palmas de Gran Canaria, Spain; martablanconuez@hotmail.com
6 Lipid Clinic, Hospital Clinic, CIBEROBN, 08036 Barcelona, Spain; EORTEGA1@clinic.cat (E.O.-M.d.V.); MSITGES@clinic.cat (M.S.)
7 Lipid Unit, Hospital del Mar, 08003 Barcelona, Spain; JPedrobotet@parcdesalutmar.cat
8 Lipid Unit, Hospital San Jorge, 22004 Huesca, Spain; teresabv11@hotmail.com
9 Cardiology Department, Hospital San Jorge, 22004 Huesca, Spain
* Correspondence: martin.laclaustra@unizar.es (M.L.); civeira@unizar.es (F.C.)

Received: 14 November 2019; Accepted: 12 December 2019; Published: 14 December 2019

Abstract: Hypercholesterolemia and statins are risk factors for aortic stenosis (AS) and vascular calcification, respectively. Whether heterozygous subjects with familial hypercholesterolemia (HeFH) treated with statins are at risk of AS is unknown. We study the prevalence of AS, aortic valve calcification (AoVC), and aortic sclerosis (ASc) in elderly subjects with HeFH in a prolonged statin treatment. Case-control study, cases were adults ≥65 years of age with a genetic diagnosis of HeFH, LDLc >220 mg/dl, and statin treatment ≥5 years. Controls were relatives of HeFH patients, with LDLc <190 mg/dl. Participants underwent a cardiac ultrasound for aortic valve analysis. We studied 205 subjects, 112 HeFH and 93 controls, with mean age 71.8(6.5) years and 70.0(7.3) years, respectively. HeHF, with respect to controls, presented greater gradients of aortic transvalvular pressure, 7.4(7.3) mmHg versus 5.0(2.8) mmHg, and maximum aortic velocity, 1.7(0.7) m/s versus 1.5(0.4) m/s, and lower aortic valve opening area, 2.0(0.7) cm^2 versus 2.4(0.6) cm^2 (all $p < 0.05$). AoVC and ASc were also more prevalent in HeFH ($p < 0.05$ between groups). Moderate/severe AS prevalence was higher among HeFH: 7.1% versus 1.1% (age- and sex-adjusted odds ratio (OR) 8.33, $p = 0.03$). Independent risk factors for aortic valve disease in HeFH were age and LDLc before treatment. The number of years under statin treatment was not associated with any aortic valve measurement. Subjects ≥65 years with HeFH in prolonged statin treatment show more aortic valvular disease and higher frequency of AS than controls. Life-long elevated LDLc exposure, rather than time of exposure to statins, explains this higher risk.

Keywords: aortic stenosis; aortic sclerosis; heterozygous familial hypercholesterolemia; statins; aortic valve calcification; LDL cholesterol

1. Introduction

Familial hypercholesterolemia is a common autosomal codominant disease mostly caused by mutations in the *LDLR* gene. The prevalence of heterozygous familial hypercholesterolemia (HeFH) is approximately of 1/200–500 in most countries [1]. Patients with HeFH show very high plasma concentration of low-density lipoprotein cholesterol (LDLc) [1–3] and, without lipid-lowering treatment, approximately 50% of HeFH men and 30% of HeFH women will develop coronary heart disease by the age of 50 years [1,4,5]. Fortunately, lipid-lowering treatment has decreased coronary heart disease in HeFH, and many patients now have almost a normal life expectancy [5]. Other diseases related to hypercholesterolemia, or to defects in the LDL receptor pathway, that are not reversed by statins or that may go unnoticed with shorter life-spans may appear nowadays thanks to the greater survival rate. This surge of new phenotypes has already been described in homozygous familial hypercholesterolemia. In the latter condition, these pediatric patients used to die from extremely premature coronary atherosclerosis. Given that the risk of coronary heart disease has been substantially reduced thanks to starting LDL apheresis from childhood [6–8], when homozygous familial hypercholesterolemia patients get older, they often show calcification of the aortic annulus and ascending aorta and an increased risk of severe aortic stenosis (AS) [7]. AS is an inflammatory and degenerative process caused by endothelial damage. The disease involves lipid infiltration, progressive fibrosis, and calcification, and ends up narrowing the aortic valve area [9]. Interestingly, AS prevalence has increased steadily in recent years in most countries, including Spain [10]. Risk factors for AS include age, hypercholesterolemia, diabetes mellitus, and hypertension, which are also traditional risk factors for arteriosclerosis [11]. Unfortunately, treatment for hypercholesterolemia with statins and ezetimibe has not proven to reduce AS progression in the long-term, although some benefit was observed only in a subset of patients with mild AS and high pretreatment LDLc levels [12,13]. Several factors may predispose HeFH patients to AS. They usually have very high LDLc concentration from childhood, a known risk factor for AS. In addition, chronic treatment with statins favors vascular calcification, which occurs when the lipidic and inflammatory components of the atheroma plaques are reduced by these drugs [14]. Besides, many HeFH patients have elevated lipoprotein(a) (Lp(a)) concentration, another well-known independent risk factor for vascular and aortic valve calcification (AoVC) [15,16]. Success of current treatment for HeFH may have allowed that these factors combine with the effects of aging. Nearly half of the elderly general population (>75 years old) have AoVC to some extent, and a fraction of them suffer AS [15]. Consequently, previous high early coronary heart disease (CHD) mortality in HeFH [17] could have hidden AoVC and AS that tend to appear at older ages. *LDLR* mutations strongly predict AoVC [18], but whether elderly HeFH patients are at higher risk of AS is still unknown. We hypothesized that many subjects with HeFH under chronic treatment with statins have not only AoVC, but also impaired hemodynamic parameters of the aortic valve function, even reaching AS diagnostic criteria. We aimed to study these functional differences by comparing HeFH patients with controls, to assess, in addition, the current prevalence of AS in HeFH subjects ≥65 years in chronic treatment with statins, and to explore potential risk factors for the development of AS in HeFH.

2. Methods

2.1. Study Characteristics

This is an observational, multicenter, case-control study. Five lipid clinics throughout Spain took part in the study. Consecutive HeFH cases were recruited with the following criteria: Age ≥65 years; a pathogenic mutation in a candidate gene for familial hypercholesterolemia (*LDLR*, *APOB,* or *PCSK9*) in the subject or in a first-degree relative; historic LDLc levels ≥220 mg/dl without lipid-lowering therapy; and statin treatment ≥5 years in all cases. Controls were selected from relatives of HeFH patients from the lipid clinics, requiring: Absence of hypercholesterolemia (LDLc <190 mg/dl without lipid-lowering treatment); age ≥55 years; and being either HeFH partners who cohabited >25 years or HeFH siblings.

Additional HeFH cases were recruited from relatives of cases when they fulfilled the inclusion criteria. Participants were excluded if they had a personal history of rheumatic heart disease.

The key data collection component of the study was a transthoracic echocardiogram. Laboratory data were obtained from records in the lipid clinics from dates as close as possible (<1 year) to the cardiac ultrasound, if they were on stable lipid-lowering treatment. When these were not available, a blood sample was drawn during the visit. All procedures were carried out locally, at each lipid clinic.

All subjects gave a written informed consent to the protocol, which was approved by a central ethical committee (Comité Ético de Investigación Clínica de Aragón, CEICA).

2.2. Transthoracic Echocardiography

Transthoracic conventional echocardiograms were performed by cardiologists who were nationally certified for echocardiography. Echocardiogram studies were focused on the aortic valve at the same position for all patients. The following variables were measured: Mean aortic valve pressure gradient; maximum aortic velocity (Vmax); aortic valve area; aortic valve area indexed to body surface area; bicuspid or tricuspid aorta valve; valvular thickening >3 mm; and calcification of the aortic valve leaflets. The cardiologists performing echocardiograms were blinded to the diagnosis of HeFH. Degree of calcification of the aortic valve was scored as follows: 1, no calcification; 2, mildly calcified (small isolated spots); 3, moderately calcified (multiple larger spots); and 4, heavily calcified (extensive thickening and calcification of all cusps) [19]. AS was diagnosed as defined by the American College of Cardiology/American Heart Association Task Force on Practice Guidelines [11]. AS is considered present with any of the following findings: Mean aortic valve pressure gradient ≥20 mm; Vmax ≥2 m/s; and aortic valve area ≤1 cm^2. Identified AS stages were mild (Vmax 2–2.9 m/s, valve pressure gradient <20 mm, and aortic valve area >1cm^2); moderate (Vmax 3-3.9 m/s or valve pressure gradient 20–39 mm and aortic valve area >1 cm^2), and severe (Vmax ≥4 m/s, valve pressure gradient ≥40 mm, or aortic valve area ≤1 cm^2) [11]. Aortic valve sclerosis (ASc), a milder aortic condition, was defined in the presence of thickening (>3 mm) and/or calcification of the aortic valve without significant obstruction of flow (Vmax <2 m/s) or AS criteria [20].

2.3. Clinical Interview

Within clinical data information, we collected age, gender, level of education, history of smoking, hypertension, diabetes, personal history of cardiovascular disease and familial history of cardiovascular disease in first-degree relatives, age at which cardiovascular events occurred, lipid values without treatment, and history of lipid-lowering treatment. Level of education was classified as primary school, secondary school, and higher education. Current smoking was defined by smoking in the present or having smoked in the last year. Former smokers were defined as subjects having smoked at least 50 cigarettes in their lifetime, but not having smoked in the last year. Smoking burden was recorded as the number of daily packets smoked multiplied by the number of years smoked.

About the lipid-lowering treatment, we recorded age at which statin treatment began, statin most commonly prescribed, statin dose, ezetimibe use, age at which ezetimibe treatment began, and which statin and dose are prescribed as current treatment.

In participants with previous cardiovascular disease, age of first event and kind of event was recorded: Myocardial infarction; acute coronary syndrome requiring hospitalization; ischemic stroke; coronary, carotid, or peripheral revascularization; recovered sudden death; or aortic aneurysm.

2.4. Physical Exam

In the physical exam, we recorded height, weight, systolic and diastolic blood pressure, and presence of tendon xanthomas. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

2.5. Laboratory Tests

When current lipid values (<1 year) were not available, then a blood sample was obtained to determine cholesterol, triglycerides, HDLc, apolipoprotein B (apo B), Lp(a), glucose, and HbA1c. Laboratory measurements and sample preservation were performed at each center.

2.6. Definitions

Arterial hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or current use of antihypertensive medication. Diabetes was defined as fasting plasma glucose ≥126 mg/dl, HbA1c ≥6.5%, or current use of antidiabetic medication.

2.7. Statistical Analysis

Summary data are expressed as mean (standard deviation) or percentage. For comparisons between cases and controls, aortic valve ultrasound variables and presence of aortic valve affectation levels are modeled in linear and logistic regressions based on generalized estimating equations (GEE) with exchangeable variance structure (to account for family links) with different levels of adjustment: Unadjusted, adjusted for sex and age, and additionally adjusted for untreated LDLc concentration. Analyses stratified by cases and controls and those restricted to cases were based on generalized linear models, and they included, in order to estimate their influence, the variable untreated LDLc concentration or years of life treated with lipid-lowering drugs. Influence of LDLc and lipid-lowering treatment were studied separately in cases and controls strata. All of the analyses were performed with the statistical software R version 3.4.4. and the package "gee" version 4.13.19.

3. Results

The research team recruited 205 subjects, 112 cases and 93 controls. Mean age was 71.8 years and 70.0 years in the case and control groups, respectively. Besides untreated total and LDLc, age, previous cardiovascular disease prevalence, and family history of premature cardiovascular disease prevalence were also higher in the HeFH group than in the control group. BMI was similar in both groups. There were no differences in smoking, hypertension, or type 2 diabetes mellitus (Table 1). All cases were on lipid-lowering treatment with a mean treatment time of 22.5 (8.7) years. No case or control had a history of aortic valve replacement.

3.1. Aortic Valve Characterization

Mean aortic valve pressure gradient was higher in cases (7.4 mmHg) than in controls (5.0 mmHg), after adjusting for age and sex (p = 0.002). HeFH patients, compared with controls, had greater maximum aortic velocity (Vmax) (1.7 m/s and 1.5 m/s, respectively, p = 0.011), and lower aortic valve area (2.0 cm^2 and 2.4 cm^2, respectively, $p < 0.001$). Among HeFH patients, average valvular calcification score of the aortic valve leaflets was higher and valvular thickening was more prevalent (p = 0.004). Left ventricular ejection fraction tended to be lower in cases (65.7% vs. 67.2%, p = 0.056). All studied valves were tricuspid. AS with moderate or severe criteria and ASc were more prevalent among HeFH (7% vs. 1%, age- and sex-adjusted OR 8.33, 95% confidence interval (CI) 1.22, 57.10, p = 0.031; and 55% vs. 32%, age- and sex-adjusted OR 1.90, 95% CI 1.04, 3.47, p = 0.061, respectively) (Table 2) and increased with age (Figure 1). Additionally, adjusting these comparisons of aortic measurements and stenosis prevalence for untreated LDLc concentrations rendered all differences non-significant. Thus, LDLc could justify a considerable amount of the valve differences between HeFH and controls, but LDLc is part of the definition of case and control, and stratified regressions were performed to clarify the issue. They showed that age, but not LDLc, was significantly associated with all aortic valve variables among controls, but Vmax and aortic valve calcification scores were also associated with untreated LDLc concentration among HeFH cases (Supplementary Table S1). Mean aortic valve gradient increased 4.1 (2.1, 6.1) mmHg/10 year among cases, while it only increased 0.8 (0.0, 1.6) mmHg/10 year among

controls across different age groups (Supplementary Table S1 and Figure 2). All clinical and laboratory data were similar in all aortic valve stages except for the presence of tendon xanthomas (Supplementary Table S2).

3.2. Risk Factors for Valvular Disease

In order to evaluate how statin treatment could modify aortic valve parameters in HeFH patients, we used models including sex, age, and length of statin treatment among HeFH patients. Aortic valve area decreased, and aortic valve calcification score increased significantly with age ($p < 0.001$), independently of statins (Table 3). Ejection fraction was independent of age but decreased with length of statin treatment ($p = 0.005$). Mean aortic valve gradient increased with age in cases and controls, but with higher incremental rate in HeFH (Figure 2).

J. Clin. Med. 2019, 8, x FOR PEER REVIEW 5 of 15

3.2. Risk Factors for Valvular Disease

In order to evaluate how statin treatment could modify aortic valve parameters in HeFH patients, we used models including sex, age, and length of statin treatment among HeFH patients. Aortic valve area decreased, and aortic valve calcification score increased significantly with age ($p < 0.001$), independently of statins (Table 3). Ejection fraction was independent of age but decreased with length of statin treatment ($p = 0.005$). Mean aortic valve gradient increased with age in cases and controls, but with higher incremental rate in HeFH (Figure 2).

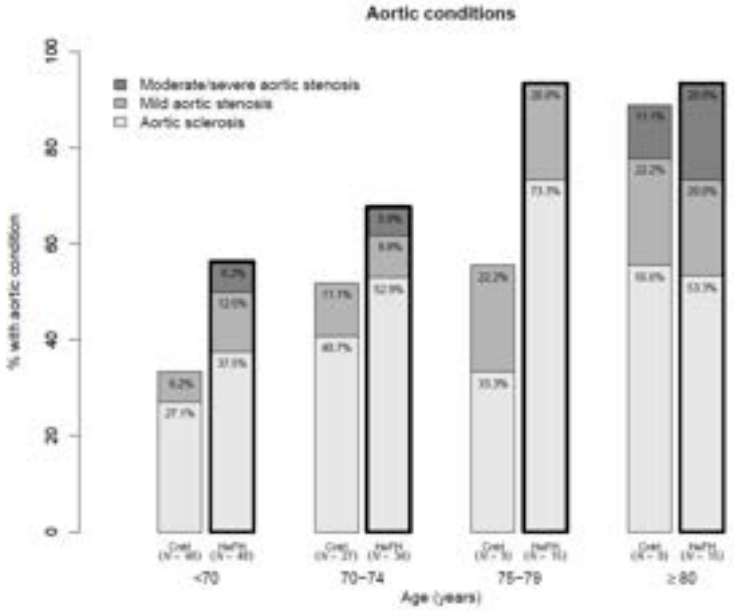


Figure 1. Prevalence of aortic stenosis and aortic sclerosis in heterozygous familial hypercholesterolemia (HeFH) and controls by age groups.

Figure 1. Prevalence of aortic stenosis and aortic sclerosis in heterozygous familial hypercholesterolemia (HeFH) and controls by age groups.

J. Clin. Med. 2019, 8, x FOR PEER REVIEW 6 of 15

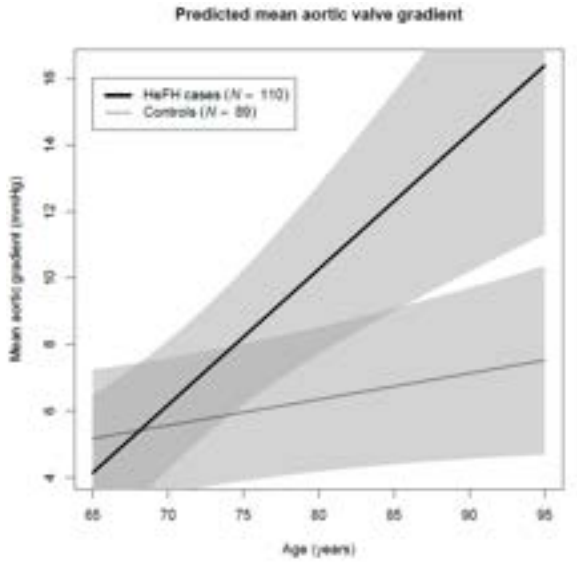


Figure 2. Predicted aortic valve gradient in HeFH and controls as a function of age. Models adjusted for sex and low-density lipoprotein cholesterol (LDLc) and stratified for the case and control groups.

Figure 2. Predicted aortic valve gradient in HeFH and controls as a function of age. Models adjusted for sex and low-density lipoprotein cholesterol (LDLc) and stratified for the case and control groups.

Table 1. Baseline clinical and laboratory characteristics of cases and controls.

Mean (SD) / proportion (*n* = count)	Controls		Cases		
	n	**Mean/%**	***n***	**Mean/%**	***p*-value**
Age (years)	93	70.0 (7.3)	112	71.8 (6.5)	0.038
Sex, women % (*n* = count)	93	51.6 (*n* = 48)	112	66.1 (*n* = 74)	0.050
Weight (Kg)	92	75.9 (14.3)	110	72.1 (13.5)	0.054
Height (cm)	92	161.7 (8.5)	110	159.4 (9.9)	0.075
Systolic blood pressure (mmHg)	92	136.7 (14.9)	110	134.1 (16.9)	0.199
Diastolic blood pressure (mmHg)	92	79.5 (9.7)	110	77.1 (9.7)	0.089
Body mass index (Kg/m^2)	92	29.0 (4.7)	110	28.3 (4.0)	0.265
Tendon xanthomas, % (*n* = count)	93	0.0 (*n* = 0)	102	39.2 (*n* = 40)	<0.001
Hypertension, % (*n* = count)	93	55.9 (*n* = 52)	112	54.5 (*n* = 61)	0.769
Type 2 diabetes, % (*n* = count)	93	14.0 (*n* = 13)	112	21.4 (*n* = 24)	0.139
Previous cardiovascular disease, % (*n* = count)	93	16.1 (*n* = 15)	112	27.7 (*n* = 31)	0.036
Family history of premature cardiovascular disease, % (*n* = count)	89	25.8 (*n* = 23)	100	45.0 (*n* = 45)	<0.001
Packages/day × number of years smoking	90	15.2 (25.3)	108	9.8 (21.6)	0.120
Lipid-lowering treatment, % (*n* = count)	93	51.6 (*n* = 48)	112	100.0 (*n* = 112)	<0.001
Statin treatment (years)	48	8.3 (7.2)	110	22.5 (8.7)	<0.001
Ezetimibe treatment, % (*n* = count)	68	8.8 (*n* = 6)	112	83.0 (*n* = 93)	<0.001
Untreated total cholesterol (mg/dL)	93	223.8 (43.3)	111	395.9 (73.0)	<0.001
Untreated triglycerides (mg/dL)	93	145.7 (119.0)	112	139.7 (76.3)	0.669
Untreated HDLc (mg/dL)	91	56.9 (15.3)	112	55.7 (13.6)	0.621
Untreated LDLc (mg/dL)	90	138.0 (31.7)	111	314.2 (71.4)	<0.001

Continuous data are expressed as mean (standard deviation, SD); categorical data are expressed as percentages (*n* = count). HDLc: High-density lipoprotein cholesterol; LDLc: Low-density lipoprotein cholesterol. *p*-values from linear and logistic regressions based on generalized estimating equations (GEE) with exchangeable variance structure, unadjusted.

Table 2. Morphological and hemodynamic parameters of aortic valve in cases and controls.

Mean (SD)/proportion (*n* = count)	Controls		Cases				
	n	**mean/%**	***n***	**mean/%**	***p*-value ***	***p*-value ****	***p*-value *****
Mean aortic valve pressure gradient (mm)	92	5.0 (2.8)	111	7.4 (7.3)	0.002	0.003	0.626
Maximum aortic velocity (Vmax) (m/s)	92	1.5 (0.4)	111	1.7 (0.7)	0.004	0.011	0.959
Aortic valve area (cm^2)	92	2.4 (0.6)	107	2.0 (0.7)	<0.001	<0.001	0.771
Left ventricular ejection fraction (%)	93	67.2 (7.0)	111	65.7 (9.5)	0.167	0.056	0.285
Aortic valve velocity ratio	61	0.73 (0.11)	61	0.70 (0.14)	0.244	0.138	0.321
Calcification of the aortic valve leaflets (score)	92	0.7 (0.9)	110	1.1 (1.0)	0.002	0.008	0.723
Valvular thickening >3 mm, % (*n* = count)	93	12.9 (*n* = 12)	112	27.7 (*n* = 31)	0.006	0.004	0.600
Aortic stenosis, % (*n* = count)	93	11.8 (*n* = 11)	112	20.5 (*n* = 23)	0.107	0.171	0.842
Aortic stenosis moderate or severe, % (*n* = count)	93	1.1 (*n* = 1)	112	7.1 (*n* = 8)	0.067	0.031	0.187
Aortic sclerosis, % (*n* = count)	92	34.8 (*n* = 32)	111	49.5 (*n* = 55)	0.046	0.061	0.337

Continuous data are expressed as mean (SD); categorical data are expressed as percentages (*n* = count). *p*-values from linear and logistic regressions based on generalized estimating equations (GEE) with exchangeable variance structure. *p*- value *: Unadjusted; *p* -value **: Adjusted for sex and age; *p*-value ***: Adjusted for sex, age, and untreated LDLc concentration.

Table 3. Influence of age and length of lipid-lowering treatment on aortic valve characteristics in HeFH.

Morphological and hemodynamic parameters	Cases			
	Per each 10 year of age Difference/OR (95% CI)	*p*-value	Per each 10 year of lipid-lowering use Difference/OR (95% CI)	*p*-value
Mean aortic valve pressure gradient (mm)	4.623 (2.518, 6.728)	<0.001	0.214 (−1.304, 1.732)	0.783
Maximum aortic velocity (Vmax) (m/s)	0.394 (0.185, 0.603)	<0.001	0.012 (−0.139, 0.162)	0.881
Aortic valve area (cm^2)	−0.373 (−0.562, −0.185)	<0.001	0.001 (−0.133, 0.135)	0.990
Left ventricular ejection fraction (%)	−1.924 (−4.634, 0.787)	0.167	−2.877 (−4.827, −0.927)	0.005
Calcification of the aortic valve leaflets (score)	0.698 (0.418, 0.979)	<0.001	−0.034 (−0.233, 0.165)	0.737
Valvular thickening >3 mm, OR	1.36 (0.69, 2.63)	0.359	0.86 (0.52, 1.41)	0.549
Aortic stenosis, OR	2.11 (1.04, 4.37)	0.038	0.94 (0.52, 1.65)	0.817
Aortic stenosis moderate or severe, OR	2.95 (1.07, 8.26)	0.032	0.82 (0.29, 2.15)	0.687
Aortic sclerosis, OR	1.23 (0.67, 2.32)	0.510	1.03 (0.66, 1.62)	0.883

Linear and logistic regressions based on generalized linear models (GLM). HeFH: heterozygous familial hypercholesterolemia; OR: odds ratio; CI: Confidence Interval. *p*-value from a single model, adjusted for sex.

4. Discussion

In the present study, we described the prevalence of aortic disease in HeFH patients ≥65 years under long-term treatment with lipid-lowering drugs. Aortic valve involvement in HeFH has been previously explored, but, to our knowledge, this is the first work focused on elderly subjects, the most clinically relevant population due to the close relationship of AS with age, and the first to describe the prevalence of AS and to evaluate the potential role of statin treatment in development of aortic disease in HeFH. Our results are in agreement with those recently published from a registry-based prospective cohort study in Norway, in which a marked increased risk of AS in HeFH was found compared with the general Norwegian population [21].

4.1. Prevalence of AS in HeFH

In our study, AS prevalence is more than three times higher than that reported for this group of age in the general population, 1.5–3%, and also higher than in our related control sample [22–25]. In view of this substantially elevated prevalence, a systematic transthoracic echocardiography is probably justified for the screening of AS in elderly HeFH over 65 years.

Several studies have previously analyzed aortic valve thickening or calcification in HeFH. Ten Kake et al. compared a cohort of 59 HeFH subjects from the Netherlands with controls and showed that the patients with HeFH, especially those with *LDLR*-negative mutations, showed higher AoVC prevalence (41% versus 21%, respectively, $p < 0.001$) [18]. This ratio is similar to the one observed in our study. However, the authors did not report data on AS, probably because their sample was smaller than ours and it included younger patients. In the Cardiovascular Health Study, clinically defined familial hypercholesterolemia was associated with AoVC and ASc, but an association with AS could not be demonstrated [26,27]. In our study, in addition to fulfilling clinical lipidic criteria, all cases were defined by having a genetic mutation in a gene that causes familial hypercholesterolemia, ascertained directly in them or in a blood-relative. It is well established that familial hypercholesterolemia definition based exclusively on clinical criteria includes other forms of genetic hypercholesteremia [28,29], and hypercholesterolemic subjects with a genetic mutation have more severe cardiovascular phenotypes than in the absence of mutation, even with a similar LDLc concentration at the moment of diagnosis [30].

4.2. Risk Factors for Aortic Valvular Disease

Our results support that atherosclerosis risks factors, including high LDLc throughout life, are major risk factors for AS [31]. AS is probably produced by a combination of mechanical stress and endothelial damage of the aortic valves, driving to subsequent valve inflammation, fibrosis, calcification, and progressive valve narrowing [9]. Mechanical stress affects the endothelial function and facilitates infiltration of lipids and inflammatory cells (T-cells) into the valve. All of these mechanisms are involved in the inflammatory activity [32,33]. As a result, fibroblasts differentiate into myofibroblasts, which, under the influence of angiotensin, promote thickening of the valve [9]. Hypercholesterolemia treatment does not prevent progression from moderate to severe AS once AS is already established [34], suggesting that hypercholesterolemia plays a role in the startup process but has little effect in the advanced disease [35]. Our study would support current guidelines, which recommend early and intensive treatment of HeFH to prevent not only coronary disease, but also valvular disease later in life [5].

4.3. Statins and Valvular Disease

Lipid-lowering treatment is associated with increased vascular calcification [36] due, at least in part, to statins-enhanced new bone formation in bone cells, via increased expression of gene *BMP*-2 [37]. Furthermore, the reduction in inflammatory markers associated with statins is significantly associated with the percentage of calcium volume within the coronary plaques [38]. Consequently, it has been speculated that lipid-lowering treatment, especially prolonged statin treatment, may favor

development of AoVC and AS [39]. Although, other studies such as Al Kindi's showed that vascular calcification associated to statins is not related to valve calcification [40]. Currently, HeFH are the patients to whom earlier, more intense, and more prolonged lipid-lowering drugs are prescribed. Hence, our elderly HeFH sample, exposed to statins for a mean of 22.5 years, is excellent to study this potential association. We found that the number of years under statin treatment is not a risk factor for AS, indicating that if there is any risk of AS with statin, it is highly compensated by the beneficial effect on LDLc, as it has been previously suggested [22]. These results are in full agreement with those found in the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) study, where, in a non-prespecified post-hoc analysis, lipid-lowering treatment impeded the progression of AS in patients with the highest LDL cholesterol concentration (>160 mg/dL) and mild AS at baseline [41]. Statin therapy was also significantly associated with a lesser change in aortic valve area in SALTIRE and RAAVE trials [42].

4.4. Study Limitations

This was a multicenter project in which echocardiographic studies were performed by different researchers, and thus a certain degree of variability may exist in the measurements. However, all ultrasound scans were performed by cardiologists who were experts in echocardiography, with practice within hospitals of the Spanish National Health System, and all of them certified at the national level with homogeneous and strict criteria. In addition, echocardiographers were blinded to the diagnosis of HeFH to avoid bias.

Measurements of aortic valve functionality may vary in the presence of abnormal systolic function [43]. Although it was not an exclusion criterion, none of our cases and controls had left ventricular ejection fraction (LVEF) <40% or hypertrophic cardiomyopathy, thus, this limitation does not play an important role in our study, and all measurements of the valvular surface could be performed by continuity equation, the best standardized procedure. Finally, some data on the personal history of lipid-lowering drug use and family history of cardiovascular disease were self-reported by the participants, and they may have been affected by some degree of recall inaccuracy. However, the main outcome and the inclusion and exclusion criteria for the cases and controls groups were based on objective data, collected or verified by the research team.

It would had been important to have the lifetime LDLc exposure to study the effect of cumulative LDLc on AS. Considering that we are dealing with elderly patients, this is not feasible. However, we considered LDLc at diagnosis and the number of years under statin treatment, and we believe that both variables are good subrogates of lifetime LDLc exposure.

5. Conclusions.

Subjects ≥65 years with HeFH in prolonged statin treatment for a mean period of over 22 years show more aortic valvular disease and higher frequency of AS than controls. Independent risk factors for aortic valve disease in HeFH were age and LDLc before treatment. Duration of statin treatment was not associated with any aortic valve measurement. Hence, cumulative LDLc exposure, rather than time of exposure to statins, explains this higher risk. These results suggest that elderly HeFH should be monitored for the presence of aortic disease, and emphasize the importance of early lipid-lowering treatment in HeFH population to prevent not only coronary disease, but also aortic valvular disease. Furthermore, our study provides additional support to the already established body of work on the role of hypercholesterolemia on AS.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0383/8/12/2209/s1, Table S1: Influence of age and low-density lipoprotein cholesterol (LDLc) on morphological and hemodynamic parameters of aortic valve in cases and controls, Table S2: Baseline clinical and laboratory characteristics of HeFH with aortic valve conditions.

Author Contributions: All authors listed meet the International Committee of Medical Journal Editors criteria for authorship. Conceptualization, F.C.; Methodology, V-M-B., M.L. and F.C.; Software, V.M-B. and M.L.; Validation, F.C.; Formal Analysis, V.M-B. and M.L.; Investigation, all authors.; Resources, F.C.; Data Curation, V.M-B.; Writing-Original Draft Preparation, V.M-B., M-L. and F.C.; Writing-Review & Editing, all authors.; Visualization,

all authors; Supervision, M-L. and F.C.; Project Administration, V.M-B.; Funding Acquisition, F.C. All authors take responsibility for the integrity of the work as a whole and have given final approval for the version to be published.

Funding: This work was supported by grants from the Spanish Ministry of Economy and Competitiveness PI15/01983, Gobierno de Aragón and CIBERCV. These projects were co-financed by Instituto de Salud Carlos III and the European Regional Development Fund (ERDF) of the European Union "A way to make Europe". Martin. Laclaustra's research activity was funded by Agencia Aragonesa para la Investigación y el Desarrollo (ARAID).

Conflicts of Interest: E.O. received advisory and/or lecture fees from Lilly, Sanofi, Amgen, Novo, MSD; J.P-B. received advisory and/or lecture fees from Amgen, Astra-Zeneca, Esteve, Ferrer, Mylan, MSD, and Sanofi. F.C received grants, consulting fees, and/or honoraria from Amgen, Merck, Pfizer, and Sanofi-Aventis. All identified disclosures are modest. The other researcher did not receive any specific grant from agencies in the public, commercial, or not-for-profit sectors.

Abbreviations

AoVC	Aortic valve calcification
AS	Aortic stenosis
ASc	Aortic sclerosis
BMI	Body mass index
GEE	Generalized estimating equations
HeFH	Heterozygous familial hypercholesterolemia
HDLc	High-density lipoprotein cholesterol
LDLc	Low-density lipoprotein cholesterol
Lp(a)	Lipoprotein(a)
Vmax	Maximum aortic velocity

References

1. Scriver, C.R. *The Metabolic & Molecular Bases of Inherited Disease*, 8th ed.; McGraw-Hill: New York, NY, USA, 2001; ISBN 978-0-07-913035-8.
2. Austin, M.A.; Hutter, C.M.; Zimmern, R.L.; Humphries, S.E. Genetic causes of monogenic heterozygous familial hypercholesterolemia: A HuGE prevalence review. *Am. J. Epidemiol.* **2004**, *160*, 407–420. [CrossRef] [PubMed]
3. de Ferranti, S.D.; Rodday, A.M.; Mendelson, M.M.; Wong, J.B.; Leslie, L.K.; Sheldrick, R.C. Prevalence of Familial Hypercholesterolemia in the 1999 to 2012 United States National Health and Nutrition Examination Surveys (NHANES). *Circulation* **2016**, *133*, 1067–1072. [CrossRef] [PubMed]
4. Civeira, F. International Panel on Management of Familial Hypercholesterolemia Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. *Atherosclerosis* **2004**, *173*, 55–68. [CrossRef]
5. Nordestgaard, B.G.; Chapman, M.J.; Humphries, S.E.; Ginsberg, H.N.; Masana, L.; Descamps, O.S.; Wiklund, O.; Hegele, R.A.; Raal, F.J.; Defesche, J.C.; et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: Guidance for clinicians to prevent coronary heart disease: Consensus statement of the European Atherosclerosis Society. *Eur. Heart J.* **2013**, *34*, 3478–3490. [CrossRef] [PubMed]
6. Cuchel, M.; Bruckert, E.; Ginsberg, H.N.; Raal, F.J.; Santos, R.D.; Hegele, R.A.; Kuivenhoven, J.A.; Nordestgaard, B.G.; Descamps, O.S.; Steinhagen-Thiessen, E.; et al. Homozygous familial hypercholesterolaemia: New insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *Eur. Heart J.* **2014**, *35*, 2146–2157. [CrossRef] [PubMed]
7. Palcoux, J.-B.; Atassi-Dumont, M.; Lefevre, P.; Hequet, O.; Schlienger, J.-L.; Brignon, P.; Roussel, B. Low-density lipoprotein apheresis in children with familial hypercholesterolemia: Follow-up to 21 years. *Ther. Apher. Dial.* **2008**, *12*, 195–201. [CrossRef] [PubMed]
8. Raal, F.J.; Santos, R.D. Homozygous familial hypercholesterolemia: Current perspectives on diagnosis and treatment. *Atherosclerosis* **2012**, *223*, 262–268. [CrossRef] [PubMed]
9. Dweck, M.R.; Boon, N.A.; Newby, D.E. Calcific aortic stenosis: A disease of the valve and the myocardium. *J. Am. Coll. Cardiol.* **2012**, *60*, 1854–1863. [CrossRef]

10. Osnabrugge, R.L.J.; Mylotte, D.; Head, S.J.; Van Mieghem, N.M.; Nkomo, V.T.; LeReun, C.M.; Bogers, A.J.J.C.; Piazza, N.; Kappetein, A.P. Aortic stenosis in the elderly: Disease prevalence and number of candidates for transcatheter aortic valve replacement: A meta-analysis and modeling study. *J. Am. Coll. Cardiol.* **2013**, *62*, 1002–1012. [CrossRef]
11. Nishimura, R.A.; Otto, C.M.; Bonow, R.O.; Carabello, B.A.; Erwin, J.P.; Guyton, R.A.; O'Gara, P.T.; Ruiz, C.E.; Skubas, N.J.; Sorajja, P.; et al. 2014 AHA/ACC guideline for the management of patients with valvular heart disease: Executive summary: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J. Am. Coll. Cardiol.* **2014**, *63*, 2438–2488. [CrossRef]
12. Rossebø, A.B.; Pedersen, T.R.; Boman, K.; Brudi, P.; Chambers, J.B.; Egstrup, K.; Gerdts, E.; Gohlke-Bärwolf, C.; Holme, I.; Kesäniemi, Y.A.; et al. Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis. *N. Engl. J. Med.* **2008**, *359*, 1343–1356. [CrossRef] [PubMed]
13. Greve, A.M.; Bang, C.N.; Boman, K.; Egstrup, K.; Kesäniemi, Y.A.; Ray, S.; Pedersen, T.R.; Wachtell, K. Relation of Lipid-Lowering Therapy to Need for Aortic Valve Replacement in Patients With Asymptomatic Mild to Moderate Aortic Stenosis. *Am. J. Cardiol.* **2019**, *124*, 1736–1740. [CrossRef] [PubMed]
14. Banach, M.; Serban, C.; Sahebkar, A.; Mikhailidis, D.P.; Ursoniu, S.; Ray, K.K.; Rysz, J.; Toth, P.P.; Muntner, P.; Mosteoru, S.; et al. Impact of statin therapy on coronary plaque composition: A systematic review and meta-analysis of virtual histology intravascular ultrasound studies. *BMC Med.* **2015**, *13*, 229. [CrossRef] [PubMed]
15. Lindroos, M.; Kupari, M.; Heikkilä, J.; Tilvis, R. Prevalence of aortic valve abnormalities in the elderly: An echocardiographic study of a random population sample. *J. Am. Coll. Cardiol.* **1993**, *21*, 1220–1225. [CrossRef]
16. Vongpromek, R.; Bos, S.; Ten Kate, G.-J.R.; Yahya, R.; Verhoeven, A.J.M.; de Feyter, P.J.; Kronenberg, F.; Roeters van Lennep, J.E.; Sijbrands, E.J.G.; Mulder, M.T. Lipoprotein(a) levels are associated with aortic valve calcification in asymptomatic patients with familial hypercholesterolaemia. *J. Intern. Med.* **2015**, *278*, 166–173. [CrossRef] [PubMed]
17. Perak, A.M.; Ning, H.; de Ferranti, S.D.; Gooding, H.C.; Wilkins, J.T.; Lloyd-Jones, D.M. Long-Term Risk of Atherosclerotic Cardiovascular Disease in US Adults With the Familial Hypercholesterolemia Phenotype. *Circulation* **2016**, *134*, 9–19. [CrossRef] [PubMed]
18. Ten Kate, G.-J.R.; Bos, S.; Dedic, A.; Neefjes, L.A.; Kurata, A.; Langendonk, J.G.; Liem, A.; Moelker, A.; Krestin, G.P.; de Feyter, P.J.; et al. Increased Aortic Valve Calcification in Familial Hypercholesterolemia: Prevalence, Extent, and Associated Risk Factors. *J. Am. Coll. Cardiol.* **2015**, *66*, 2687–2695. [CrossRef]
19. Rosenhek, R.; Binder, T.; Porenta, G.; Lang, I.; Christ, G.; Schemper, M.; Maurer, G.; Baumgartner, H. Predictors of outcome in severe, asymptomatic aortic stenosis. *N. Engl. J. Med.* **2000**, *343*, 611–617. [CrossRef]
20. Coffey, S.; Cox, B.; Williams, M.J.A. The prevalence, incidence, progression, and risks of aortic valve sclerosis: A systematic review and meta-analysis. *J. Am. Coll. Cardiol.* **2014**, *63*, 2852–2861. [CrossRef]
21. Mundal, L.J.; Hovland, A.; Igland, J.; Veierød, M.B.; Holven, K.B.; Bogsrud, M.P.; Tell, G.S.; Leren, T.P.; Retterstøl, K. Association of Low-Density Lipoprotein Cholesterol With Risk of Aortic Valve Stenosis in Familial Hypercholesterolemia. *JAMA Cardiol.* **2019**, *4*, 1156–1159. [CrossRef]
22. Pohle, K.; Mäffert, R.; Ropers, D.; Moshage, W.; Stilianakis, N.; Daniel, W.G.; Achenbach, S. Progression of aortic valve calcification: Association with coronary atherosclerosis and cardiovascular risk factors. *Circulation* **2001**, *104*, 1927–1932. [CrossRef] [PubMed]
23. Nkomo, V.T.; Gardin, J.M.; Skelton, T.N.; Gottdiener, J.S.; Scott, C.G.; Enriquez-Sarano, M. Burden of valvular heart diseases: A population-based study. *Lancet* **2006**, *368*, 1005–1011. [CrossRef]
24. Cosmi, J.E.; Kort, S.; Tunick, P.A.; Rosenzweig, B.P.; Freedberg, R.S.; Katz, E.S.; Applebaum, R.M.; Kronzon, I. The risk of the development of aortic stenosis in patients with "benign" aortic valve thickening. *Arch. Intern. Med.* **2002**, *162*, 2345–2347. [CrossRef] [PubMed]
25. Supino, P.G.; Borer, J.S.; Preibisz, J.; Bornstein, A. The epidemiology of valvular heart disease: A growing public health problem. *Heart Fail. Clin.* **2006**, *2*, 379–393. [CrossRef]
26. Rallidis, L.; Naoumova, R.P.; Thompson, G.R.; Nihoyannopoulos, P. Extent and severity of atherosclerotic involvement of the aortic valve and root in familial hypercholesterolaemia. *Heart* **1998**, *80*, 583–590. [CrossRef] [PubMed]

27. Sprecher, D.L.; Schaefer, E.J.; Kent, K.M.; Gregg, R.E.; Zech, L.A.; Hoeg, J.M.; McManus, B.; Roberts, W.C.; Brewer, H.B. Cardiovascular features of homozygous familial hypercholesterolemia: Analysis of 16 patients. *Am. J. Cardiol.* **1984**, *54*, 20–30. [CrossRef]
28. Civeira, F.; Ros, E.; Jarauta, E.; Plana, N.; Zambon, D.; Puzo, J.; Martinez de Esteban, J.P.; Ferrando, J.; Zabala, S.; Almagro, F.; et al. Comparison of genetic versus clinical diagnosis in familial hypercholesterolemia. *Am. J. Cardiol.* **2008**, *102*, 1187–1193. [CrossRef]
29. Talmud, P.J.; Shah, S.; Whittall, R.; Futema, M.; Howard, P.; Cooper, J.A.; Harrison, S.C.; Li, K.; Drenos, F.; Karpe, F.; et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: A case-control study. *Lancet* **2013**, *381*, 1293–1301. [CrossRef]
30. Khera, A.V.; Won, H.-H.; Peloso, G.M.; Lawson, K.S.; Bartz, T.M.; Deng, X.; van Leeuwen, E.M.; Natarajan, P.; Emdin, C.A.; Bick, A.G.; et al. Diagnostic Yield and Clinical Utility of Sequencing Familial Hypercholesterolemia Genes in Patients With Severe Hypercholesterolemia. *J. Am. Coll. Cardiol.* **2016**, *67*, 2578–2589. [CrossRef]
31. Rajamannan, N.M. Calcific Aortic Stenosis: Lessons Learned from Experimental and Clinical Studies. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 162–168. [CrossRef]
32. Lindman, B.R.; Clavel, M.-A.; Mathieu, P.; Iung, B.; Lancellotti, P.; Otto, C.M.; Pibarot, P. Calcific aortic stenosis. *Nat. Rev. Dis. Primers* **2016**, *2*, 16006. [CrossRef] [PubMed]
33. O'Brien, K.D.; Reichenbach, D.D.; Marcovina, S.M.; Kuusisto, J.; Alpers, C.E.; Otto, C.M. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of "degenerative" valvular aortic stenosis. *Arterioscler. Thromb. Vasc. Biol.* **1996**, *16*, 523–532. [CrossRef] [PubMed]
34. Chan, K.L.; Teo, K.; Dumesnil, J.G.; Ni, A.; Tam, J. ASTRONOMER Investigators Effect of Lipid lowering with rosuvastatin on progression of aortic stenosis: Results of the aortic stenosis progression observation: Measuring effects of rosuvastatin (ASTRONOMER) trial. *Circulation* **2010**, *121*, 306–314. [CrossRef] [PubMed]
35. Moura, L.M.; Ramos, S.F.; Zamorano, J.L.; Barros, I.M.; Azevedo, L.F.; Rocha-Gonçalves, F.; Rajamannan, N.M. Rosuvastatin affecting aortic valve endothelium to slow the progression of aortic stenosis. *J. Am. Coll. Cardiol.* **2007**, *49*, 554–561. [CrossRef] [PubMed]
36. Saremi, A.; Bahn, G.; Reaven, P.D. VADT Investigators Progression of vascular calcification is increased with statin use in the Veterans Affairs Diabetes Trial (VADT). *Diabetes Care* **2012**, *35*, 2390–2392. [CrossRef] [PubMed]
37. Mundy, G.; Garrett, R.; Harris, S.; Chan, J.; Chen, D.; Rossini, G.; Boyce, B.; Zhao, M.; Gutierrez, G. Stimulation of bone formation in vitro and in rodents by statins. *Science* **1999**, *286*, 1946–1949. [CrossRef]
38. Kwon, O.; Kang, S.-J.; Kang, S.H.; Lee, P.H.; Yun, S.-C.; Ahn, J.-M.; Park, D.-W.; Lee, S.-W.; Kim, Y.-H.; Lee, C.W.; et al. Relationship Between Serum Inflammatory Marker Levels and the Dynamic Changes in Coronary Plaque Characteristics After Statin Therapy. *Circ. Cardiovasc. Imaging* **2017**, *10*, e005934. [CrossRef]
39. Cho, K.I.; Sakuma, I.; Sohn, I.S.; Jo, S.-H.; Koh, K.K. Inflammatory and metabolic mechanisms underlying the calcific aortic valve disease. *Atherosclerosis* **2018**, *277*, 60–65. [CrossRef]
40. Al Kindi, M.; Bélanger, A.M.; Sayegh, K.; Senouci, S.; Aljenedil, S.; Sivakumaran, L.; Ruel, I.; Al Rasadi, K.; Al Waili, K.; Awan, Z.; et al. Aortic Calcification Progression in Heterozygote Familial Hypercholesterolemia. *Can. J. Cardiol.* **2017**, *33*, 658–665. [CrossRef]
41. Greve, A.M.; Bang, C.N.; Boman, K.; Egstrup, K.; Forman, J.L.; Kesäniemi, Y.A.; Ray, S.; Pedersen, T.R.; Best, P.; Rajamannan, N.M.; et al. Effect Modifications of Lipid-Lowering Therapy on Progression of Aortic Stenosis (from the Simvastatin and Ezetimibe in Aortic Stenosis [SEAS] Study). *Am. J. Cardiol.* **2018**, *121*, 739–745. [CrossRef]
42. Rajamannan, N.M.; Greve, A.M.; Moura, L.M.; Best, P.; Wachtell, K. SALTIRE-RAAVE: Targeting calcific aortic valve disease LDL-density-radius theory. *Expert Rev. Cardiovasc. Ther.* **2015**, *13*, 355–367. [CrossRef] [PubMed]
43. Alskaf, E.; Kardos, A. The mystery of defining aortic valve area: What have we learnt from three-dimensional imaging modalities? *J. Echocardiogr.* **2018**, *16*, 130–138. [CrossRef] [PubMed]

Atherosclerosis 320 (2021) 47–52

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Maternally inherited hypercholesterolemia does not modify the cardiovascular phenotype in familial hypercholesterolemia

Victoria Marco-Benedí [a], Martín Laclaustra [a,b,**], Ana M. Bea [a], Manuel Suarez-Tembra [d], Núria Plana [e], Xavier Pinto [f], Angel Brea [g], Rosa M. Sanchez-Hernandez [h], Fernando Civeira [a,c,*]

[a] Hospital Universitario Miguel Servet, IIS Aragón, Cibercv, Spain
[b] Fundación Agencia Aragonesa para La Investigación y El Desarrollo (ARAID), Zaragoza, Spain
[c] Universidad de Zaragoza, Zaragoza, Spain
[d] Servicio de Medicina Interna, Unidad de Lípidos y Riesgo Cardiovascular, Hospital San Rafael, A Coruña, Spain
[e] Unitat de Medicina Vascular I Metabolisme (UVASMET) Hospital Universitari Sant Joan, IISPV, CIBERDEM, Universitat Rovira I Virgili, Reus, Tarragona, Spain
[f] Unidad de Lípidos. Servicio de Medicina Interna. Hospital Universitario de Bellvitge-Idibell. Universidad de Barcelona, CiberObn, Spain
[g] Unidad de Lípidos. Servicio de Medicina Interna. Hospital San Pedro, Logroño, Spain
[h] Sección de Endocrinología y Nutrición, Complejo Hospitalario Universitario Insular Materno Infantil de Gran Canaria, Instituto Universitario de Investigaciones Biomédicas y Sanitarias (IUIBS) de La Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

ARTICLE INFO

Keywords:
Heterozygous familial hypercholesterolemia
Low-density lipoprotein receptor
HeFH phenotype
Mother-offspring

ABSTRACT

Background and aims: Familial hypercholesterolemia (FH) is a codominant autosomal disease characterized by a high risk of cardiovascular disease when not in lipid-lowering treatment. However, there is a large variability in the clinical presentation in heterozygous subjects (HeFH). Maternal hypercholesterolemia has been proposed as a cardiometabolic risk factor later in life. Whether this phenotype variability depends on the mother or father origin of hypercholesterolemia is unknown.

The objective of this study was to analyze potential differences in anthropometry, superficial lipid deposits, comorbidities, and lipid concentrations depending on the parental origin of hypercholesterolemia within a large group of HeFH.

Methods: This is a cross-sectional observational, multicenter, nation-wide study in Spain. We recruited adults with HeFH to study clinical differences according to the parental origin. Data on HeFH patients were obtained from the Dyslipidemia Registry of the Spanish Atherosclerosis Society.

Results: HeFH patients were grouped in 1231 HeFH-mother-offspring aged 45.7 (16.3) years and 1174 HeFH-father-offspring aged 44.8 (16.7) years. We did not find any difference in lipid parameters (total cholesterol, triglycerides, LDLc, HDLc, and Lp(a)), nor in the comorbidities studied (cardiovascular disease prevalence, age of onset of cardiovascular disease, obesity, diabetes, and hypertension) between groups. Lipid-lowering treatment did not differ between groups. The prevalence of comorbidities did not show differences when they were studied by age groups.

Conclusions: Our research with a large group of subjects with HeFH shows that a potential maternal effect is not relevant in FH. However, due to the size of our sample, potential differences between genders cannot be completely ruled out. This implies that severe maternal hypercholesterolemia during pregnancy is not associated with additional risk in the FH affected offspring.

1. Introduction

Familial hypercholesterolemia (FH) is a codominant autosomal disease characterized by very high concentrations of low-density lipoprotein cholesterol (LDLc), superficial deposits of cholesterol in the form of corneal arcus and tendon xanthomas, and high risk of premature cardiovascular disease (CVD) in the absence of adequate lipid-lowering treatment [1,2]. LDLc concentrations of heterozygous FH (HeFH) tend to

* Corresponding author. Unidad de Lípidos, Hospital Universitario Miguel Servet, Avda Isabel La Catolica 1-3, 50009, Zaragoza, Spain.
** Corresponding author. Unidad de Lípidos, Hospital Universitario Miguel Servet, Avda Isabel La Catolica 1-3, 50009, Zaragoza, Spain.
E-mail addresses: martin.laclaustra@unizar.es (M. Laclaustra), civeira@unizar.es (F. Civeira).

https://doi.org/10.1016/j.atherosclerosis.2021.01.015
Received 30 October 2020; Received in revised form 30 December 2020; Accepted 12 January 2021
Available online 18 January 2021

be approximately twice those of subjects in the general population and their CVD risk in the first decades of life, especially coronary disease, is up to 100 times higher [3]. However, a characteristic of HeFH is the great variability in clinical presentation, including LDLc concentrations, and the presence of tendon xanthomas or coronary artery disease [4]. This variability is multifactorial and has been associated with the gene responsible for FH, with a more severe phenotype in carriers of *LDLR* mutations than in those with a mutation in *APOB*, *PCSK9,* or *APOE* [5,6]; the type of causal mutation, with worse phenotype in null-allele carriers than in defective allele carriers [7]; the interaction with other genes, such as *ABCA1* or *PSCK9* [8,9]; and the presence of CVD risk factors common to the general population, such as smoking, diabetes, low high-density lipoprotein cholesterol (HDLc), and high lipoprotein(a) (Lp (a)) levels [10]. Despite all this, the origin of this clinical variation in HeFH remains unknown [1,4].

One of the potential factors associated with the clinical variation of HeFH subjects is the parental origin of the genetic defect. Some relatively frequent phenomena in nature that could explain differences in the phenotype in monogenic diseases are the so-called genomic imprinting where the expression level of the alleles of a gene depends upon their parental origin [11]; and a maternal effect, where the phenotype of the offspring is determined not only by the postnatal environment and genotype but also by the environment during gestation [12]. These epigenetic phenomena are produced by modifications to chromatin mainly DNA methylation, histone acetylation, or the interaction of non-coding RNAs with DNA. The induction of DNA methylation is highly influenced by the maternal environment [13]. Genomic imprinting genes have not been associated with FH [11]. However, a maternal effect in HeFH has been attributed to a possible effect of maternal hypercholesterolemia during pregnancy that would condition a metabolic memory during adulthood [14]. It has been reported that maternally derived HeFH subjects may have higher LDLc levels [15] and higher CVD mortality than paternally derived HeFH [16]. It would be similar to what happens with the mother smoking or the diet rich in saturated fat during pregnancy [17], or low birth weight, with the risk of diabetes [18], arterial hypertension, or atheromatous cardiovascular disease in adulthood [19].

The effect of hypercholesterolemia during pregnancy favours the early development of arteriosclerosis lesions in newborns and an increased risk of diabetes and arterial hypertension in adulthood in different animal models [20,21]. Whether this effect exists in humans is not known. Lipid-lowering treatment is contraindicated during pregnancy and, given that cholesterol levels physiologically rise during the second and third trimesters of pregnancy, cholesterol rise is substantial in pregnant women with HeFH [22]. Therefore, FH is a good model to identify whether severe hypercholesterolemia during pregnancy in HeFH women conditions the phenotype in the offspring and explains, at least in part, the differences that we find among adult subjects with HeFH.

The objective of this analysis was to identify potential differences in anthropometry, superficial lipid deposits, comorbidities, and lipid concentrations between subjects with maternal or paternal origin of hypercholesterolemia within a large group of HeFH.

2. Patients and methods

2.1. Aim, design and participants

This cross-sectional observational multicenter nation-wide study in Spain was designed to identify differences in HeFH according to the parental origin of hypercholesterolemia. Data on HeFH patients were obtained from the Dyslipidemia Registry of the Spanish Atherosclerosis Society (SEA). The Dyslipidemia Registry of the SEA is an active online registry, where 65 certified lipid clinics across all regions of Spain report cases of various types of primary hyperlipidemias [23]. Inclusion criteria and data collection were standardized among clinicians in 5 training sessions before case recruitment. Written informed consent was obtained from each patient included in the study; the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki; and the study protocol has been priorly approved by the Institution's ethics committee on research on humans (Comité Ético de Investigación Clínica de Aragón).

HeFH subjects were eligible for inclusion in this analysis if they had a clinical or genetic diagnosis of HeFH. Clinical diagnosis was based on the diagnostic criteria proposed by the Dutch Lipid Clinics Network: 6–8 points (probable) and >8 points (definitive). Genetic diagnosis was based on tested carrier status of a known pathogenic mutation for FH. Pathogenicity definition of mutations followed the American College of Medical Genetics ACMG recommendations [1]. Homozygous FH subjects were excluded for this study. Patients in whom the parental inheritance of FH was unknown were not included in the final analysis.

2.2. Study variables

2.2.1. Clinical interview

For HeFH, the registry includes, among other data, personal and family health history, anthropometry, physical examination, laboratory data, presence of CVD, age at which statin treatment began, history of lipid-lowering treatment, and genetic data regarding mutations in *LDLR*, *APOB,* or *PCSK9* (positive, negative or unknown).

2.2.2. Family health history

Information about parental transmission of hypercholesterolemia was self-reported and confirmed from the patient's medical records. CVD is defined as coronary (myocardial infarction, coronary revascularization procedure, sudden death); cerebral (stroke with>24 h neurological deficit without evidence of bleeding in brain imaging tests); peripheral vascular disease (intermittent claudication with ankle arm index <0.9, or arterial revascularization of lower limbs) or symptomatic or asymptomatic abdominal aortic aneurysm.

2.2.3. Laboratory tests

Lipid and lipoprotein levels are included in fasting state not using lipid-lowering medication for at least 6 weeks.

2.2.4. Definitions

Arterial hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or self-reported use of antihypertensive medication. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Diabetes mellitus (DM) was defined as the use of antidiabetic medications. Current smoker was defined as being currently smoking or having smoked in the last year. Former smoker was defined as a subject having smoked at least 50 cigarettes in his lifetime, but not having smoked in the last year.

We conducted this study in accordance with the Declaration of Helsinki for the protection of the rights and welfare of people participating in biomedical research.

2.3. Statistical analyses

Variables were summarized as mean (standard deviation) or percentage. Unadjusted differences between parental groups were tested with the Student-t test or the Chi-squared test as appropriate. Linear and logistic regression models adjusted for age and sex were used to model the observed clinical characteristics, and to test the differences between parental origins. Differences in prevalence of comorbidities were tested with logistic regression models adjusted for age, sex, and BMI. A sensitivity analysis was performed restricting the dataset to those subjects with confirmed genetic mutation. All data analyses were performed with SPSS version 22 and R version 3.6.0. A *post -hoc* power calculation was performed to analyze for cardiovascular disease prevalence difference according to the parental origin of FH. $p < 0.05$ is considered statistically

significant.

3. Results

3.1. Clinical characteristics

HeFH patients were grouped into 1231 HeFH-mother-offspring and 1174 HeFH-father-offspring, aged 45.7 (16.3) years and 44.8 (16.7) years, respectively. In the registry, the parental origin of the disease could not be determined in 884 subjects. The main characteristics of the three groups are presented in Table 1 and Supplemental Table 1. Subjects without information on the parental origin were older than the other two groups. No other differences were found between parental origins in the rest of the studied variables including total cholesterol, triglycerides, LDLc, HDLc, and Lp(a)), DLCN scores, or lipid-lowering treatment intensity or duration. There were no differences between HeFH with maternal or paternal origin in all these variables after age and gender adjustment (Table 2), and when only HeFH subjects with genetic confirmation were considered (Table 3). All variables were analyzed stratifying by sex without statistical differences between men and women. (Supplemental Tables 4-7).

3.2. Prevalence of cardiovascular disease, obesity, diabetes and hypertension

The prevalence of these morbidities is presented in Tables 4 and 5. They do not differ between groups even after adjustment for age, gender, and BMI when appropriate (not adjusted for BMI in anthropometric results). As expected, the prevalence of DM was low in both groups and there were no hints of differences between groups in the studied diseases. We estimate that our sample has 80% power to detect a relative risk of 1.367 between two groups of 1202 subjects when the overall prevalence is 12 cases per 100 with an alpha threshold of 0.05.

3.3. Prevalence of cardiovascular disease, obesity, diabetes and hypertension by age group

To further identify potential differences in morbidity prevalence and different evolution according to age, we studied all variables and morbidities by age decades. None of the studied variables show differences between groups of parental origin. The prevalence of DM, CVD, LDLc concentration and blood pressure increased in a similar magnitude as age increased (Fig. 1).

Table 1
Clinical and biochemical characteristics of the HeFH subjects according to the FH parental origin.

FH parental origin	Mother	Father	Unknown
N	1231	1174	884
Age (years)	45.7 (16.3)	44.8 (16.7)	54.7 (13.9)
Sex (women), N (%)	646 (52.5)	569 (48.5)	504 (57.0)
Tobacco (current smoker), N (%)	278 (23.0)	267 (23.2)	187 (21.8)
BMI (Kg/m^2)[a]	25.5 (4.6)	25.7 (4.8)	26.9 (4.5)
Waist circumference (cm)	78.9 (61.7)	78.3 (56.8)	81.1 (45.4)
Systolic blood pressure (mmHg)	124.9 (17.2)	125.7 (16.5)	129.8 (17.2)
Diastolic blood pressure (mmHg)	76.0 (11.4)	76.0 (10.8)	79.0 (10.4)
Corneal arcus, N (%)	331 (28.8)	336 (30.5)	290 (34.6)
Tendon xanthoma, N (%)	109 (9.2)	110 (9.8)	88 (10.4)
Total cholesterol (mg/dL)	384.5 (183.5)	381.8 (176.9)	397.6 (184.8)
Triglycerides (mg/dL)	126.2 (97.6)	124.6 (79.5)	144.2 (118.9)
LDL cholesterol (mg/dL)[b]	303.4 (180.5)	301.9 (175.2)	313.4 (182.7)
HDL cholesterol (mg/dL)[c]	55.8 (15.8)	55.0 (14.6)	55.4 (15.9)
Lipoprotein(a) (mg/dL)	46.6 (51.8)	47.2 (53.1)	55.6 (63.2)
DLCN score (points)[d]	14.0 (5.3)	13.7 (5.4)	12.8 (5.3)
Positive genetic diagnosis, N (%)	868 (70.5)	792 (67.5)	513 (58.0)
Statin treatment duration (years)	8.8 (7.9)	8.7 (7.9)	8.8 (7.5)

[a] BMI, body mass index.
[b] LDL, low-density lipoprotein.
[c] HDL, high-density lipoprotein.
[d] DLCN, Dutch lipid clinics network. Data are summarized as mean (SD) or N (percentage).

Table 2
Sex and age adjusted comparison of clinical and biochemical characteristics of the HeFH subjects according to the FH parental origin.

FH parental origin	Mother	Father	*p*
N	1231	1174	
Tobacco (smoker) N (%)	278 (23.0)	267 (23.2)	0.93
BMI (Kg/m^2)[a]	25.4	25.6	0.34
Waist circumference (cm)	81.2	80.6	0.81
Systolic blood pressure (mmHg)	125.1	126.1	0.14
Diastolic blood pressure (mmHg)	75.5	75.6	0.85
Corneal arcus, N (%)	292 (25.4)	283 (27.5)	0.26
Tendon xanthoma, N (%)	106 [9]	109 (9.7)	0.59
Total cholesterol (mg/dL)	372.2	371.9	0.97
Triglycerides (mg/dL)	132.1	130.6	0.68
LDL cholesterol (mg/dL)[b]	295.0	295.4	0.95
HDL cholesterol (mg/dL)[c]	50.8	50.4	0.48
Lipoprotein(a) (mg/dL)	44.6	45.9	0.61
DLCN score (points)[d]	14.2	13.9	0.10
Positive genetic diagnosis, N (%)	868 (70.5)	792 (67.5)	0.256
LDLR mutation, N (%)	814 (93.8)	749 (94.6)	0.492
APOB mutation, N (%)	44 (5.1)	36 (4.5)	0.619
Statin treatment duration (years)	7.5	7.6	0.69
Age at first CVD event (years)	34.5	35.5	0.29
High-intensity statin N (%)	811 (65.9)	750 (63.9)	0.59
Ezetimibe use, N (%)	661 (53.7)	621 (52.9)	0.71

Data are summarized as mean (SD) or N (percentage).
[a] BMI denotes body mass index.
[b] LDL, low-density lipoprotein.
[c] HDL, high-density lipoprotein.
[d] DLCN, Dutch lipid clinics network. Linear and logistic regression models were used to calculate conditionally age and sex adjusted estimates for a 40 years old man (values in cells) and to test for differences.

Table 3
Sex and age adjusted comparison of clinical and biochemical characteristics of the genetically confirmed HeFH subjects according to the FH parental origin.

FH parental origin	Mother	Father	*p*
N	868	792	
Tobacco (smoker), N (%)	207 (24.4)	171 (22.0)	0.28
BMI (Kg/m^2)[a]	25.3	25.4	0.55
Waist circumference (cm)	83.0	82.4	0.85
Systolic blood pressure (mmHg)	125.1	126.1	0.19
Diastolic blood pressure (mmHg)	74.9	74.7	0.68
Corneal arcus, N (%)	202 (24.9)	192 (25.9)	0.68
Tendon xanthoma, N (%)	83 (10.0)	84 (11.0)	0.54
Total cholesterol (mg/dL)	373.6	372.7	0.92
Triglycerides (mg/dL)	123.1	124.2	0.77
LDL cholesterol (mg/dL)[b]	297.8	297.7	0.99
HDL cholesterol (mg/dL)[c]	51.2	50.1	0.15
Lipoprotein(a) (mg/dL)	42.6	43.8	0.64
DLCN score[d]	16.4	16.4	0.74
Statin treatment duration (years)	8.0	8.6	0.10
Age at first CVD event (years)	33.9	34.6	0.61
High-intensity statin, N (%)	582 (67.1)	508 (64.2)	0.51
Ezetimibe use, N (%)	495 (57.0)	430 (54.3)	0.29

Data are summarized as mean (SD), median (interquartile range) or N (percentage).
[a] BMI denotes body mass index.
[b] LDL, low-density lipoprotein.
[c] HDL, high-density lipoprotein.
[d] DLCN, Dutch lipid clinics network. Linear and logistic regression models were used to calculate conditionally age and sex adjusted estimates for a 40-year-old man (values in cells) and to test for differences.

4. Discussion

Several studies had suggested that maternal hypercholesterolemia might increase adult CVD in the offspring [20]. We studied this issue in a

Table 4
Sex and age adjusted comparison of comorbidities of subjects according to the FH parental origin.

FH parental origin	Mother	Father	*p* (adj.)
N	1231	1174	
Diabetes, N (%)	22 (1.8)	25 (2.2)	0.43
High blood pressure, N (%)	73 (6.2)	59 (5.2)	0.19
Cardiovascular disease, N (%)	113 (9.2)	109 (9.3)	0.92
Overweight or obesity, N (%)	700 (57.1)	676 (57.9)	0.68
Obesity, N (%)	136 (11.1)	147 (12.7)	0.21

Conditionally age and sex adjusted estimates for a 40-year-old man. Test for raw differences using Chi^2 test and regression tests (*p* (adj.)) from adjusted logistic models.

Table 5
Sex and age adjusted comparison of comorbidities of the genetically confirmed HeFH subjects according to the FH paternal origin.

FH parental origin	Mother	Father	*p*
N	868	792	
Diabetes	13 (1.6)	17 (2.2)	0.29
High blood pressure	43 (5.3)	37 (4.9)	0.68
Cardiovascular disease	77 (8.9)	67 (8.4)	0.75
Overweight or obesity	464 (53.8)	434 (55.3)	0.58
Obesity	80 (9.3)	85 (10.8)	0.28

Conditionally age and sex adjusted estimates for a 40-year-old man. Test for raw differences using Chi^2 test and regression tests (*p* (adj.)) from adjusted logistic models.

large group of HeFH and we did not find any significant differences in the phenotype including CVD, DM, hypertension or plasma levels of lipids according to the parental origin of the genetic defect. FH is a good model to study the effect of hypercholesterolemia in the offspring due to the large increase in lipid levels that women with FH have during pregnancy, often with concentrations of total cholesterol twice those of mothers without FH and higher than 400 mg/dL. Our findings do not support that maternal hypercholesterolemia has a deleterious effect on the offspring.

These results are in line with other studies that did not find any difference in lipids and lipoprotein levels between HeFH who had inherited FH maternally or paternally [24]. In addition, Tonstad et al. did not observe any difference in the carotid intima-media thickness and prevalence of plaque between HeFH children in spite of the parental origin [25]. However, Van der Graf et al. had previously observed that maternal hereditary hypercholesterolemia slightly increases TC, LDLc and apolipoprotein B levels in their offspring later in life [15]; and maternally inherited FH was associated with a significantly higher mortality than FH transmitted by fathers (relative risk 2.2; $p = 0.048$) in HeFH carrying the V408 M mutation in the *LDLR* gene [16]. Probably the different inclusion criteria between studies or the number of subjects studied can explain the differences found.

Our study also provides relevant information regarding the role of hypercholesterolemia during pregnancy, regardless of its cause, in the subsequent development of cardiovascular complications. Previous information in models suggests that maternal hypercholesterolemia accelerates the development of arteriosclerosis in offspring in both rabbits [26] and mice [27], regardless of whether hypercholesterolemia in the mother was induced by genetic manipulation, diet, or both, and independent of postnatal LDLc concentration [19]. The effect of hypercholesterolemia during pregnancy in humans has been much less studied. In a postmortem study of the aortic arch and abdominal aorta of 156 normocholesterolaemic children aged 1–13 years, who died of trauma and other causes, the Fate of Early Lesions in Children Study showed an association between maternal cholesterol and the presence of initial lesions of arteriosclerosis in children [20]. However, this has not been subsequently confirmed.

During pregnancy, a physiological increase in maternal cholesterol levels occurs. It is an adaptive mechanism responding to the higher demands of cholesterol during prenancy and it is known as “maternal physiological hypercholesterolemia” [28]. In addition, some women have an alteration called “maternal supraphysiological hypercholesterolemia” which is associated with fetoplacental vascular modifications.

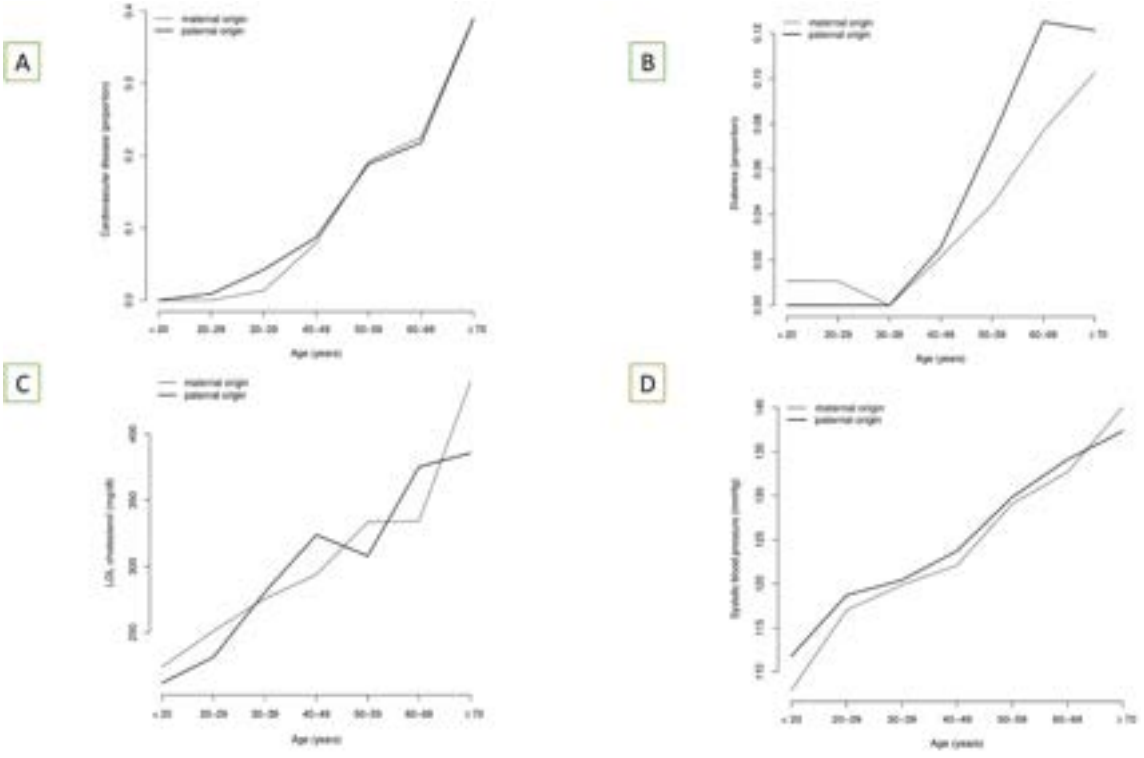


Fig. 1. Prevalence of cardiovascular disease (A), and diabetes (B), LDL cholesterol concentration (C) and systolic blood pressure (D) according to age decades.

Nevertheless, maternal hypercholesterolemia does not affect neonatal lipid levels [29,30] because cholesterol plasma concentration in the fetus is a highly regulated process mostly independent of maternal plasma cholesterol. The cholesterol in the fetus comes from *de novo* synthesis or placenta transport. Cholesterol is transported in the human placenta from mother to fetus through cholesterol uptake by the placenta from maternal lipoproteins, crossing trophoblast and endothelium and via efflux from it to acceptors in the fetus. In the apical side of the syncytiotrophoblast (STB), the cholesterol uptake from the maternal circulation comes from LDL and HDL particles through the low-density lipoprotein and SR-BI receptors, respectively. It is secreted at the basal side facing the villous stroma [12,31,32]. The mechanisms by which the cholesterol is transported to the endothelial cells to finally reach the fetal circulation are mostly unknown [33,34] but two highly regulated proteins, ABCA1 and ABCG1, are responsible for translocating placenta cholesterol to lipid-free apolipoproteins A1 and HDL particles [35] without the participation of the LDL receptor, which is poorly expressed at the basal side of the syncytiotrophoblast [30].

The main clinical implication of our results is that the clinical management of subjects with HeFH should not be different depending on whether the inheritance is maternal or paternal, since the lipid phenotype and long-term complications are similar in both groups. There is a tendency in the clinical practice of cardiovascular diseases to underuse effective cardiac medications among women than among men [36–38] and this could be accentuated in FH since family history of premature cardiovascular disease is a risk-enhancing factor in the general population [39], and premature cardiovascular disease is less common in HeFH women [10]. Therefore, the risk of having a history of early disease is greater if the inheritance is paternal. This potential bias is not observed in our study since clinical management is very similar between subjects with paternal or maternal inheritance. Neither the years of statin nor the percentage of subjects with high-intensity lipid-lowering treatment was different depending on paternal inheritance. This is most likely due to the fact that the patients in our study come from specialized units [23] where therapeutic recommendations are mostly based on individual risk factors according to current guidelines [1]. We think our data are solid about the absence of a relevant clinical effect in hypercholesterolemia of monogenic origin. However, whether other forms of hypercholesterolemia during pregnancy play a relevant role later in life should be explored.

Some aspects of our study should be discussed. First, the parental assignment has been self-reported, although it was rechecked in the medical records. For this reason, 27% of the subjects were excluded from the analysis since the allocation could not be verified. Second, the diagnosis of HeFH was based on clinical criteria and genetic diagnosis was not available in 10.1% and 11.5% of HeFH with maternal and paternal origin, respectively, although we did not find any difference when considering only those subjects with a positive genetic diagnosis. Finally, our study is underpowered to see gender-associated differences and might miss gender-associated differences in the phenotype.

In conclusion, our results from a large group of subjects with HeFH do not support differences in the lipid phenotype, cardiovascular disease prevalence, age of onset of cardiovascular disease or cardiometabolic complications such as DM and hypertension in relation to the maternal or paternal origin of hypercholesterolemia. These findings imply that maternal hypercholesterolemia does not confer an additional risk to offspring later in life, and that a potential maternal effect is not relevant in FH.

CRediT authorship contribution statement

Victoria Marco-Benedí: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Martín Laclaustra:** Methodology. **Ana M. Bea:** Data curation, Investigation. **Manuel Suarez-Tembra:** Data curation, Formal analysis, Investigation, Software. **Núria Plana:** Data curation. **Xavier Pinto:** Data curation, Investigation. **Angel Brea:** Data curation, Investigation. **Rosa M. Sanchez-Hernandez:** Data curation, Investigation. **Fernando Civeira:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Financial support

This study was supported by grants from the Spanish Ministry of Economy and Competitiveness PI 18/01777 PI 19/00694 and CIBERCV; and Gobierno de Aragón B-14 These projects are co-financed by Instituto de Salud Carlos III and the European Regional Development Fund (ERDF) of the European Union "A way to make Europe". Dr. Laclaustra's research activity is funded by Agencia Aragonesa para la Investigación y el Desarrollo (ARAID). Sanofi provided a research grant to support the trial, but had no role in the study design, data collection, analysis, and interpretation, report writing, or decision to submit for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2021.01.015.

References

[1] B.G. Nordestgaard, M.J. Chapman, S.E. Humphries, et al., Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society, Eur. Heart J. 34 (2013) 3478–3490a.
[2] F. Civeira, E. Ros, E. Jarauta, et al., Comparison of genetic versus clinical diagnosis in familial hypercholesterolemia, Am. J. Cardiol. 102 (2008) 1187–1193, 1193.e1.
[3] Anon, Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group, BMJ 303 (1991) 893–896.
[4] M.D. Di Taranto, C. Giacobbe, G. Fortunato, Familial hypercholesterolemia: a complex genetic disease with variable phenotypes, Eur. J. Med. Genet. (2019) 103831.
[5] S. Bertolini, L. Pisciotta, C. Rabacchi, et al., Spectrum of mutations and phenotypic expression in patients with autosomal dominant hypercholesterolemia identified in Italy, Atherosclerosis 227 (2013) 342–348.
[6] N.S. Abul-Husn, K. Manickam, L.K. Jones, et al., Genetic identification of familial hypercholesterolemia within a single U.S. health care system, Science 354 (2016).
[7] M. Junyent, R. Gilabert, E. Jarauta, et al., Impact of low-density lipoprotein receptor mutational class on carotid atherosclerosis in patients with familial hypercholesterolemia, Atherosclerosis 208 (2010) 437–441.
[8] A. Cenarro, M. Artieda, S. Castillo, et al., A common variant in the ABCA1 gene is associated with a lower risk for premature coronary heart disease in familial hypercholesterolaemia, J. Med. Genet. 40 (2003) 163–168.
[9] N. Ohta, M. Hori, A. Takahashi, et al., Proprotein convertase subtilisin/kexin 9 V4I variant with LDLR mutations modifies the phenotype of familial hypercholesterolemia, J Clin Lipidol 10 (2016) 547–555, e5.
[10] S. Perez-Calahorra, M. Laclaustra, V. Marco-Benedí, et al., Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia, Atherosclerosis 284 (2019) 245–252.
[11] W. Reik, The Wellcome Prize Lecture. Genetic imprinting: the battle of the sexes rages on, Exp. Physiol. 81 (1996) 161–172.
[12] W. Palinski, E. Nicolaides, A. Liguori, C. Napoli, Influence of maternal dysmetabolic conditions during pregnancy on cardiovascular disease, J Cardiovasc Transl Res 2 (2009) 277–285.
[13] C. Allard, V. Desgagné, J. Patenaude, et al., Mendelian randomization supports causality between maternal hyperglycemia and epigenetic regulation of leptin gene in newborns, Epigenetics 10 (2015) 342–351.
[14] W. Palinski, T. Yamashita, S. Freigang, C. Napoli, Developmental programming: maternal hypercholesterolemia and immunity influence susceptibility to atherosclerosis, Nutr. Rev. 65 (2007) S182–S187.
[15] A. van der Graaf, M.N. Vissers, D. Gaudet, et al., Dyslipidemia of mothers with familial hypercholesterolemia deteriorates lipids in adult offspring, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 2673–2677.

[16] J. Versmissen, I.P.G. Botden, R. Huijgen, et al., Maternal inheritance of familial hypercholesterolemia caused by the V408M low-density lipoprotein receptor mutation increases mortality, Atherosclerosis 219 (2011) 690–693.
[17] D. Zhou, Y.-X. Pan, Pathophysiological basis for compromised health beyond generations: role of maternal high-fat diet and low-grade chronic inflammation, J. Nutr. Biochem. 26 (2015) 1–8.
[18] D.M. Cerqueira, S.L. Hemker, A.J. Bodnar, et al., In utero exposure to maternal diabetes impairs nephron progenitor differentiation, Am. J. Physiol. Ren. Physiol. 317 (2019) F1318–F1330.
[19] F.E. Alkemade, A.C. Gittenberger-de Groot, A.E. Schiel, et al., Intrauterine exposure to maternal atherosclerotic risk factors increases the susceptibility to atherosclerosis in adult life, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 2228–2235.
[20] C. Napoli, C.K. Glass, J.L. Witztum, R. Deutsch, F.P. D'Armiento, W. Palinski, Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: fate of Early Lesions in Children (FELIC) study, Lancet 354 (1999) 1234–1241.
[21] C. Napoli, F.P. D'Armiento, F.P. Mancini, et al., Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions, J. Clin. Invest. 100 (1997) 2680–2690.
[22] A.L. Amundsen, J. Khoury, P.O. Iversen, et al., Marked changes in plasma lipids and lipoproteins during pregnancy in women with familial hypercholesterolemia, Atherosclerosis 189 (2006) 451–457.
[23] S. Pérez-Calahorra, R.M. Sánchez-Hernández, N. Plana, P. Valdivielso, F. Civeira, National dyslipidemia registry of the Spanish arteriosclerosis society: current status, Clín. Invest. Arterioscler. 29 (2017) 248–253.
[24] D.M. Kusters, H.J. Avis, M.J. Braamskamp, et al., Inheritance pattern of familial hypercholesterolemia and markers of cardiovascular risk, J. Lipid Res. 54 (2013) 2543–2549.
[25] S. Tonstad, O. Joakimsen, T.P. Leren, L. Ose, Does maternal or paternal heredity affect carotid atherosclerosis in children with familial hypercholesterolaemia? Acta Paediatr. 89 (2000) 1490–1492.
[26] C. Napoli, J.L. Witztum, F. Calara, F. de Nigris, W. Palinski, Maternal hypercholesterolemia enhances atherogenesis in normocholesterolemic rabbits, which is inhibited by antioxidant or lipid-lowering intervention during pregnancy: an experimental model of atherogenic mechanisms in human fetuses, Circ. Res. 87 (2000) 946–952.
[27] C. Napoli, F. de Nigris, J.S. Welch, et al., Maternal hypercholesterolemia during pregnancy promotes early atherogenesis in LDL receptor-deficient mice and alters aortic gene expression determined by microarray, Circulation 105 (2002) 1360–1367.
[28] P. Brizzi, G. Tonolo, F. Esposito, et al., Lipoprotein metabolism during normal pregnancy, Am. J. Obstet. Gynecol. 181 (1999) 430–434.
[29] M. Ethier-Chiasson, A. Duchesne, J.-C. Forest, et al., Influence of maternal lipid profile on placental protein expression of LDLr and SR-BI, Biochem. Biophys. Res. Commun. 359 (2007) 8–14.
[30] B. Fuenzalida, C. Cantin, S. Kallol, et al., Cholesterol uptake and efflux are impaired in human trophoblast cells from pregnancies with maternal supraphysiological hypercholesterolemia, Sci. Rep. 10 (2020) 5264.
[31] C. Cantin, B. Fuenzalida, A. Leiva, Maternal hypercholesterolemia during pregnancy: potential modulation of cholesterol transport through the human placenta and lipoprotein profile in maternal and neonatal circulation, Placenta 94 (2020) 26–33.
[32] L.A. Woollett, Maternal cholesterol in fetal development: transport of cholesterol from the maternal to the fetal circulation, Am. J. Clin. Nutr. 82 (2005) 1155–1161.
[33] M. Furuhashi, H. Seo, S. Mizutani, O. Narita, Y. Tomoda, N. Matsui, Expression of low density lipoprotein receptor gene in human placenta during pregnancy, Mol. Endocrinol. 3 (1989) 1252–1256.
[34] C. Wadsack, S. Tabano, A. Maier, et al., Intrauterine growth restriction is associated with alterations in placental lipoprotein receptors and maternal lipoprotein composition, Am. J. Physiol. Endocrinol. Metab. 292 (2007) E476–E484.
[35] J. Stefulj, U. Panzenboeck, T. Becker, et al., Human endothelial cells of the placental barrier efficiently deliver cholesterol to the fetal circulation via ABCA1 and ABCG1, Circ. Res. 104 (2009) 600–608.
[36] H.V. Tran, M.E. Waring, D.D. McManus, et al., Underuse of effective cardiac medications among women, middle-aged adults, and racial/ethnic minorities with coronary artery disease (from the national health and nutrition examination survey 2005 to 2014), Am. J. Cardiol. 120 (2017) 1223–1229.
[37] A. Sabbag, S. Matetzky, A. Porter, et al., Sex differences in the management and 5-year outcome of young patients (<55 Years) with acute coronary syndromes, Am. J. Med. 130 (2017) 1324.e15–1324.e22.
[38] Q. Ngo-Metzger, S. Zuvekas, P. Shafer, H. Tracer, A.E. Borsky, A.S. Bierman, Statin use in the U.S. For secondary prevention of cardiovascular disease remains suboptimal, J. Am. Board Fam. Med. 32 (2019) 807–817.
[39] S.M. Grundy, N.J. Stone, A.L. Bailey, et al., AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: executive summary: a report of the American College of cardiology/American heart association task force on clinical practice guidelines, 2019, Circulation 139 (2018) e1046–e1081.

Atherosclerosis xxx (xxxx) xxx

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Lipoprotein(a) in hereditary hypercholesterolemia: Influence of the genetic cause, defective gene and type of mutation

Victoria Marco-Benedí [a,b], Ana Cenarro [a], Martín Laclaustra [a,b,**], Asier Larrea-Sebal [c,d], Estíbaliz Jarauta [a,b], Itziar Lamiquiz-Moneo [a,b], Pilar Calmarza [a], Ana M. Bea [a], Núria Plana [e], Xavier Pintó [f], César Martín [c,d], Fernando Civeira [a,b,*]

[a] Hospital Universitario Miguel Servet, IIS Aragón, CIBERCV, Zaragoza, Spain
[b] Department of Medicine, Psychiatry and Dermatology, Universidad de Zaragoza, Zaragoza, Spain
[c] Fundación Biofisika Bizkaia, Leioa, Spain
[d] Biofisika Institute (UPV/EHU, CSIC), Leioa, Spain, Department of Biochemistry and Molecular Biology, Universidad del País Vasco UPV/EHU, Bilbao, Spain
[e] Unitat de Medicina Vascular i Metabolisme (UVASMET) Hospital Universitari Sant Joan, IISPV, CIBERDEM, Universitat Rovira i Virgili, Reus, Tarragona, Spain
[f] Unidad de Lípidos, Servicio de Medicina Interna, Hospital Universitario de Bellvitge-Idibell, Universidad de Barcelona, CiberObn, Barcelona, Spain

ARTICLE INFO

Keywords:
Lipoprotein(a)
Heterozygous familial hypercholesterolemia
Polygenic hypercholesterolemia
Low-density lipoprotein receptor
Apolipoprotein B
Polygenic
Score

ABSTRACT

Background and aims: Lipoprotein(a) [Lp(a)] concentration in heterozygous familial hypercholesterolemia (heFH) is not well established. Whether the genetic defect responsible for heFH plays a role in Lp(a) concentration is unknown.

We aimed to compare Lp(a) in controls from a healthy population, in genetically diagnosed heFH and mutation-negative hypercholesterolemia subjects, and to assess the influence on Lp(a) of the genetic defect responsible for heFH.

Methods: We conducted a cross-sectional study, performed in a lipid clinic in Spain. We studied adults with suspected heFH and a genetic study of FH genes (*LDLR*, *APOB*, *APOE* and *PCSK9*) and controls from de Aragon Workers' Health Study. HeFH patients from the Dyslipidemia Registry of the Spanish Atherosclerosis Society (SEA) were used as validation cohort.

Results: Adjusted geometric means (95% confidence interval) of Lp(a) in controls (n = 1059), heFH (n = 500), and mutation-negative subjects (n = 860) were 14.9 mg/dL (13.6, 16.4), 21.9 mg/dL (18.1, 25.6) and 37.4 mg/dL (33.3, 42.1), $p < 0.001$ in all comparisons. Among heFH subjects, *APOB*-dependent FH showed the highest Lp(a), 36.5 mg/dL (22.0, 60.8), followed by *LDLR*-dependent FH, 21.7 mg/dL (17.9, 26.4).

These differences were also observed in heFH from the SEA cohort. The number of plasminogen-like kringle IV type−2 repeats of *LPA*, the hypercholesterolemia polygenic score or LDLc concentration did not explain these differences. In *LDLR*-dependent FH, Lp(a) levels were not different depending on the affected protein domain.

Conclusions: Lp(a) is elevated in mutation-negative subjects and in heFH. The concentration of Lp(a) in heFH varies in relation to the responsible gene. Higher Lp(a) in heFH is not explained by their higher LDLc.

1. Introduction

Lipoprotein(a) [Lp(a)] is a variant of low-density lipoproteins (LDL) [1] due to an additional glycoprotein, called apolipoprotein(a) [apo(a)], covalently linked to apolipoprotein B100 (apoB) [2,3]. Apo(a) is encoded by the *LPA* gene, evolutionarily derived from the plasminogen gene. Apo(a) contains 10 different subtypes of plasminogen kringle IV (KIV), 1 copy of kringle V and an inactive protease domain [4]. Apo(a) isoform size varies, depending on the number of KIV type 2 copies (from 1 to 40) and it is inversely related to plasma Lp(a) concentration [5]. About 25% of subjects have Lp(a) concentration >50 mg/dL, a figure considered clinically relevant, increasing cardiovascular risk [5,6]. *LPA* gene determines over 90% of variability in the Lp(a) plasma levels, which suffer little influence of environmental factors, including diet [7].A high Lp(a)

* Corresponding author. Unidad de Lípidos, Hospital Universitario Miguel Servet, Avda Isabel La Catolica 1-3, 50009, Zaragoza, Spain.
** Corresponding author. Unidad de Lípidos, Hospital Universitario Miguel Servet, Avda Isabel La Católica 1-3, 50009, Zaragoza, Spain.
E-mail addresses: Martin.Laclaustra@unizar.es (M. Laclaustra), civeira@unizar.es (F. Civeira).

https://doi.org/10.1016/j.atherosclerosis.2021.08.009
Received 20 May 2021; Received in revised form 27 July 2021; Accepted 5 August 2021
Available online 23 August 2021

concentration is an independent risk factor for cardiovascular disease (CVD), including heterozygous familial hypercholesterolemia (heFH) [6,8,9]. Epidemiological, Mendelian randomization, genome-wide association and, very recently, intervention studies with PCSK9 inhibitors have shown a linear and positive relationship between Lp(a) concentration and the risk of myocardial infarction and ischemic stroke [2, 9–11]. In addition, aortic valve stenosis increases in subjects with high Lp(a) concentrations [11,12]. However, the mechanism responsible for the proatherogenic effect of Lp(a) is mostly unknown [2,13]. Actually, many other aspects of the physiopathology of Lp(a) are poorly understood, [1,2,14]. The LDL receptor is the main receptor responsible for the LDL catabolism, but its involvement in Lp(a) catabolism is controversial. Homozygous FH patients carrying two null *LDLR* alleles have a two-fold higher Lp(a) concentration than non-FH family members, with a clear gene-dosage effect [15], while studies in heterozygous familial hypercholesterolemia (heFH) subjects have shown inconclusive results. However, other *in vitro* and *in vivo* studies have discarded the LDL receptor as a significant pathway for Lp(a) catabolism [16]. Clinical studies are not entirely consistent, and they suffer from relevant limitation biases, mainly heterogeneity in the criteria used for FH diagnosis, the population studied, the genes responsible for the disease, or the protein domain affected by the genetic defect.

The objective of the present study is to compare the Lp(a) concentration in controls drawn from a healthy population with subjects with different genetic hypercholesterolemias to explore whether the concentration of LDLc, the defective gene involved, and the domain of the

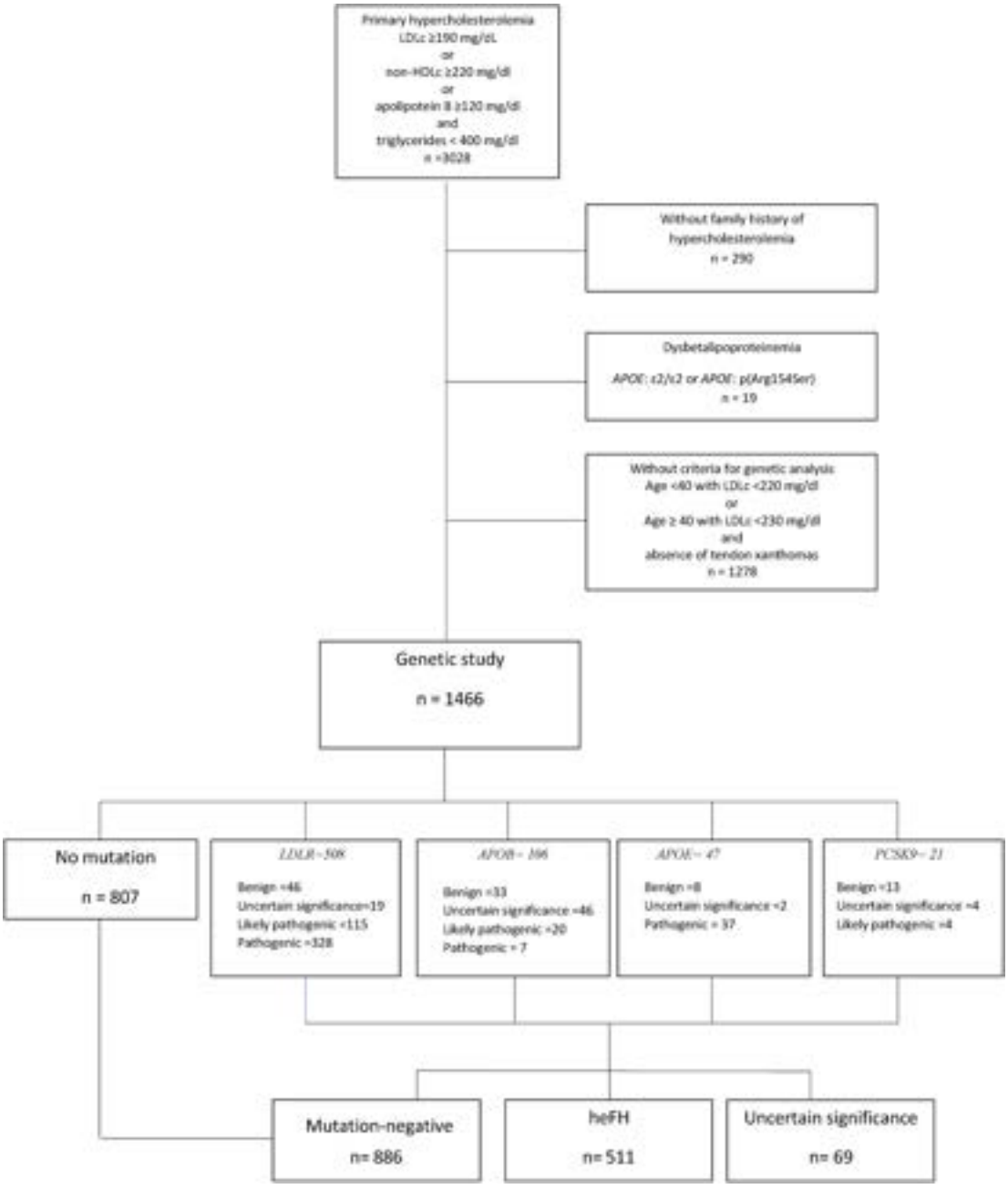


Fig. 1. Study flow diagram detailing the number of participants who were screened, analyzed and classified according to the genetic analysis.
Multiple mutations are possible in the same subject. LDLc, low-density lipoprotein cholesterol; HDLc, high-density lipoprotein cholesterol; AWHS, Aragon Workers' Health Study; heFH, heterozygous familial hypercholesterolemia; uncertain, patients with a mutation of uncertain functional significance. *LDLR, APOB, APOE, PCSK9,* genes responsible for FH. More than one mutation benign and/or of uncertain significance coincided in a single subject.

protein where the defect occurs are associated with Lp(a) concentration.

2. Patients and methods

2.1. Subjects with hereditary hypercholesterolemia

Patients are referred to the Lipid Unit at Hospital Universitario Miguel Servet, Zaragoza, for the study of their hypercholesterolemia by their primary care physicians. All patients referred from January 2006 to March 2020, ≥18 years, with the clinical diagnosis of primary hypercholesterolemia, with suspected heFH, and with complete genetic study of the responsible genes for FH, were included. The protocol for referral to our Unit is based on previously agreed criteria and includes total cholesterol concentrations >300 mg/dL once secondary causes have been excluded.

Primary hypercholesterolemia was defined when LDLc ≥190 mg/dL or non- HDLc ≥220 mg/dL or apoB ≥120 mg/dL and triglycerides <400 mg/dL in absence of secondary causes of hypercholesterolemia: body mass index >35 kg/m^2, thyroid-stimulating hormone >6 mU/L, creatinine >2.0 mg/dL, poorly controlled diabetes (HbA1c >7.5%), cholestasis (direct bilirubin >1 mg/dL)) or use of drugs that promote disorders of lipid metabolism. Patients with suspicion of genetic hypercholesterolemia included all subjects with severe primary hypercholesterolemia (LDLc ≥220 mg/dL if age <40 years or ≥230 mg/dL if age ≥40 years), vertical transmission of hypercholesterolemia in the family, and LDLc >95th percentile in at least one first-degree relative (Fig. 1). The results of the genetic analysis (detailed below) allowed classifying the patients in heFH (those with "pathogenic" and "likely pathogenic" mutations in canonical FH genes (N = 511), patients with a mutation of uncertain significance (N = 69), and patients mutation-negative (those without functional mutation in genes with a known role in FH) (N = 886) (Fig. 1).

2.2. Controls

Controls were selected from the Aragon Workers' Health Study (AWHS). The AWHS is a longitudinal cohort study of cardiovascular risk factors and subclinical atherosclerosis in patients followed since 2009 to March 2020 [17]. Lp(a) was determined at baseline in a random subset of participants. All individuals ≥18 years from AWHS, with Lp(a) determination were included as controls (N = 1221).

All participants signed an informed consent, approved by the Aragón Clinical Research Ethics Committee before being included in the study.

2.3. Clinical data

Personal history of diabetes, hypertension, smoking, cardiovascular disease, and drugs treatment, family history of hypercholesterolemia and cardiovascular disease was collected in cases and controls. During the same visit, a physical examination including height, weight, waist circumference, and the presence of corneal arcus and tendon xanthomas was completed.

2.3.1. Laboratory measurements

A blood sample was obtained after 10 h of fasting and after the withdrawal of any lipid lowering treatment for at least 5 weeks except for those subjects with a personal history of cardiovascular disease or very high CVD risk. No patient was being treated with PCSK9 inhibitors, a treatment that could decrease Lp(a) concentration.

Total cholesterol, triglycerides, HDL cholesterol (HDLc), Lp(a), apo A1 and B, glucose, uric acid, creatinine, and liver and muscle enzymes levels were determined in cases and controls. Values of LDLc were calculated with the Friedewald equation and LDLc corrected by Lp(a) was calculated by subtracting 1/3 of the concentration of Lp(a) from the LDLc value [18]. All biochemical measurements were performed in a central laboratory as previously described [19]. Lipid values were adjusted according to statin therapy in those participants taking lipid-lowering drugs [20].

Lp(a) concentration was measured in fresh plasma samples, during the recruitment period, by rate nephelometry using LPAX reagent in conjunction with IMMAGE Immunochemistry Systems following manufacturer instructions. Samples were analyzed the same day of sampling. Four quality control samples were used daily with a coefficient of variation <12% in all cases. Lp(a) results below the detection threshold were imputed to 0.5 mg/dL, half of that threshold.

Samples from subjects included in this study were provided by the Biobank of the Aragon Health System (PT17/0015/0039) with the appropriate approval of the Ethics and Scientific Committees.

2.3.2. Genetic study

In all subjects with a clinical suspicion of FH, *LDLR* (NM_000527.4), *APOB* (NM_000384.2), and *PCSK9* (NM_174936.3) genes were studied with the Progenika Biopharma Grifols (Derio, Spain) [21] or GEN inCode (Terrassa-Barcelona, Spain) [22] platforms, as previously described. These platforms include point mutations, large rearrangements and copy number variations. Besides, all subjects underwent sequencing of exon 4 of *APOE* (NM_000041.4)), since it has been described as a cause of FH [23]. The *LPA* (NM_005577.4) genetic polymorphism responsible for the Lp(a) size variability was analyzed with a real-time PCR-based methodology. Briefly, a multiplexed qPCR was carried out using TaqMan probes for LPA KIV2 (exon 4) and an endogenous single-copy control gene, RNase P [24]. The result is the mean LPA KIV2 of the two LPA alleles.

2.4. Functionality of the mutations

Genetic variants in *LDLR*, *APOB* and *PSCK9* genes were classified as "pathogenic", "likely pathogenic", "uncertain significance", "likely benign", and "benign" according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) [25].

2.5. LDL receptor defective domains

Mutations were classified according to the affected domain and gene location (exon, intron or UTR). Briefly, mutations on *LDLR* were divided into exonic, intronic and UTR and additionally, the exonic mutations were also classified in their corresponding protein domains: Signal sequence, Ligand Binding Domain (LBD), Epidermal Growth Factor A-precursor homology domain (EGF)-A, EGF-B, β-propeller, EGF-C, O-linked domain, transmembrane domain and cytosolic domain. *APOB* mutants were divided into exonic, intronic and UTR categories. PCSK9 mutants were divided in protein domains: Signal sequence, prodomain, catalytic domain, and C-terminal domain.

2.6. Polygenic score

The polygenic score (PS) was based on 12 common single nucleotide variations (SNVs) (Supplementary Table 1) identified as LDLc-raising from genome wide association consortium studies from European-Caucasian populations [26]

2.6.1. Validation cohort

All unrelated subjects from the Dyslipidemia Registry of the Spanish Atherosclerosis Society (SEA), excluding those subjects from Hospital Universitario Miguel Servet, with the same inclusion and exclusion criteria that the main study group were analyzed as validation cohort. The Dyslipidemia Registry of the SEA is an active online registry, in which certified lipid clinics across all regions of Spain report cases of various types of primary hyperlipidemias [27]. Subjects were defined with the same criteria. Written informed consent was obtained from each patient included in the registry.

2.7. Statistical analysis

Variables were summarized as mean (standard deviation), median (interquartile range), or percentage. Unadjusted differences between groups were tested with the Kruskal- Wallis Rank Sum Test or Chi-squared Test as appropriate. Linear regression models adjusted for age, sex, and BMI were used to model plasma lipids. Lp(a), kringle repeats, and triglycerides were logarithmically-transformed for the regressions. These analyses were restricted to complete-variable cases for the main variables and adjustment variables. Test for relevance of the groups variables were performed with likelihood ratio tests (test for heterogeneity among groups). For Lp(a) for which these tests were highly significant, post-hoc pairwise tests were performed for appropriate line combinations of the regression coefficients. An additional regression model was created to study interaction of LDLc and group (controls *vs* FH) on Lp(a).

3. Results

3.1. Clinical characteristics of hypercholesterolemic patients and controls

The study included 1466 subjects with hereditary hypercholesterolemia referred for genetic study to the lipid clinic and 1221 control subjects from the AWHS study. After the genetic study, those subjects with hypercholesterolemia were classified in three groups: heFH with a pathogenic mutation (n = 511); subjects with a mutation, but with functional implications of uncertain significance (n = 69); and subjects without any identified mutation in any of the FH genes (n = 886) (Fig. 1). As expected, hypercholesterolemia groups had higher concentrations of total cholesterol, LDLc, apoB, and HDLc, whereas BMI was significantly lower than that of the control group, according to inclusion criteria for primary hypercholesterolemia. The main clinical characteristics of the different groups are presented in Supplementary Table 2 for the whole group and including only probands in Supplementary Table 3. The differences in lipid variables were statistically significant after adjusting for age, sex, and BMI (Table 1, groups restricted to complete-variable cases, see Supplementary Table 4). Regarding Lp(a) concentration, mutation-negative subjects had a significantly higher concentration than heFH, and both higher than the control group (Fig. 2A). The percentage of subjects with Lp(a) concentration ≥50 mg/dL in heFH with a pathogenic mutation, subjects with a mutation, but with functional implications of uncertain significance, and in mutation-negative subjects were 31.1%, 43.5%, and 52.3% respectively. These percentages were all significantly higher than in controls (23.1%), ($p < 0.01$) (Supplementary Table 5).

3.2. Lp(a) concentration in FH according to the responsible gene

The etiology of the 511 heFH, according to the responsible gene was: *LDLR* 443 subjects, *APOB* 27 subjects, carriers of the p.(Leu167del) mutation in *APOE* 37 subjects, and *PCSK9* 4 subjects. The complete list of mutations is presented in Supplementary Table 6. Lipids differed across gene groups after adjusting for age, sex, and BMI (Table 2, groups restricted to complete-variable cases). Lp(a) differed among subjects with *LDLR*, *APOB* and *APOE* mutation ($p < 0.001$). Age-, sex-, and BMI-adjusted geometric mean Lp(a) concentration was greatest in *APOB*-dependent FH, 36.5 mg/dL (95%CI 22.0, 60.8), intermediate in *LDLR*-dependent FH, 21.7 mg/dL (95%CI 17.9, 26.4) and lowest in carriers of the p.(Leu167del) mutation in *APOE,* 7.99 mg/dL (95%CI 4.9, 12.7). All Lp(a) differences between groups with a reasonable number of cases (all except mutations in *PCSK9* gene, N = 4), were statistically significant pairwise (Fig. 2B). The geometric mean of *LPA* KIV-2 repeats did not differ among the FH gene subgroups and the estimations and differences for Lp(a) remained unchanged after adjustment for the number of KIV-2 repeats.

3.3. Effect of the LDLR mutations on Lp(a) concentration according to the protein domain affected in the LDL receptor

The location of *LDLR* mutations in the 443 subjects is showed in Supplementary Table 6. There were no differences in Lp(a) concentration among groups, neither after grouping the mutations in 4 major groups: null alleles, ligand binding domain, beta-propeller + EGF, and splicing mutations; nor 2 groups: defective alleles vs null defects (Supplementary Table 7).

3.4. Association of Lp(a) concentration with LDLc

There was a positive association between LDLc and Lp(a) concentration (Fig. 2C). However, this association was more intense in the control (AWHS) group than in heFH, with a highly significant difference in slopes between groups ($p = 0.006$). At the same LDLc concentration (e.g. 200 mg/dL), estimated Lp(a) was significantly lower in heHF than in control subjects ($p = 0.025$). A similar pattern was observed for apoB, although the slope did not reach statistical significance ($p = 0.08$) (Fig. 2D).

3.5. Effect of the polygenic score

The PS was performed on a subset of 216 subjects corresponding to all consecutive subjects studied from January 2018: 137 in the mutation-negative group, 32 with a FH gene mutation of uncertain significance and 47 subjects with heFH. The clinical and biochemical characteristics were similar to those of the main group. (Supplementary Table 8). The differences in Lp(a) remained around 15 mg/dL higher in the mutation-negative group compared to heFH, 50.4 mg/dL (IQR 19.0, 119.0) *vs* 36.5 mg/dL (IQR 7.94, 58.4), respectively ($p = 0.071$). The PS was significantly higher in mutation-negative subjects than in subjects with heHF (Supplementary Table 8). When the mutation-negative group was divided by tertiles of PS, the subjects in the highest tertile had a non-

Table 1
Laboratory lipid characteristics of study groups adjusted for gender, age, and body mass index.

	AWHS N = 1059	Heterozygous familial hypercholesterolemia N = 500	Uncertain significance N = 66	Mutation-negative N = 860	*p*
Total cholesterol (mg/dL)	218 (215, 222)	362 (357, 368)	329 (317, 341)	309 (305, 313)	<0.001
Triglycerides (mg/dL)	122 (118, 126)	131 (124, 139)	152 (134, 173)	151 (145, 158)	<0.001
LDL cholesterol (mg/dL)	137 (134, 140)	287 (282, 292)	245 (234, 256)	224 (220, 227)	<0.001
HDL cholesterol (mg/dL)	54.1 (53.2, 55.0)	46.1 (44.7, 47.6)	51.0 (47.7, 54.3)	50.7 (49.6, 51.8)	<0.001
Lp(a) (mg/dL)	14.9 (13.6, 16.4)	21.9 (18.7, 25.6)	30.3 (21.2, 43.4)	37.4 (33.3, 42.1)	<0.001
Apolipoprotein B (mg/dL)	101 (98, 104)	186 (181, 190)	176 (165, 186)	167 (163, 170)	<0.001

Means and 95%CI for each group conditionally adjusted for men, 48.9 years with BMI 27.2. Lp(a) and triglycerides are geometric means (the logarithmically-transformed variable was modeled in the regressions). Adjusted tests were performed to calculate *p* for heterogeneity between groups. All estimations and tests are calculated from linear regression models adjusted for age, sex, and BMI, using LRTs and linear combination of coefficients as appropriate.
AWHS, Aragon worker's health study; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Lp(a), lipoprotein(a).

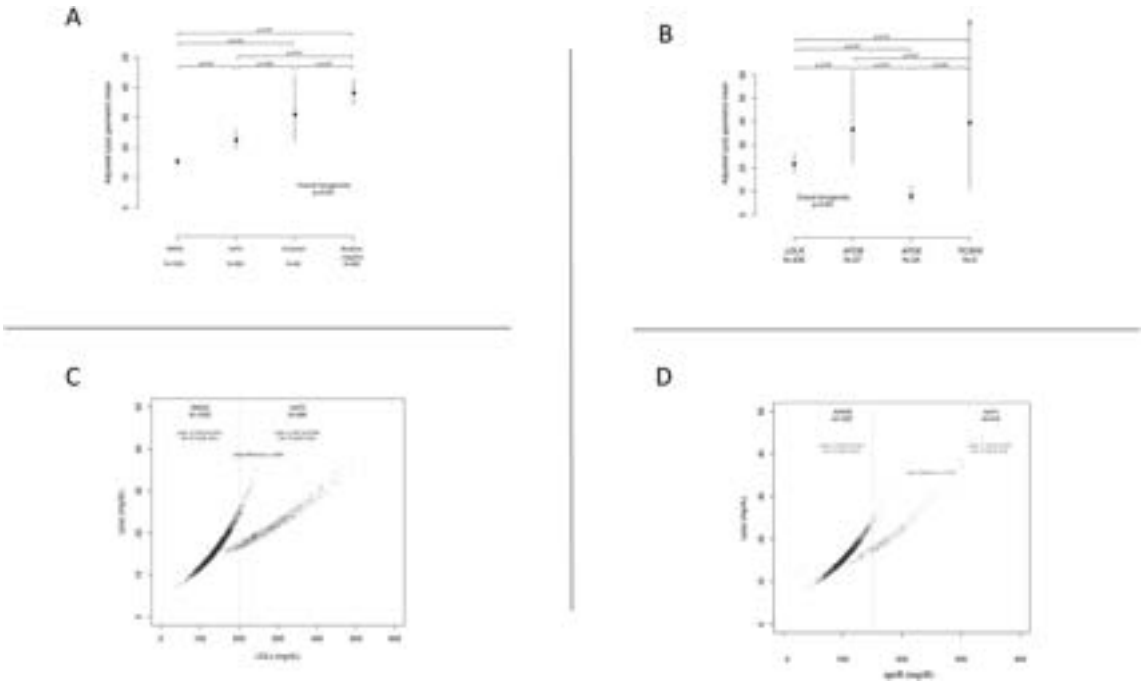


Fig. 2. (A) Adjusted Lp(a) geometric means (95% CI) across study groups. Logarithm of Lp(a) was modeled with linear regression and predictions are conditionally adjusted for men, 48.9 years with BMI 27.2 kg/m^2. All estimations and tests are calculated from linear regression models adjusted for age, sex, and BMI, using LRTs and linear combination of coefficients as appropriate. (B) Adjusted Lp(a) geometric means (95% CI) in heFH according to the responsible gene. Logarithm of Lp(a) was modeled with linear regression and predictions are conditionally adjusted for men, 48.9 years with BMI 27.2 kg/m^2. All estimations and tests are calculated from linear regression models adjusted for age, sex, and BMI, using LRTs and linear combination of coefficients as appropriate. (C) Adjusted Lp(a) geometric mean association with LDLc in AWHS and heFH. Logarithm of Lp(a) was modeled with linear regression with an interaction term between LDLc and group (AWHS or heFH) and predictions are conditionally adjusted for men, 48.9 years with BMI 27.2 kg/m^2. All estimations and tests are calculated from linear regression models adjusted for age, sex, and BMI, using LRTs and linear combination of coefficients as appropriate. (D) Adjusted Lp(a) geometric mean association with apoB in AWHS and heFH. Logarithm of Lp(a) was modeled with linear regression with an interaction term between apoB and group (AWHS or heFH) and predictions are conditionally adjusted for men, 48.9 years with BMI 27.2 kg/m^2. All estimations and tests are calculated from linear regression models adjusted for age, sex, and BMI, using LRTs and linear combination of coefficients as appropriate. LDLc denotes low-density lipoprotein cholesterol; HDLc, high-density lipoprotein cholesterol; AWHS, Aragon Workers' Health Study; heFH, heterozygous familial hypercholesterolemia; Uncertain, patients with a mutation of uncertain functional significance. *LDLR, APOB, APOE, PCSK9,* genes responsible of FH.

significant tendency to higher concentrations of LDLc and apoB. However, the concentration of Lp(a) was homogeneously elevated in the three groups, with no differences among them (Supplementary Table 9).

3.6. Validation cohort

The hypercholesterolemic group from the Dyslipidemia Registry of the SEA was composed of 707 heFH with a pathogenic mutation; 74 patients with a mutation of uncertain significance; and 398 mutation-negative subjects (Table 3, groups restricted to complete-variable cases). Similar to what occurred in the main study group, mutation-negative subjects had significantly higher Lp(a) concentration than heFH, and both higher than the control group (Table 3 and Supplementary Fig. 1A). In this validation group, there were 671 heFH with a pathogenic mutation in *LDLR,* and 36 heFH with mutation in *APOB,* all with the p.(Arg3527Gln*)* mutation, usually named as APOB-3500. Adjusted Lp(a) geometric means were 22.2 mg/dL (95%CI 19.2, 25.6) and 34.9 mg/dL (95%CI 22.2, 55.0), respectively ($p = 0.045$) (Supplementary Table 10). and Supplementary Fig. 1B). The association between LDLc and with Lp(a) showed a similar pattern than the main cohort (Supplementary Fig. 1C). In this group, no *APOE* or *PCSK9* pathogenic mutations were reported.

4. Discussion

We describe Lp(a) concentrations in different types of hereditary hypercholesterolemias, including two large independent cohorts of patients with monogenic heFH and mutation-negative, some of them with demonstrated polygenic hypercholesterolemia.

We have also analyzed the impact of the gene responsible for FH on Lp(a) concentration, and the potential implication of the different protein domains affected by the gene defect. The highest Lp(a) concentration was observed in subjects with hereditary hypercholesterolemia but without a pathogenic mutation in FH genes independently of the hypercholesterolemia PS. FH subjects had a higher Lp(a) concentration than the general population but lower than mutation-negative subjects; and this increase of Lp(a) in heFH was related to the gene involved, but their differences are not explained by the number of plasminogen-like kringle IV type-2 of *LPA*. Higher Lp(a) in heFH is not explained by their higher LDLc and, in *LDLR*-dependent FH, Lp(a) levels are not different depending on domain of the LDL receptor protein affected.

Several studies have analyzed Lp(a) concentration in subjects with FH, obtaining a wide range of results: from higher values [28–32] to no difference regarding a control population [33–35]. Kraft et al. compared Lp(a) concentration in homozygous FH, heFH, and relatives without FH finding a dose-dependent effect on the Lp(a) concentration. Homozygous FH had higher concentration than heterozygote subjects and those, in turn, higher concentration than controls, without differences in the *LPA* genotype [36]. The same dose-effect was found by Sjouke et al. [31]. Our study agrees with the finding of higher Lp(a) concentrations in heFH. However, there are, at least, two important potential biases in the previously described association of Lp(a) and FH.

First, Lp(a) is an independent risk factor for CVD. In previous studies, the selection criteria for the diagnosis of FH included coronary heart

Table 2
Laboratory lipid characteristics of HeFH according to the gene causing defect adjusted for gender, age, and body mass index.

	LDLR N = 435	APOB N = 27	APOE N = 34	PCSK9 N = 4	*p*
Total cholesterol (mg/dL)	369 (360, 379)	359 (333, 385)	344 (320, 368)	322 (255, 389)	0.079
Triglycerides (mg/dL)	128 (119, 137)	128 (107, 155)	192 (162, 228)	132 (82, 212)	<0.001
LDL cholesterol (mg/dL)	294 (285, 304)	275 (250, 300)	260 (235, 285)	239 (175, 304)	0.007
HDL cholesterol (mg/dL)	46.2 (44.3, 48.1)	54.6 (49.7, 59.6)	53.8 (49.3, 58.4)	55.2 (42.6, 67.8)	<0.001
Lp(a) (mg/dL)	21.7 (17.9, 26.4)	36.5 (22.0, 60.8)	7.9 (4.9, 12.7)	39.3 (10.7, 144.1)	<0.001
LPA Kringle IV-2 repeats (N)*	22.8 (21.4, 24.3)	23.3 (20.3, 26.7)	25.7 (22.6, 29.2)	22.8 (16.8, 31.1)	0.299
Apolipoprotein B (mg/dL)	188 (182, 195)	187 (170, 204)	167 (151, 182)	160 (117, 204)	0.028

[b]Means and 95%CI for each group. Lipids parameters conditionally adjusted for men, 48.9 years with BMI 27.2. Lp(a), kringle repeats, and triglycerides are geometric means (the logarithmically-transformed variable was modeled in the regressions). Adjusted tests were performed to calculate *p* for heterogeneity between groups. All estimations and tests are calculated from linear regression models adjusted for age, sex, and BMI, using LRTs and linear combination of coefficients as appropriate. LDL, low-density lipoprotein; HDL, high-density lipoprotein; Lp(a), lipoprotein(a).
*LPA Kringle IV-2 repeats available for *LDLR* = 180; *APOB* = 17; *APOE* = 22; *PCKS9* = 3.

disease (CHD) and ischaemic heart disease [8,9], as it is set out in the Dutch Lipid Clinic Network Score or the Simon Broome criteria for FH [28–30], which implies an obvious bias towards higher Lp(a) that has recently been pointed out [32]. We have used diagnostic criteria for FH based on LDLc, and not on CHD. Actually, only 8% of our heFH subjects had CHD, a much lower percentage than previously published [37]. The second potential bias is the use of genetic analysis in the definition of heFH in some studies, while including genetic variants of uncertain significance [34,38], whereas other studies were only based on clinical criteria [28,29,39]. The present report encompasses all mutations in all recognized responsible genes of FH in all subjects, as well as considering their functionality. Moreover, we have excluded those subjects with an uncertain genetic diagnosis.

Lp(a) concentration plays an important role in determining LDLc concentration in the general concentration and in subjects with very high Lp(a) may be classified as heFH [30]. Due to the overlapping between FH and hypercholesterolemia that is secondary to high levels of Lp(a), it is important to precise the genetic diagnosis of FH to evaluate Lp(a) concentration in these patients. For the same reason, the measurement of Lp(a) is highly recommended in subjects with the clinical diagnosis of heFH [6] but also in subjects with the suspicion of polygenic hypercholesterolemia. One of the most remarkable results of our study is the different concentration of Lp(a) in FH according to the gene responsible for FH. Subjects with *APOB* mutations have higher concentrations of Lp(a) whereas subjects with *APOE* mutation have lower concentrations in comparison with *LDLR* mutant carriers, without differences or confusion by the number of KIV type 2 repetitions. Sjouke et al. studied Lp(a) concentrations in 13 heterozygous and 2 homozygous for *APOB* mutations, yielding median values of 50.3 mg/dL(IQR 18.7; 120.9) and 205.5 mg/dL, respectively, both values higher than those in control subjects [31]. In the same vein, Van der Kooek et al. demonstrated that the FDB status increases Lp(a) level and variability, and suggested that *APOB* may be a variability gene for Lp(a) levels in plasma [40]. The mechanism explaining apoB elevation in *APOB* dependent heFH is unknown. Knight et al. compared the apoB composition of LDL and Lp(a) particles in FDB heterozygous subjects. They showed that the proportions of the mutant and wild-type apoB were similar in Lp(a) particles indicating that, in heFH due to mutation in *APOB,* an increased synthesis is the probable mechanism involved in the increased Lp(a) rather than a decreased catabolism [41].

Another important finding was that carriers of the p.(Leu167del) *APOE* mutation had lower Lp(a) than other forms of FH. The genetic variation in *APOE* is involved in Lp(a) concentration: Moriarty et al. analyzed *APOE* common isoforms and demonstrated a 65% increase in Lp(a) in ϵ4/ϵ4 compared to ϵ2/ϵ2 subjects [18]. Recently, Croyal et al. have demonstrated the close association between VLDL-apoE particles and Lp(a) production [42]. Our group has recently described the higher hepatic uptake of VLDL particles containing p.(Leu167del) in *APOE* [23], hence we speculate that the probable mechanisms for the reduction in Lp(a) concentration associated with a decreased normal apoE containing VLDL may be a reduced Lp(a) production and/or assembly. The results of our study reinforce the fact that LDL receptor plays hardly any role in the catabolism of Lp(a). Several *in vitro* cell culture, mouse, and human studies have discarded a significant role for the LDL receptor in Lp(a) catabolism [1]. We demonstrate that Lp(a) concentration in heFH is independent of the type of LDL receptor defect, supporting that the receptor is not involved in Lp(a) catabolism. Several receptors for Lp (a) particle, other than LDL receptor, using either apoB, apo(a), or oxidized phospholipids (OxPL) as ligands have been demonstrated [1], and whether they could be involved in the increased Lp(a) concentration in heFH cannot be discarded.

4.1. Study limitations

It is an observational, retrospective study, carried out in a single center, with a limited number of patients with mutations other than *LDLR,* especially in *APOE.* The control population was mainly male, and the *LPA* genotyping was limited to the number of KIV type 2 and it was

Table 3
Laboratory lipid characteristics of the control group and the validation cohort from the Dyslipemia Registry of the Spanish Atherosclerosis Society adjusted for gender, age, and body mass index.

	AWHS N = 1059	Heterozygous familial hypercholesterolemia N = 707	Uncertain significance N = 74	Mutation-negative N = 398	*p*
Total cholesterol (mg/dL)	220 (216, 225)	361 (355, 368)	365 (349, 381)	355 (347, 363)	<0.001
Triglycerides (mg/dL)	126 (122, 130)	124 (118, 129)	133 (118, 150)	150 (142, 158)	<0.001
LDL cholesterol (mg/dL)	138 (133, 142)	284 (277, 290)	285 (270, 301)	270 (263, 277)	<0.001
HDL cholesterol (mg/dL)	54.0 (53.1, 54.8)	49.4 (48.2, 50.6)	48.6 (45.6, 51.6)	50.6 (49.2, 52.0)	<0.001
Lp(a) (mg/dL)	14.6 (13.4, 16.1)	22.2 (19.4, 25.3)	21.3 (15.3, 29.7)	27.8 (23.8, 32.6)	<0.001

[c]Means and 95%CI for each group conditionally adjusted for men, 49.4 years with BMI 27.6. Lp(a) and triglycerides are geometric means (the logarithmically-transformed variable was modeled in the regressions). Adjusted tests were performed to calculate *p* for heterogeneity between groups. All estimations and tests are calculated from linear regression models adjusted for age, sex, and BMI, using LRTs and linear combination of coefficients as appropriate.
AWH, Aragon worker's health study; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Lp(a), lipoprotein(a).

not available in all subjects, although those with the measurement were randomly selected. The quantification method of Lp(a) in our study is not totally independent of apo(a) isoform size and could have influenced the results. Not all study subjects underwent the PS analysis; nevertheless, it was obtained in a representative subgroup without showing any relationship between the concentration of Lp(a) and the PS. Two thirds of the mutation-negative subjects had a PS above 0.93, which has been used as a criterion for the diagnosis of PH, in the absence of mutations in the FH genes [43]. The manner of selecting patients based on elevated LDLc may enrich for those subjects with high Lp(a), which contributed to calculated LDLc [33]. We cannot fully exclude this selection bias although seems unlikely because the minor contribution of cholesterol transported in Lp(a) to total LDLc in heFH in our study. The method used to analyze the number of LPA KIV-2 repeats averages the number of repeats of the two alleles, but this average does not take into account that the effect on Lp(a) concentration may be different between alleles. Finally, our results are representative of the Spanish population with a Caucasian genetic background.

Luckily, our work has also several strengths and main differences with respect previous work. All subjects have been genetically analyzed, only subjects with clearly pathogenic mutations have been included, the effect on the Lp(a) concentration is analyzed according to the responsible gene and in the case of LDLR the domain of the affected protein and finally the selection of the HF is not carried out based on cardiovascular disease. Furthermore, results have validated in an independent cohort.

In conclusion, our results show that Lp(a) is elevated in FH mutation-negative hypercholesterolemia, PH and heFH; that the concentration of Lp(a) in heFH is variable depending on the FH-responsible gene; and, that their differences are not explained by the number of plasminogen-like kringle IV type-2 of *LPA* or the hypercholesterolemia PS. Higher Lp(a) in heFH is not explained by their higher LDLc, and in *LDLR*-dependent FH, Lp(a) levels are not different depending on the affected protein domain.

Financial support

This study was supported by grants from the Spanish Ministry of Economy and Competitiveness PI 18/01777, PI 19/00694 and CIBERCV; and Gobierno de Aragón B-14. These projects are co-financed by Instituto de Salud Carlos III and the European Regional Development Fund (ERDF) of the European Union "A way to make Europe". Sanofi provided a research grant to support the Dyslipemia Registry of the Spanish Atherosclerosis Society, but had no role in the study design, data collection, analysis, and interpretation, report writing, or decision to submit for publication.

CRediT authorship contribution statement

Victoria Marco-Benedí: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Ana Cenarro:** Methodology, Investigation, Resources, Writing – review & editing. **Martín Laclaustra:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Asier Larrea-Sebal:** Investigation, Resources, Writing – review & editing. **Estíbaliz Jarauta:** Investigation, Resources, Writing – review & editing. **Itziar Lamiquiz-Moneo:** Investigation, Resources, Writing – review & editing. **Pilar Calmarza:** Investigation, Resources, Writing – review & editing. **Ana M. Bea:** Investigation, Resources, Writing – review & editing. **Núria Plana:** Validation, Investigation, Resources, Writing – review & editing. **Xavier Pintó:** Validation, Investigation, Resources, Writing – review & editing. **César Martín:** Methodology, Investigation, Resources, Writing – review & editing. **Fernando Civeira:** Term, Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by grants from the Spanish Ministry of Economy and Competitiveness PI 18/01777, PI 19/00694 and CIBERCV; and Gobierno de Aragón B-14. These projects are co-financed by Instituto de Salud Carlos III and the European Regional Development Fund (ERDF) of the European Union "A way to make Europe". Sanofi provided a research grant to support the Dyslipemia Registry of the Spanish Atherosclerosis Society, but had no role in the study design, data collection, analysis, and interpretation, report writing, or decision to submit for publication. We want to particularly acknowledge the patients and the Biobank of the Aragon Health System (PT17/0015/0039) integrated in the Spanish National Biobanks Network for their collaboration.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2021.08.009.

References

[1] S.P.A. McCormick, W.J. Schneider, Lipoprotein(a) catabolism: a case of multiple receptors, Pathology 51 (2019) 155–164.
[2] S. Tsimikas, A test in context: lipoprotein(a): diagnosis, prognosis, controversies, and emerging therapies, J. Am. Coll. Cardiol. 69 (2017) 692–711.
[3] S.M. Marcovina, M.L. Koschinsky, Lipoprotein(a) as a risk factor for coronary artery disease, Am. J. Cardiol. 82 (1998) 57U–66U, discussion 86U.
[4] K. Schmidt, A. Noureen, F. Kronenberg, G. Utermann, Structure, function, and genetics of lipoprotein (a), J. Lipid Res. 57 (2016) 1339–1359.
[5] S.M. Marcovina, J.J. Albers, Lipoprotein (a) measurements for clinical application, J. Lipid Res. 57 (2016) 526–537.
[6] B.G. Nordestgaard, M.J. Chapman, K. Ray, et al., Lipoprotein(a) as a cardiovascular risk factor: current status, Eur. Heart J. 31 (2010) 2844–2853.
[7] E. Boerwinkle, C.C. Leffert, J. Lin, C. Lackner, G. Chiesa, H.H. Hobbs, Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations, J. Clin. Invest. 90 (1992) 52–60.
[8] M. Seed, F. Hoppichler, D. Reaveley, et al., Relation of serum lipoprotein(a) concentration and apolipoprotein(a) phenotype to coronary heart disease in patients with familial hypercholesterolemia, N. Engl. J. Med. 322 (1990) 1494–1499.
[9] O. Wiklund, B. Angelin, S.O. Olofsson, et al., Apolipoprotein(a) and ischaemic heart disease in familial hypercholesterolaemia, Lancet 335 (1990) 1360–1363.
[10] Emerging Risk Factors Collaboration, S. Erqou, S. Kaptoge, et al., Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality, J. Am. Med. Assoc. 302 (2009) 412–423.
[11] P.R. Kamstrup, A. Tybjærg-Hansen, B.G. Nordestgaard, Extreme lipoprotein(a) levels and improved cardiovascular risk prediction, J. Am. Coll. Cardiol. 61 (2013) 1146–1156.
[12] B.J. Arsenault, S.M. Boekholdt, M.-P. Dubé, et al., Lipoprotein(a) levels, genotype, and inciden aortic valve stenosis: a prospective Mendelian randomization study and replication in a case-control cohort, Circ Cardiovasc Genet 7 (2014) 304–310.
[13] K.H. Zheng, S. Tsimikas, T. Pawade, et al., Lipoprotein(a) and oxidized phospholipids promote valve calcification in patients with aortic stenosis, J. Am. Coll. Cardiol. 73 (2019) 2150–2162.
[14] C. Yeang, P.L.S.M. Gordts, S. Tsimikas, Novel lipoprotein(a) catabolism pathway via apolipoprotein(a) recycling: adding the plasminogen receptor PlgRKT to the list, Circ. Res. 120 (2017) 1050–1052.
[15] A. Vuorio, G.F. Watts, W.J. Schneider, S. Tsimikas, P.T. Kovanen, Familial hypercholesterolemia and elevated lipoprotein(a): double heritable risk and new therapeutic opportunities, J. Intern. Med. 287 (2020) 2–18.
[16] D.J. Rader, W.A. Mann, W. Cain, et al., The low density lipoprotein receptor is not required for normal catabolism of Lp(a) in humans, J. Clin. Invest. 95 (1995) 1403–1408.
[17] J.A. Casasnovas, V. Alcaide, F. Civeira, et al., Aragon workers' health study–design and cohort description, BMC Cardiovasc. Disord. 12 (2012) 45.
[18] P.M. Moriarty, S.A. Varvel, P.L.S.M. Gordts, J.P. McConnell, S. Tsimikas, Lipoprotein(a) mass level increase significantly according to APOE genotype: an analysis of 431 239 patients, Arterioscler. Thromb. Vasc. Biol. 37 (2017) 580–588.

[19] M. Solanas-Barca, I. de Castro-Orós, R. Mateo-Gallego, et al., Apolipoprotein E gene mutations in subjects with mixed hyperlipidemia and a clinical diagnosis of familial combined hyperlipidemia, Atherosclerosis 222 (2012) 449–455.
[20] N.J. Stone, J.G. Robinson, A.H. Lichtenstein, et al., ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, J. Am. Coll. Cardiol. 63 (2013) 2889–2934, 2014.
[21] L. Palacios, L. Grandoso, N. Cuevas, et al., Molecular characterization of familial hypercholesterolemia in Spain, Atherosclerosis 221 (2012) 137–142.
[22] A. Amor-Salamanca, S. Castillo, E. Gonzalez-Vioque, et al., Genetically confirmed familial hypercholesterolemia in patients with acute coronary syndrome, J. Am. Coll. Cardiol. 70 (2017) 1732–1740.
[23] A. Cenarro, A. Etxebarria, I. de Castro-Orós, et al., The p.Leu167del Mutation in APOE Gen Causes Autosomal Dominant Hypercholesterolemia by Down-regulation of LDL Receptor Expression in Hepatocytes, J. Clin. Endocrinol. Metab. 101 (2016) 2113–2121.
[24] M.B. Lanktree, C. Rajakumar, J.H. Brunt, M.L. Koschinsky, P.W. Connelly, R. A. Hegele, Determination of lipoprotein(a) kringle repeat number from genomic DNA: copy number variation genotyping using qPCR, J. Lipid Res. 50 (2009) 768–772.
[25] S. Richards, N. Aziz, S. Bale, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and Genomics and the association for molecular pathology, Genet. Med. 17 (2015) 405–424.
[26] M. Futema, M. Bourbon, M. Williams, S.E. Humphries, Clinical utility of the polygenic LDL-C SNP score in familial hypercholesterolemia, Atherosclerosis 277 (2018) 457–463.
[27] S. Pérez-Calahorra, R.M. Sánchez-Hernández, N. Plana, P. Valdivielso, F. Civeira, National dyslipidemia registry of the Spanish arteriosclerosis society: current status, Clín. Invest. Arterioscler. 29 (2017) 248–253.
[28] S. Li, N.-Q. Wu, C.-G. Zhu, et al., Significance of lipoprotein(a) levels in familial hypercholesterolemia and coronary artery disease, Atherosclerosis 260 (2017) 67–74.
[29] A.D. Mbewu, D. Bhatnagar, P.N. Durrington, et al., Serum lipoprotein(a) in patients heterozygous for familial hypercholesterolemia, their relatives, and unrelated control populations, Arterioscler. Thromb. 11 (1991) 940–946.
[30] E. Meriño-Ibarra, J. Puzo, E. Jarauta, et al., Hyperlipoproteinaemia(a) is a common cause of autosomal dominant hypercholesterolaemia, J. Inherit. Metab. Dis. 30 (2007) 970–977.
[31] B. Sjouke, R. Yahya, M.W.T. Tanck, et al., Plasma lipoprotein(a) levels in patients with homozygous autosomal dominant hypercholesterolemia, J Clin Lipidol 11 (2017) 507–514.
[32] M. Trinder, M.L. DeCastro, H. Azizi, et al., Ascertainment bias in the association between elevated lipoprotein(a) and familial hypercholesterolemia, J. Am. Coll. Cardiol. 75 (2020) 2682–2693.
[33] O.I. Afanasieva, M.V. Ezhov, O.A. Razova, M.I. Afanasieva, E.A. Utkina, S. N. Pokrovsky, Apolipoprotein(a) phenotype determines the correlations of lipoprotein(a) and proprotein convertase subtilisin/kexin type 9 levels in patients with potential familial hypercholesterolemia, Atherosclerosis 277 (2018) 477–482.
[34] R. Alonso, E. Andres, N. Mata, et al., Lipoprotein(a) levels in familial hypercholesterolemia: an important predictor of cardiovascular disease independent of the type of LDL receptor mutation, J. Am. Coll. Cardiol. 63 (2014) 1982–1989.
[35] R. Carmena, S. Lussier-Cacan, M. Roy, et al., Lp(a) levels and atherosclerotic vascular disease in a sample of patients with familial hypercholesterolemia sharing the same gene defect, Arterioscler. Thromb. Vasc. Biol. 16 (1996) 129–136.
[36] H.G. Kraft, A. Lingenhel, F.J. Raal, M. Hohenegger, G. Utermann, Lipoprotein(a) in homozygous familial hypercholesterolemia, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 522–528.
[37] B.G. Nordestgaard, M.J. Chapman, S.E. Humphries, et al., Familial hypercholesterolaemia isunderdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society, Eur. Heart J. 34 (2013) 3478–3490a.
[38] C. Pavanello, C. Pirazzi, K. Bjorkman, et al., Individuals with familial hypercholesterolemia and cardiovascular events have higher circulating Lp(a) levels, J Clin Lipidol 13 (2019), 778-787.e6.
[39] K.L. Ellis, L. Pérez de Isla, R. Alonso, F. Fuentes, G.F. Watts, P. Mata, Value of measuring lipoprotein(a) during cascade testing for familial hypercholesterolemia, J. Am. Coll. Cardiol. 73 (2019) 1029–1039.
[40] Y.Y. van der Hoek, A. Lingenhel, H.G. Kraft, J.C. Defesche, J.J. Kastelein, G. Utermann, Sib-pair analysis detects elevated Lp(a) levels and large variation of Lp(a) concentration in subjects with familial defective ApoB, J. Clin. Invest. 99 (1997) 2269–2273.
[41] B.L. Knight, Lp(a) catabolism in hypercholesterolaemic individuals, Chem. Phys. Lipids 67–68 (1994) 233–239.
[42] M. Croyal, V. Blanchard, K. Ouguerram, et al., VLDL (Very-Low-Density Lipoprotein)-apo E (apolipoprotein E) may influence Lp(a) (lipoprotein [a]) synthesis or assembly, Arterioscler. Thromb. Vasc. Biol. 40 (2020) 819–829.
[43] M. Futema, S. Shah, J.A. Cooper, et al., Refinement of variant selection for the LDL cholesterol genetic risk score in the diagnosis of the polygenic form of clinical familial hypercholesterolemia and replication in samples from 6 countries, Clin. Chem. 61 (2015) 231–238.

Journal of
Clinical Medicine

MDPI

Article

Cataract Surgery in Elderly Subjects with Heterozygous Familial Hypercholesterolemia in Prolonged Treatment with Statins

Victoria Marco-Benedí [1,2], Martín Laclaustra [1,2,*], Rosa M. Sánchez-Hernández [3], Emilio Ortega-Martínez de Victoria [4], Juan Pedro-Botet [5], Jose Puzo [6] and Fernando Civeira [1,2,*]

1 Lipid Unit, Hospital Universitario Miguel Servet, IIS Aragón, CIBERCV, 50009 Zaragoza, Spain; vmarcobenedi@gmail.com
2 Departamento de Medicina, Psiquiatría y Dermatología, Universidad de Zaragoza, 50009 Zaragoza, Spain
3 Endocrinology Department, Hospital Insular Universitario de Gran Canaria, 35016 Las Palmas de Gran Canaria, Spain; rosamariasanher@gmail.com
4 Lipid Clinic, Endocrinology Department, Hospital Clinic, CIBEROBN, 08036 Barcelona, Spain; EORTEGA1@clinic.cat
5 Lipid Unit, Hospital del Mar, Universitat Autònoma de Barcelona, 08003 Barcelona, Spain; JPedrobotet@parcdesalutmar.cat
6 Lipid Unit, Hospital San Jorge, 22004 Huesca, Spain; josepuzo@gmail.com
* Correspondence: martin.laclaustra@unizar.es (M.L.); civeira@unizar.es (F.C.); Tel.: +34-976765500 (ext. 14288) (M.L.); +34-976765500 (ext. 142895) (F.C.)

Citation: Marco-Benedí, V.; Laclaustra, M.; Sánchez-Hernández, R.M.; Ortega-Martínez de Victoria, E.; Pedro-Botet, J.; Puzo, J.; Civeira, F. Cataract Surgery in Elderly Subjects with Heterozygous Familial Hypercholesterolemia in Prolonged Treatment with Statins. *J. Clin. Med.* **2021**, *10*, 3494. https://doi.org/10.3390/jcm10163494

Academic Editor: Francisco Javier Ascaso

Received: 2 July 2021
Accepted: 6 August 2021
Published: 8 August 2021

Abstract: Background: Cataracts are the main cause of blindness and represent one fifth of visual problems worldwide. It is still unknown whether prolonged statin treatment favors the development of cataracts. We aimed to ascertain the prevalence of cataract surgery in elderly subjects with genetically diagnosed heterozygous familial hypercholesterolemia (HeFH) receiving statin treatment for ≥5 years, and compare this with controls. Methods: This is an observational, multicenter, case–control study from five lipid clinics in Spain. We collected data with the following inclusion criteria: age ≥65 years, LDL cholesterol levels ≥220 mg/dL without lipid-lowering drugs, a pathogenic mutation in a candidate gene for HeFH (LDLR, APOB, or PCSK9) and statin treatment for ≥5 years. Controls were selected from relatives of HeFH patients without hypercholesterolemia. Linear and logistic regressions based on generalized linear models and generalized estimating equations (GEE) were used. Cataract surgery was used as a proxy for cataract development. Results: We analyzed 205 subjects, 112 HeFH, and 93 controls, with a mean age of 71.8 (6.5) and 70.0 (7.3) years, respectively. HeFH subjects presented no difference in clinical characteristics, including smoking, hypertension, and type 2 diabetes mellitus, compared with controls. The mean duration of lipid-lowering treatment in HeFH was 22.5 (8.7) years. Cataract surgery prevalence was not significantly different between cases and controls. The presence of cataracts was associated neither with LDLc nor with the length of the statin therapy. Conclusion: In the present study, HeFH was not a risk factor for cataract surgery and prolonged statin treatment did not favor it either. These findings suggest that statin treatment is not related with cataracts.

Keywords: cataracts; heterozygous familial hypercholesterolemia; statins; lipid-lowering treatment

1. Introduction

Cataracts cause one third of blindness worldwide, sharing the leading position with uncorrected refractive errors and glaucoma [1], and 20–25 million cataract surgical interventions are performed worldwide each year [2]. Cataracts are defined as a degradation of the optical quality of the eye due to the clouding of the crystalline lens. Several properties of the lens gradually decline with age, and accordingly, old age is the most important risk factor in cataract formation. Other common risk factors include diabetes mellitus

(DM); long-term use of topical, systemic, intravitreal, inhaled, or oral corticosteroids; prior intraocular surgery; trauma; smoking; and exposition to ultraviolet-B light [3,4].

Statins are inhibitors of the enzyme HMG CoA reductase commonly used as lipid-lowering drugs [5]. They are used to lower blood cholesterol, which has proved to be a highly effective strategy to prevent cardiovascular disease in high-risk subjects. Since the description of vision loss due to irreversible cataracts caused by triparanol, the first synthetic cholesterol-lowering drug [6], some reports have associated the use of statins with the development of cataracts, although with conflicting results. A recent meta-analysis including observational studies concluded that statins slightly increase the risk of cataracts [5,7], whereas in randomized clinical trials, statins did not increase the risk of cataracts [8]. This topic was recently reviewed by several councils of the American Heart Association, and their conclusion was that statins in clinical use do not increase the risk of cataracts [9]. However, these observational studies and clinical trials were performed in populations in which the prevalence of cataracts is not very high, since they excluded patients $\geq$75 years of age, and in which the use of statins is limited to only a few years, usually less than 5 years.

Familial hypercholesterolemia (FH) is a monogenic disease characterized by an abnormal increase in low-density lipoprotein cholesterol (LDLc) levels from birth and subsequent high-risk of coronary disease. Only heterozygous FH (HeFH) patients reach older age. For this reason, HeFH is a group of patients in whom a high dose of potent statins has been used for decades. Thus, elderly HeFH subjects, having undergone decades of potent lipid-lowering therapy, may be an attractive population model to explore unexpected side effects [10,11]. The development of cataracts in HeFH has not been studied. We aimed to study the association between cataracts and statin use in a group of elderly HeFH under prolonged statin treatment, and compare this with controls.

2. Methods

2.1. Study Characteristics

This is an observational, multicenter, case–control study. We studied HeFH cases and controls from five lipid clinics in Spain. The protocol has been previously published [12] and was designed to explore non-coronary morbidities in elder HeFH. In brief, we recruited subjects with age $\geq$65 years; men and women, with a pathogenic mutation in a candidate gene for FH (*LDLR, APOB, or PCSK9*) in the subject or in a first-degree relative; LDLc levels $\geq$220 mg/dL without lipid-lowering therapy; and statin treatment for $\geq$5 years. Controls were selected from relatives of HeFH patients, requiring the absence of hypercholesterolemia (LDLc <190 mg/dL without lipid-lowering treatment). All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Aragon (CEICA) #PI19/440.

2.2. Assessments

In a clinical interview, we collected age, gender, ethnicity, smoking habit, and personal history of hypertension, diabetes mellitus, cataract surgery and cardiovascular heart disease. Cataract surgery was confirmed through reviewing the medical records. In addition, information of lipid-lowering treatment, such as statins, ezetimibe, and subtilisin-convertase proprotein/kexin type 9 inhibitors (PCSK9i), was collected. The recorded data included the type of drug and dose prescribed in the current treatment, the most common treatment used during the subject's lifetime, dose, and time when lipid-lowering treatment started. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

2.3. Statistical Analyses

We analyzed the association of cataracts with HeFH using generalized estimating equations (GEE), using logistic models with several levels of adjustment: unadjusted, adjusted for sex and age, and additionally adjusted for untreated LDLc concentration. Data for cases and controls are shown as mean (standard deviation) or percentage. The study of the influence of LDLc and statin treatment was analyzed within strata (cases and controls separately) with generalized linear models. All the analyses were performed with the statistical software R version 3.4.4. and the package "gee" version 4.13.19 (R Foundation, Auckland, New Zealand).

3. Results

Data were collected for 205 subjects (112 HeFH and 93 controls) aged 71.8 (6.5) and 70.0 (7.3) years, respectively. All cases and controls were Caucasian. The number (percentage) of women and men was 74 (66.1%) and 38 (33.9%) in cases and 48 (51.6%) and 45 (48.4%) in controls, respectively. There were no differences in clinical characteristics between cases and controls, with the exception of data on history of cardiovascular diseases, which were more frequent in HeFH subjects' blood-relatives (45.0% vs. 25.8%) and in themselves (27.7% vs. 16.1%); $p < 0.05$ in both cases. Similarly, the concentration of LDLc was higher in HeFH cases: 314 vs. 138 mg/dL ($p < 0.01$). The mean duration of statin treatment use in HeFH was 22.5 (8.7) years. Ninety-nine out of the 112 (88.4%) HeFH patients were on high-potency statins (atorvastatin 40–80 mg and rosuvastatin 20–40 mg). We did not observe any differences in smoking, hypertension, and DM between cases and controls [12]. A history of cataract surgery was present in 25.2 % of cases and 16.1% of controls, without difference in gender distribution. This difference was not statistically different either in the unadjusted test or after adjusting for age and sex, or additionally for LDLc (Table 1). When cases were classified according to the presence or absence of cataract surgery, there were no differences in clinical variables, except for age, which was higher in HeFH with cataract surgery (Table S1). We also analyzed the association of age, duration of statin treatment, and LDLc without lipid-lowering treatment with cataract surgery. Age was strongly associated with a relative risk of 2.06 (CI 1.09, 4.02) per 10 years among cases and 2.57 (CI 1.13, 6.28) among controls. The duration of statin treatment (studied among cases) and LDLc without lipid-lowering treatment did not show any association with cataract surgery (Table 2).

Table 1. Clinical and laboratory characteristics of controls and cases.

	Controls ***N* = 93**	**Cases** ***N* = 112**	***p*-Value**
Age (years)	70.0 (7.3)	71.8 (6.5)	0.038
Sex, women % (*n*)	51.6 [48]	66.1 [74]	0.050
Body mass index (Kg/m^2)	29.0 (4.7)	28.3 (4.0)	0.265
Untreated LDLc (mg/dL)	138.0 (31.7)	314.2 (71.4)	<0.001
Cataract surgery % (*n*)	16.1 [15]	25.2 [28]	0.121

Continuous data expressed as mean (SD); categorical data are expressed as percentages [count]. LDLc: low-density lipoprotein cholesterol. *p*-values from linear and logistic regressions based on generalized estimating equations (GEE) with exchangeable variance structure, unadjusted.

Table 2. Influence of age, LDL-cholesterol, and years under statin treatment on cataract surgery in controls and in HeFH.

	Controls		Cases	
	OR (95% CI)	***p*-Value**	**OR (95% CI)**	***p*-Value**
Model 1				
Per each 10 year of age	2.49 (1.12,5.87)	0.028	2.03 (1.07,3.96)	0.031
Model 2				
Per each 10 year of age	2.57 (1.13,6.28)	0.028	2.06 (1.09,4.02)	0.028
Per each 10 mg/dL of cLDL	0.89 (0.74,1.07)	0.228	1.00 (0.94,1.07)	0.905
Model 3				
Per each 10 year of age	-	-	1.83 (0.92,3.69)	0.082
Per each 10 year of lipid-lowering use	-	-	1.01 (0.59,1.53)	0.967

Linear and logistic regressions based on generalized linear models (GLM) adjusted for sex. CI: confidence interval. *p*-values in each section obtained from a single model, adjusted for sex.

4. Discussion

Our study shows that the prevalence of cataract surgery is not increased in elderly people with HeFH undergoing lipid-lowering treatment for more than 20 years. This suggests that neither severe hypercholesterolemia nor prolonged use of statins are relevant risk factors in the development of cataracts. To our knowledge, this is the first study that explores the presence of cataracts in this population, a paradigmatic subgroup of patients in whom treatment with a high dose of potent statins is the first line of treatment.

The present study has the strength of being carried out in a group of patients with three main differential characteristics with respect to previous studies: the studied subjects had very high concentrations of LDL-C from birth, they used high doses of statins for >20 years, and they are all aged ≥65 years—that is, they are a group with a high incidence of cataracts. Our study agrees that age is the main factor associated with the development of cataracts. Importantly, our data confirm the results of a previous analysis on the safety of prolonged used statins with respect to cataract development and they pose doubts on the suggested association of cholesterol concentration with cataracts. Preclinical studies showed that cholesterol has an important role in membrane integrity, and it was supposed that the inhibition of cholesterol synthesis caused cataract development [7]. In addition, it must be taken into account that statins exert their benefits across a wide spectrum of ophthalmic conditions through its hypocholesterolemic and pleiotropic effects, that may contribute to making them safe with respect to cataracts [5].

5. Conclusions

Cataract surgery prevalence was not significantly different between HeFH and controls. The presence of cataracts was associated neither with LDLc nor with the length of the statin therapy. In the present study, HeFH was not a risk factor for cataracts and prolonged statin treatment did not favor cataract development.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10163494/s1, Table S1: Clinical and laboratory characteristics of controls and cases according to previous cataract surgery.

Author Contributions: F.C., V.M.-B. and M.L. wrote the manuscript; F.C. designed the research and performed the research; M.L. analyzed the data; R.M.S.-H., E.O.-M.d.V., J.P.-B., J.P. performed the research and review the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This is work was supported by Spanish Ministry of Economy and Competitiveness: PI19/00694, Gobierno de Aragón and CIBERCV. These projects were co-financed by Instituto de Salud Carlos III and the European Regional Development Fund (ERDF) of the European Union "A way to make Europe". Authors thank Mariah Carmichael for her technical assistance in English language.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and all participants signed an informed consent, approved by the Aragón Clinical Research Ethics Committee before being included in the study.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: Data available on request from the authors.

Conflicts of Interest: None of the following authors have any proprietary interests or conflicts of interest related to this submission.

Trial Registration: ClinicalTrials.gov Identifier: NCT03310671.

Abbreviations

BMI	body mass index
GEE	generalized estimating equations
FH	familial hypercholesterolemia
HeFH	heterozygous familial hypercholesterolemia
LDLc	low-density lipoprotein cholesterol

References

1. Flaxman, S.R.; Bourne, R.R.; Resnikoff, S.; Ackland, P.; Braithwaite, T.; Cicinelli, M.V.; Das, A.; Jonas, J.B.; Keeffe, P.J.; Kempen, J.H.; et al. Global causes of blindness and distance vision impairment 1990–2020: A system-atic review and meta-analysis. *Lancet Glob. Health* **2017**, *5*, e1221–e1234. [CrossRef]
2. Kohnen, T. Treating inflammation after lens surgery. *J. Cataract. Refract. Surg.* **2015**, *41*, 2035. [CrossRef] [PubMed]
3. Olson, R.J.; Braga-Mele, R.; Chen, S.; Miller, K.M.; Pineda, R.; Tweeten, J.P.; Musch, D. Cataract in the Adult Eye Preferred Practice Pattern®. *Ophthalmology* **2017**, *124*, P1–P119. [CrossRef] [PubMed]
4. Thompson, J.; Lakhani, N. Cataracts. *Prim. Care* **2015**, *42*, 409–423. [CrossRef] [PubMed]
5. Ooi, K.G.-J.; Khoo, P.; Vaclavik, V.; Watson, S.L. Statins in ophthalmology. *Surv. Ophthalmol.* **2019**, *64*, 401–432. [CrossRef] [PubMed]
6. Kirby, T.J. Cataracts produced by triparanol. (MER-29). *Trans. Am. Ophthalmol. Soc.* **1967**, *65*, 494–543. [PubMed]
7. Alves, C.; Mendes, D.; Marques, F.B. Statins and risk of cataracts: A systematic review and meta-analysis of observational studies. *Cardiovasc. Ther.* **2018**, *36*, e12480. [CrossRef] [PubMed]
8. Yu, S.; Chu, Y.; Li, G.; Ren, L.; Zhang, Q.; Wu, L. Statin Use and the Risk of Cataracts: A Systematic Review and Meta-Analysis. *J. Am. Hear. Assoc.* **2017**, *6*, e004180. [CrossRef] [PubMed]
9. Newman, C.B.; Preiss, D.; Tobert, J.A.; Jacobson, T.A.; Page, I.R.L.; Goldstein, L.B.; Chin, C.; Tannock, L.R.; Miller, M.; Raghuveer, G.; et al. Statin Safety and Associated Adverse Events: A Scientific Statement From the American Heart Association. *Arter. Thromb. Vasc. Biol.* **2019**, *39*, e38–e81. [CrossRef] [PubMed]
10. Mach, F.; Baigent, C.; Catapano, A.L.; Koskinas, K.C.; Casula, M.; Badimon, L.; Chapman, M.J.; De Backer, G.G.; Delgado, V.; Ference, B.A.; et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *Eur. Heart J.* **2020**, *41*, 111–188. [CrossRef] [PubMed]
11. Mach, F.; Ray, K.K.; Wiklund, O.; Corsini, A.; Catapano, A.L.; Bruckert, E.; De Backer, G.; A Hegele, R.; Hovingh, G.K.; A Jacobson, T.; et al. Adverse effects of statin therapy: Perception vs. the evidence—Focus on glucose homeostasis, cognitive, renal and hepatic function, haemorrhagic stroke and cataract. *Eur. Heart J.* **2018**, *39*, 2526–2539. [CrossRef] [PubMed]
12. Marco-Benedí, V.; Laclaustra, M.; Casado-Dominguez, J.M.; Villa-Pobo, R.; Mateo-Gallego, R.; Sánchez-Hernández, R.M.; Nuez, M.B.; De Victoria, E.O.-M.; Sitges, M.; Pedro-Botet, J.; et al. Aortic Valvular Disease in Elderly Subjects with Heterozygous Familial Hypercholesterolemia: Impact of Lipid-Lowering Therapy. *J. Clin. Med.* **2019**, *8*, 2209. [CrossRef] [PubMed]

Full Title: Current causes of death in familial hypercholesterolemia

Authors´names: Victoria Marco-Benedí Msc[a,b]; Ana M. Bea[a] MLT, Ana Cenarro PhD[c], Estíbaliz Jarauta MD, PhD[a,b], Martín Laclaustra MD, PhD[a,b]*, Fernando Civeira MD, PhD[a,b*]

*Both authors should be considered senior authors.

Total word count: 2532

Author affiliations: [a]Hospital Universitario Miguel Servet, IIS Aragón, CIBERCV; [b]Universidad de Zaragoza, Zaragoza, Spain, [c]Instituto Aragonés de Ciencias de la Salud (IACS), Zaragoza, Spain.

Corresponding authors:

Dr. Fernando Civeira MD, PhD[a,b],
Unidad de Lípidos, Hospital Universitario Miguel Servet
Avda Isabel La Católica 1-3, 50009, Zaragoza, Spain
Email: civeira@unizar.es Phone: +(34) 976765500 ext 142895

Dr Martín Laclaustra MD, PhD[a,b],
Unidad de Lípidos, Hospital Universitario Miguel Servet
Avda Isabel La Católica 1-3, 50009, Zaragoza, Spain
Email: martin.laclaustra@unizar.es Phone: +(34) 976765500 ext 142895

ABSTRACT

Background: Familial hypercholesterolemia (FH) is a codominant autosomal disease characterized by high low-density lipoprotein cholesterol (LDLc) and high risk of premature cardiovascular disease (CVD). The molecular bases have been well defined and effective lipid-lowering is possible. This analysis aimed to study the current major causes of death of genetically defined heFH.

Methods: Case-control study designed to analyze life-long mortality in a group of heFH and control families. Data of first-degree family members of cases and controls (non-consanguineous cohabitants), including deceased relatives, were collected from a questionnaire and review of medical records. Mortality was compared among heFH, non-heFH, and non-consanguineous family members.

Results: We analyzed 813 family members, 26.4% of them, deceased. Among deceased, mean age of death was 69.3 years in heFH, 73.5 years in non-heFH, and 73.2 years in non-consanguineous, differences that were not statistically significant. Among them, CVD cause of death was 59.7% in heFH, 37.7.% in non-heFH, and 37.4% in non-consanguineous (P=0.012). These differences were greater restricting the analyses to parents' mortality. The hazard ratio of dying from CVD was 3.02 times higher (95% CI, 1.90-4.79) in heFH members in comparison with the other two groups (non-FH and non-consanguineous), who did not differ in their risk.

Conclusions: Current CVD mortality in heFH is lower and occurs later than that described in the last century but still higher than in non-FH. This better prognosis in CVD risk is not associated with changes in non-CVD mortality.

Keywords: heterozygous familial hypercholesterolemia phenotype, parental-offspring, cardiovascular disease death.

Abbreviations:

BMI: body mass index

CHD: coronary heart disease

CVD: cardiovascular disease

DM: diabetes mellitus

FH: familial hypercholesterolemia

heFH: heterozygous familial hypercholesterolemia

HDLc: high-density lipoprotein cholesterol

LDLc: low-density lipoprotein cholesterol

LDLR: low-density lipoprotein receptor gene

Lp(a): lipoprotein(a)

PCSK9: pro-protein convertase subtilisin/kesin 9

Introduction

Familial hypercholesterolemia (FH) is a codominant autosomal disorder and the most common monogenic metabolic disease in the population. The prevalence of heterozygous FH (heFH) is approximately 1/200-500 persons in most countries (1,2). FH is caused by mutations in the genes that control the cellular uptake of plasma cholesterol and that include the LDL receptor (*LDLR)*, apolipoprotein B *(APOB)*,pro-protein convertase subtilisin/kesin 9 *(PCSK9)* and *APOE* (1). HeFH patients show very high plasma concentration of low-density lipoprotein cholesterol (LDLc), approximately twice of non-FH subjects of the general population, often ranging between 250-400 mg/dL, deposits of cholesterol in superficial tissues such as corneal arcus and tendon xanthomas, and high risk of premature cardiovascular disease (CVD) in absence of adequate lipid-lowering treatment (3,4). The risk of developing premature CVD is increased 10 times in these patients with respect to the general population, especially coronary heart disease (CHD) in young patients (4,5). International heFH registries such as the British Simon Broome show an up to 100 higher risk of CHD in heFH men under 40 years of age with heFH in the pre-statin era, treatment that was not available until the late 1980s (6). The life expectancy of the heFH subjects had been calculated between 10-30 years lower for women and men, respectively, in relation to the non-FH population (7). In recent years, there has been a decrease in CVD in heFH, as we have recently been able to verify in our environment (4,8) probably due to earlier diagnosis and intensive lipid-lowering treatment.

Two important facts have occurred in the morbidity and mortality analysis of the heFH in the last decades. First, the genetic bases of heFH have been studied in depth and the genetic study provides a certainty diagnosis that obviates the diagnostic bias based on CVD risk as one of the major criteria for heFH diagnosis (9); and, second

current lipid-lowering drugs including statins, ezetimibe and PCSK9 inhibitors have substantially modified the lipid phenotype and consequently the clinical spectrum of the disease (5,10). In this way, if the treatment is well established during the first decades of life, heFH should be less aggressive disease than before. The complexity of the genetic FH background, the use of multiple drugs for decades, a larger life-expectancy associated with treatment and changes in environmental and social factors could lead to a much more heterogeneous phenotype than that described in the past century (5). In addition, other comorbidities could be associated to the heFH phenotype that were hidden by CVD, or associated to the lipid-lowering treatment, such as diabetes favored by statins (11). Knowing the effect of the different genetic types of heFH in the long term and the impact of prolonged lipid-lowering treatment are essential for an adequate management of this disease in the coming years.

The aim of this analysis was to study the current causes of cardiovascular and non-cardiovascular death of heFH and potential differences with a control population.

Patients and Methods

Aim, design, and participants

This is an observational, case-control study designed to describe current morbidity and mortality in heFH subjects. HeFH cases were recruited from the Lipid Unit at Hospital Universitario Miguel Servet, Zaragoza, Spain, and their non-consanguineous partners were recruited as controls. Data about first-degree family members of cases and controls, including deceased relatives, were collected from a participant questionnaire and review of their medical records. Written informed consent was obtained from each case and control included in the study; the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki; and the study protocol was previously

approved by the Institution's ethics committee on research on humans (Comité Ético de Investigación Clínica de Aragón).

The inclusion criteria for cases consisted on the following requirements: age ≥ 30 and ≤ 60 years at the time of the enrollment in the study; genetically diagnosed heFH; and personal history of hypercholesterolemia with LDLc levels >220 mg/dL without lipid-lowering treatment. Controls were selected from non-consanguineous relatives of similar age (± 5 years) of heFH patients (partners of the case who cohabited with them); age ≥ 30 and ≤ 60 years at the time of inclusion in the study; and fulfilling that neither them nor any first degree relative had a clinical diagnosis of heHF and that they had LDLc <190 mg/dL without lipid-lowering drugs.

Clinical interview

Participants were interviewed to collect personal information about history of CVD disease, CV risk factors, comorbidities, medication use, lipid values, and hospitalizations, and besides, to perform a detailed family history including these data of all first-degree relatives (parents, siblings, and offspring), and age and cause of death of those deceased. Information about history of hypercholesterolemia, lipid-lowering drug use, and age and cause of death were confirmed from the patient's medical records. If a first-degree family members of a case presented LDLc >220 mg/dL in at least one occasion and/or LDLc >160 mg/dL while taking any statin they were tagged as belonging to the heFH group. The analysis groups were thus coined "heFH family members", "non-heFH family members", and "Control family members". In this report only data on family members deaths over the age of 18 years is presented.

Genetic study.

All heFH cases interviewed in this study had a genetic diagnosis of heFH and were carriers of a "pathogenic" or "likely pathogenic" variant according to the guidelines of

the American College of Medical Genetics and Genomics (ACMG) (12) in *LDLR* (NM_000527.4), *APOB* (NM_000384.2) or *PCSK9* (NM_174936.3) genes. FH gene analysis were studied with the Progenika Biopharma Grifols (Derio, Spain) (13) or GEN inCode (Terrassa-Barcelona, Spain) (14) platforms.

Statistical Analyses

Data are expressed as mean standard deviation for numerical variables with normal distribution and were analyzed with the Student's t test, while those without normal distribution are expressed as median [interquartile range] and are analyzed with the Mann-Whitney U test. Qualitative variables are expressed as a percentage and were analyzed using the X2 test. For the comparison of non-dichotomous category variables, the ANOVA and Kruskal-Wallis tests were used. The mortality rates were calculated using the Kaplan-Meier estimate based on age, and the groups were compared using log rank test. The association between heFH and CV and non-CVD mortality was calculated using multivariate Cox's regression. A model was generated that included the covariate age, and it was calculated with techniques appropriate for analyzing complex samples to consider that data was clustered in families.

Results

Clinical characteristics of cases and controls

We recruited 166 subjects, 83 heFH cases and 83 controls. The mean ages were 54.3 years and 54.5 years, respectively, without differences in age and sex between the groups. BMI, systolic blood pressure and diastolic blood pressure were similar in both groups. Hypertension and diabetes (DM2) prevalence showed no differences either.

CVD tended to be more prevalent in heFH cases than in controls 8.4% and 2.4% respectively (P=0.08). Untreated total and LDLc were higher in cases than in controls. Statin treatment was present in all cases and in 22.9% controls. The onset of the lipid-lowering treatment was 32.8 years in the heFH and 51.3 years in controls (Table 1).

Family study

We analyzed 813 first-degree family members of cases and controls within families of cases 211 members were heFH and 219 non-heFH. We discarded 11 first-degree family members of cases with an ambiguous heFH phenotype (Figure 1). The control family group was composed by 372 subjects.

CVD and non -CVD mortality among first-degree family members of cases and control

We identified 62 dead relatives in heFH family members, 53 in non-heFH and 100 in controls (Figure 1). The percentage of dead subjects and the mean age of the death were similar in the three groups, being slightly higher in the heFH family members, 29.4% compared with 24.2% in the non-heFH family members and 26.9 % in the control family members. Average death age was approximately 4 years less in the heFH group. The proportion of deaths due to CVD was higher among the heFH group (59.7% in heFH vs 37.7% in non-heFH and 37.4% in controls, P=0.012). Other causes of death, including cancer death, did not shown significant differences among the three groups (Table 2). Additionally, we studied mortality differences between men and women. The percentage of deceased subjects did not show differences between the groups, however, HeFH subjects died approximately 4 years earlier than non-heFH and controls, although the difference was not statistically significant. Death cause in men was CVD in 69% of heFH deceased versus 38.5% of non-heFH and 37.0% of controls respectively (P=0.01). The same pattern was observed in women although the age of death was about 7 years later in women than in men similarly in the 3 groups. (Table 3 and Table 4). The hazard

ratio of dying of CVD in heFH was 2.85 times higher (95% CI, 1.73-4.69) in heFH with respect to the control family group, and without differences between non-FH and controls. This hazard ratio was 2.95 in men (95% CI, 1.52-5.75) and 3.44 in females in heFH (95% CI, 1.66-7.10) (Table 5). The separation of the curves appeared at the age of 50, to continue increasing progressively with age (Figure 2). This higher risk appeared approximately 5 years earlier in heFH men than in heFH women (Supplemental Figure 1).

CV or non - CVD mortality among parental family members of cases and controls.

Since most of the deaths corresponded to the parents of cases and controls, we analyzed mortality in this group of subjects. There were 116 deaths among fathers: 24 (72.7%) heFH, 35 (72.9%) non-heFH and 57 (70.4%) controls; and 77 deaths among mothers: 33 (66.0%) heFH, 13 (39.4%) non-heFH and 31 (37.8%) controls. The percentage of deaths from CVD was higher in heFH than in the other two groups, although the difference was significant only in men, and the age of death from CVD was younger in both men and women for heFH subjects. We did not find statistically significant differences in the non-CVD death (Figure 3), but the control family mothers had a trend to higher cancer death compared to heFH mother family (P=0.092) (Supplemental Tables 1 and 2).

Discussion

In the present work, we analyze the potential differences in mortality in a group of heFH families from a Lipid Unit comparing heFH, non-heFH, and non-consanguineous family members with the aim of update CVD and non-CVD death in heFH in the era of lipid-lowering treatment. HeFH is a singular model to study the effect on mortality of hypercholesterolemia due to an increase in LDLc levels and their relationship with CVD

events (15). In addition, heFH are usually under intensive lipid-lowering chronic treatment and our results are in line with the CVD benefit observed with the LDLc lowering in the general population (16,17). We hypothesized that the prevalence of CVD death has decreased during the last years in these patients and our results seem to support the case because CVD mortality in this group of families is lower and appeared later than heFH cohorts reported in the last decades of last century (7,17). However, we still find an increase in CVD death with respect to non-heFH, especially in the heFH men, who died 6.8 years younger compared to the other family groups. Traditionally, it has been considered that in heFH without lipid -lowering treatment, approximately 50% of men and 30 % of women will develop CVD before 50 years (18,19) with the life expectancy estimated to be 20 to 30 years lower (7). However, the CVD death could have been biased in those studies: Historically, heFH has been diagnosed clinically based on LDLc elevations, premature personal and familial CVD, and presence of tendon xanthomas or arcus cornealis. The most common criteria for diagnosis including those of the Dutch Lipid Clinic Network (20) and Simon Broome registry (21) include CVD o risk factors for CVD such as tendon xanthomas (18,22,23). In this way, the heFH subjects or their families in whom CHD prevailed, had more chances to get the clinical diagnosis of FH. The genetic characterization of FH in recent years have demonstrated that the heFH phenotype is more heterogeneous than previous belief including the presence of CVD. In a recent publication from The Netherlands, CHD was present in 7.4% of 25,137 genetically diagnosed heFH, in spite of the mean age was 38 and 71.1% were not on lipid-lowering drugs (24). Consequently, a significant proportion of heFH may have gone unnoticed while applying traditional diagnostic criteria.

Our cohort is based on very high LDLc levels (>220 mg/dl without lipid-lowering therapy) and a positive genetic test for a causative mutation in a canonical gene for FH. Furthermore, patients were referred to the clinic by their general practitioners because high LDLc (25). So, we think that our cohort has overcome previous bias. Robust evidence, including heFH observational studies, has demonstrated a reduction in major CVD events in patients who are taking lipid-lowering treatment when initiated early in life and maintained for years (26,27). Accordingly, the survival without CHD, with an early onset of statins in these subjects, could be quite similar to the rest of the population (28). In our study, we showed a large group heFH who were taking lipid-lowering treatment above 25 years on average. Furthermore, the majority of their heFH family members have been taking statins at some point in their lives. In addition, the prevalence of CVD estimated in this study, 7% in heFH, are in line with other studies in genetically defined heFH (24,29).

We have also analyzed non-CVD mortality in these genetically defined heFH families with a large history of lipid lowering drugs consumption with two purposes: First, to check whether lipid-lowering therapy could play a role in other comorbidities, and second, to explore whether the FH causing mutation itself might be associated to other morbidities other than CVD once heFH subjects live long enough without CVD, something that, until now, would have been hidden in the early mortality. In this study, non-CVD mortality did not show significant differences between heFH and non-FH in either sex group. There was a tendency in the heFH females to die later from non-CVD causes than non-FH, even though the difference did not reach statically significance. We hypothesized that it could be due to healthier lifestyles in heFH subject such as our group showed previously (8).

Our study has some limitations. Its retrospective design only heFH who lived enough time are selected, so heFH subjects who died before the analysis, cannot be studied. The number of subjects studied, imposed by the difficulty to find large series of patients, allows us to identify differences in mortality in the large disease groups, if some rare disease is associated with the mutation or the treatment, this could have gone unnoticed. Finally, we have information about the time of treatment onset, but only in cases and controls could be completely corroborated. The strengths of our article include that all heFH cases have been genetically confirmed and that HeFH diagnosis was independent of CVD.

In conclusion, our results show that current CVD mortality in heFH is lower and occurs later than that described in the last century but still higher than in non-FH. Probably, this is due to better control of the risk of CVD risk factors, especially prolonged lipid-lowering treatment. This better prognosis in CVD risk is not associated with changes in non-CVD mortality.

Declarations

Ethics approval and consent to participate:

The study protocol has been previously approved by the Institution's ethics committee on research on humans (Comité Ético de Investigación Clínica de Aragón). C.I. P18/262

Consent for publication: "Not applicable" for that section

Availability of data and materials: Data available upon reasoned request.

Competing interests: The authors declare no conflict of interest

Sources of Funding

This study was supported by grants from the Spanish Ministry of Economy and Competitiveness PI 18/01777 PI 19/00694 and CIBERCV; and Gobierno de Aragón B-14. These projects are co-financed by Instituto de Salud Carlos III and the European Regional Development Fund (ERDF) of the European Union ‘‘A way to make Europe".

Author contribution statement

Conceptualization, VM-B and FC; Data Curation VM-B, AMB, EJ, AC, FC. Formal Analysis VM-B, ML, and FC; Funding Acquisition, FC; Investigation, VM-B, AC, ML, and FC; Methodology, VM-B, ML, and FC; Project Administration, FC; Resources, FC; Software ML; Writing - Original Draft Preparation: VM-B, FC; Review: all authors. All authors have read and approved the final manuscript.

Acknowledgements: We want to particularly acknowledge the patients and the Biobank of the Aragon Health System (PT17/0015/0039) integrated in the Spanish National Biobanks Network for their collaboration.

References

1. Scriver CR, editor. The metabolic & molecular bases of inherited disease. 8th ed. New York: McGraw-Hill; 2001. 4 p.

2. Brunham LR, Hegele RA. What Is the Prevalence of Familial Hypercholesterolemia? Arterioscler Thromb Vasc Biol. 2021 Oct;41(10):2629–31.

3. Civeira F, Ros E, Jarauta E, Plana N, Zambon D, Puzo J, et al. Comparison of genetic versus clinical diagnosis in familial hypercholesterolemia. Am J Cardiol. 2008 Nov 1;102(9):1187–93, 1193.e1.

4. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease. Eur Heart J. 2013 Dec 1;34(45):3478–90.

5. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2019 Aug 31;

6. Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group. BMJ. 1991 Oct 12;303(6807):893–6.

7. WHO Human Genetics Programme. Familial hypercholesterolaemia (FH): report of a WHO consultation, Paris, 3 October 1997 [Internet]. World Health Organization; 1998 [cited 2021 Nov 23]. Report No.: WHO/HGN/CONS/FH/98.7. Available from: https://apps.who.int/iris/handle/10665/64162

8. Perez-Calahorra S, Laclaustra M, Marco-Benedí V, Lamiquiz-Moneo I, Pedro-Botet J, Plana N, et al. Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia. Atherosclerosis. 2019 May;284:245–52.

9. Sánchez-Hernández RM, Tugores A, Nóvoa FJ, Brito-Casillas Y, Expósito-Montesdeoca AB, Garay P, et al. The island of Gran Canaria: A genetic isolate for familial hypercholesterolemia. J Clin Lipidol. 2019 Aug;13(4):618–26.

10. Gaudet D, Gipe DA, Pordy R, Ahmad Z, Cuchel M, Shah PK, et al. ANGPTL3 Inhibition in Homozygous Familial Hypercholesterolemia. N Engl J Med. 2017 Jul 20;377(3):296–7.

11. Sattar N, Preiss D, Murray HM, Welsh P, Buckley BM, Craen A de, et al. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials [Internet]. Centre for Reviews and Dissemination (UK); 2010 [cited 2019 Sep 20]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK78906/

12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and

the Association for Molecular Pathology. Genet Med Off J Am Coll Med Genet. 2015 May;17(5):405–24.

13. Palacios L, Grandoso L, Cuevas N, Olano-Martín E, Martinez A, Tejedor D, et al. Molecular characterization of familial hypercholesterolemia in Spain. Atherosclerosis. 2012 Mar;221(1):137–42.

14. Amor-Salamanca A, Castillo S, Gonzalez-Vioque E, Dominguez F, Quintana L, Lluís-Ganella C, et al. Genetically Confirmed Familial Hypercholesterolemia in Patients With Acute Coronary Syndrome. J Am Coll Cardiol. 2017 Oct 3;70(14):1732–40.

15. Borén J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2020 Jun 21;41(24):2313–30.

16. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017 Aug 21;38(32):2459–72.

17. Ference BA, Majeed F, Penumetcha R, Flack JM, Brook RD. Effect of Naturally Random Allocation to Lower Low-Density Lipoprotein Cholesterol on the Risk of Coronary Heart Disease Mediated by Polymorphisms in NPC1L1, HMGCR, or Both: A 2 × 2 Factorial Mendelian Randomization Study. J Am Coll Cardiol. 2015 Apr 21;65(15):1552–61.

18. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. Eur Heart J. 2013 Dec;34(45):3478–3490a.

19. Civeira F, International Panel on Management of Familial Hypercholesterolemia. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. Atherosclerosis. 2004 Mar;173(1):55–68.

20. Defesche JC, Gidding SS, Harada-Shiba M, Hegele RA, Santos RD, Wierzbicki AS. Familial hypercholesterolaemia. Nat Rev Dis Primer. 2017 Dec 7;3:17093.

21. Mortality in treated heterozygous familial hypercholesterolaemia: implications for clinical management. Scientific Steering Committee on behalf of the Simon Broome Register Group. Atherosclerosis. 1999 Jan;142(1):105–12.

22. Goldstein JL, Brown MS. Familial hypercholesterolemia: identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. Proc Natl Acad Sci U S A. 1973 Oct;70(10):2804–8.

23. Civeira F, Castillo S, Alonso R, Meriño-Ibarra E, Cenarro A, Artied M, et al. Tendon xanthomas in familial hypercholesterolemia are associated with cardiovascular risk independently of the low-density lipoprotein receptor gene mutation. Arterioscler Thromb Vasc Biol. 2005 Sep;25(9):1960–5.

24. Besseling J, Kastelein JJP, Defesche JC, Hutten BA, Hovingh GK. Association between familial hypercholesterolemia and prevalence of type 2 diabetes mellitus. JAMA. 2015 Mar 10;313(10):1029–36.

25. Marco-Benedí V, Cenarro A, Laclaustra M, Larrea-Sebal A, Jarauta E, Lamiquiz-Moneo I, et al. Lipoprotein(a) in hereditary hypercholesterolemia: Influence of the genetic cause, defective gene and type of mutation. Atherosclerosis. 2021 Aug 23;S0021-9150(21)01270-3.

26. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet Lond Engl. 2005 Oct 8;366(9493):1267–78.

27. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J. 2012 Jul;33(13):1635–701.

28. Versmissen J, Oosterveer DM, Yazdanpanah M, Defesche JC, Basart DCG, Liem AH, et al. Efficacy of statins in familial hypercholesterolaemia: a long term cohort study. BMJ. 2008 Nov 11;337:a2423.

29. Huijgen R, Kindt I, Defesche JC, Kastelein JJP. Cardiovascular risk in relation to functionality of sequence variants in the gene coding for the low-density lipoprotein receptor: a study among 29,365 individuals tested for 64 specific low-density lipoprotein-receptor sequence variants. Eur Heart J. 2012 Sep;33(18):2325–30.

Figure legends

Figure 1 Study Flowchart

Figure 2 Kaplan-Meier cumulative survival curves for cardiovascular death

Figure 3 Kaplan-Meier cumulative survival curves for non-cardiovascular death

Supplemental Figures

Supplemental figure 1. Pannel A) Kaplan-Meier cumulative survival curves for cardiovascular death in heFH men family members. B) Kaplan-Meier cumulative survival curves for cardiovascular death in heFH women family members.

Table 1. Clinical and biochemical characteristics of heFH and control probands.

	heFH Cases	**Controls**	***P***
N	83	83	
Age (years)	54.3 (10.7)	54.5 (10.5)	0.884
Women, N (%)	45 (54.2)	44 (53.0)	0.876
Current smokers, N (%)	11 (13.3)	22 (26.5)	0.098
BMI (Kg/m^2)[a]	25.8 (3.94)	26.3 (4.17)	0.479
Systolic blood pressure (mmHg)	123.1 (12.7)	123.3 (14.0)	0.956
Diastolic blood pressure (mmHg)	74.6 (8.97)	75.3 (9.97)	0.628
Hypertension, N (%)	24 (28.9)	16 (19.8)	0.172
Type 2 diabetes mellitus, N (%)	7 (8.4)	7 (8.4)	1.000
Cardiovascular disease, N (%)	7 (8.4)	2 (2.4)	0.087
Total cholesterol (mg/dL)	363 (412-306)	220 (198-252)	<0.001
LDL cholesterol (mg/dL) [b]	283 (222-339)	130 (105-154)	<0.001
HDL cholesterol (mg/dL) [c]	56.2 (13.4)	62.0 (24.2)	0.079
Triglycerides (mg/dL)	114 (52.3)	141 (154)	0.209
Glucose (mg/dL)	89.5 (18.8)	91.3 (20.1)	0.626
Statin treatment, N (%)	83 (100)	19 (22.9)	<0.001
Onset age of statin treatment (years)	32.8 (9.43)	51.3 (8.84)	<0.001

[a]BMI denotes body mass index; [b]LDL, low-density lipoprotein; [c]HDL, high-density lipoprotein; Data are summarized as mean (SD) or N (percentage).

Table 2. Mortality among heFH and control first-degree family members

	heFH family members	Non-heFH family members	Control family members	*P*
N	211	219	372	
Total death, N (%)	62 (29.4)	53 (24.2)	100 (26.9)	0.479
Age of death (years)	69.3 (13.9)	73.5 (16.2)	73.2 (13.8)	0.179
Age of Cardiovascular disease death (years)	65.9 (13)	77.5 (12)	77.5 (13.2)	0.001
Age of Non-cardiovascular death (years)	73.9 (13.8)	71.4 (17.6)	71.72 (14.6)	0.792
Cardiovascular disease death, N (%)	37 (59.7)	20 (37.7)	37 (37.4)	0.012
Non-cardiovascular death, N (%)	25 (40.3)	33 (62.3)	62 (62.6)	
Cancer death, N (%)	12 (48.0)	14 (42.0)	31 (50.0)	0.779
Other death, N (%)	13 (52.0)	19 (57.5)	31 (50.0)	
Statin treatment, N (%)	107 (50.7)	29 (13.2)	53 (14,2)	0.001

Data are summarized as mean (SD) or N (percentage). Test for raw differences using Chi^2 test.

Table 3. Mortality among heFH and control first-degree male family members

	heFH family members	Non-heFH family members	Control family members	*P*
N	100	115	187	
Total death, N (%)	29 (29.0)	39 (34.2)	65 (34.8)	0.599
Age of death (years)	65.8 (13.0)	72.2 (16.1)	70.7 (14.4)	0.099
Age of Cardiovascular disease death (years)	62.2 (11.5)	78.7 (13)	71.2 (11.6)	0.001
Age of Non-cardiovascular death (years)	73.7 (13)	68.0 (17)	70.4 (15.8)	0.650
Cardiovascular disease death, N (%)	20 (69.0)	15 (38.5)	24 (37.0)	0.010
Non- cardiovascular death, N (%)	9 (31)	24 (61.5)	41 (63.1)	
Cancer death, N (%)	6 (66.7)	12 (50)	19 (46.3)	0.543
Other death, N (%)	3 (33.3)	12 (50.0)	22 (53.6)	

Data are summarized as mean (SD) or N (percentage). Test for raw differences using Chi^2 test.

Table 4. Mortality among heFH and control first-degree female family members

	heFH family members	Non-heFH family members	Control family members	*P*
N	111	104	185	
Total death, N (%)	33 (29.7)	14 (13.5)	34 (18.4)	0.010
Age of death (years)	72.4 (14.1)	77.2 (16.6)	78.1 (11.5)	0.178
Age of Cardiovascular disease death (years)	70.2 (13.5)	74 (15.0)	82.6 (8.7)	0.032
Age of Non-cardiovascular death (years)	74.1 (14.6)	80.3 (17.6)	74.3 (11.7)	0.513
Cardiovascular disease, N (%)	17 (51.5)	5 (35.7)	13 (38.2)	0.451
Non- cardiovascular death, N (%)	16 (48.5)	9 (64.3)	21 (61.8)	
Cancer death, N (%)	6 (37.5)	2 (22.2)	12 (57.1)	0.175
Other death, N (%)	10 (62.5)	7 (77.7)	9 (42.8)	

Data are summarized as mean (SD) or N (percentage). Test for raw differences using Chi^2 test.

Table 5. Prospective multivariable Cox Regression Analysis of Predictive Factors for a cardiovascular death in the families group

heFH family members	CVD death HR (95% CI)	CVD death HR (95% CI) *
All	3.02 (1.90-4.79)	2.85 (1.73-4.69)
Males	2.90 (1.59-5.29)	2.95 (1.52- 5.71)
Females	3.20 (1.55-6.63)	3.44 (1.66-7.10)
Non-FH family members	HR (95%CI)	HR (95%CI) *
All	0.81 (0.46-1.42)	0.98 (0.58-1.65)
Males	0.79 (0.49-1.57)	0.80 (0.41-1.53)
Females	0.95 (0.33-2.67)	1.02 (0.38-2.71)

95% CI, 95% confidence interval; HR, hazard ratio. HR (95%CI) *: confidence interval estimations calculate taking into account family clusters.

Figure 1

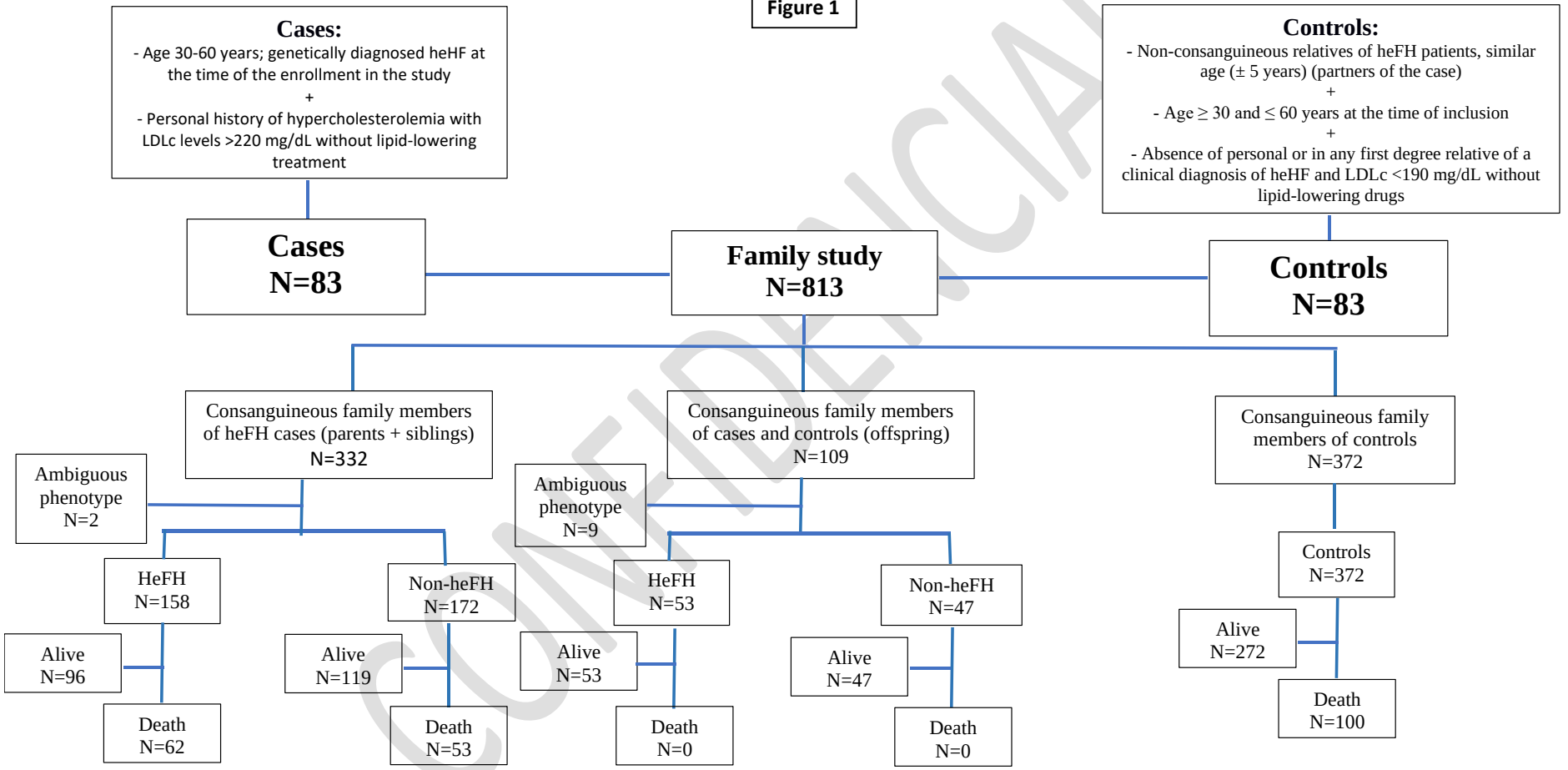
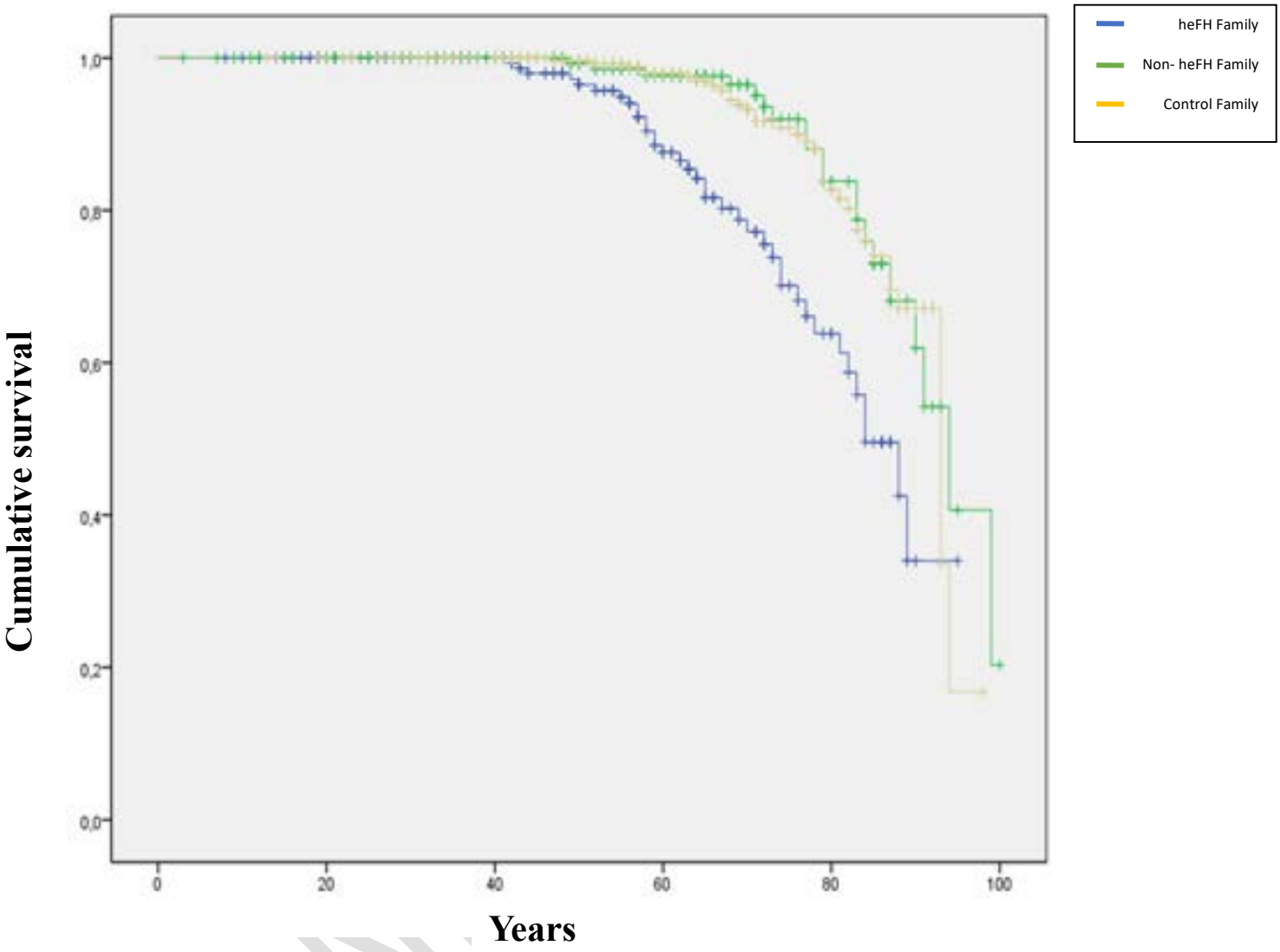


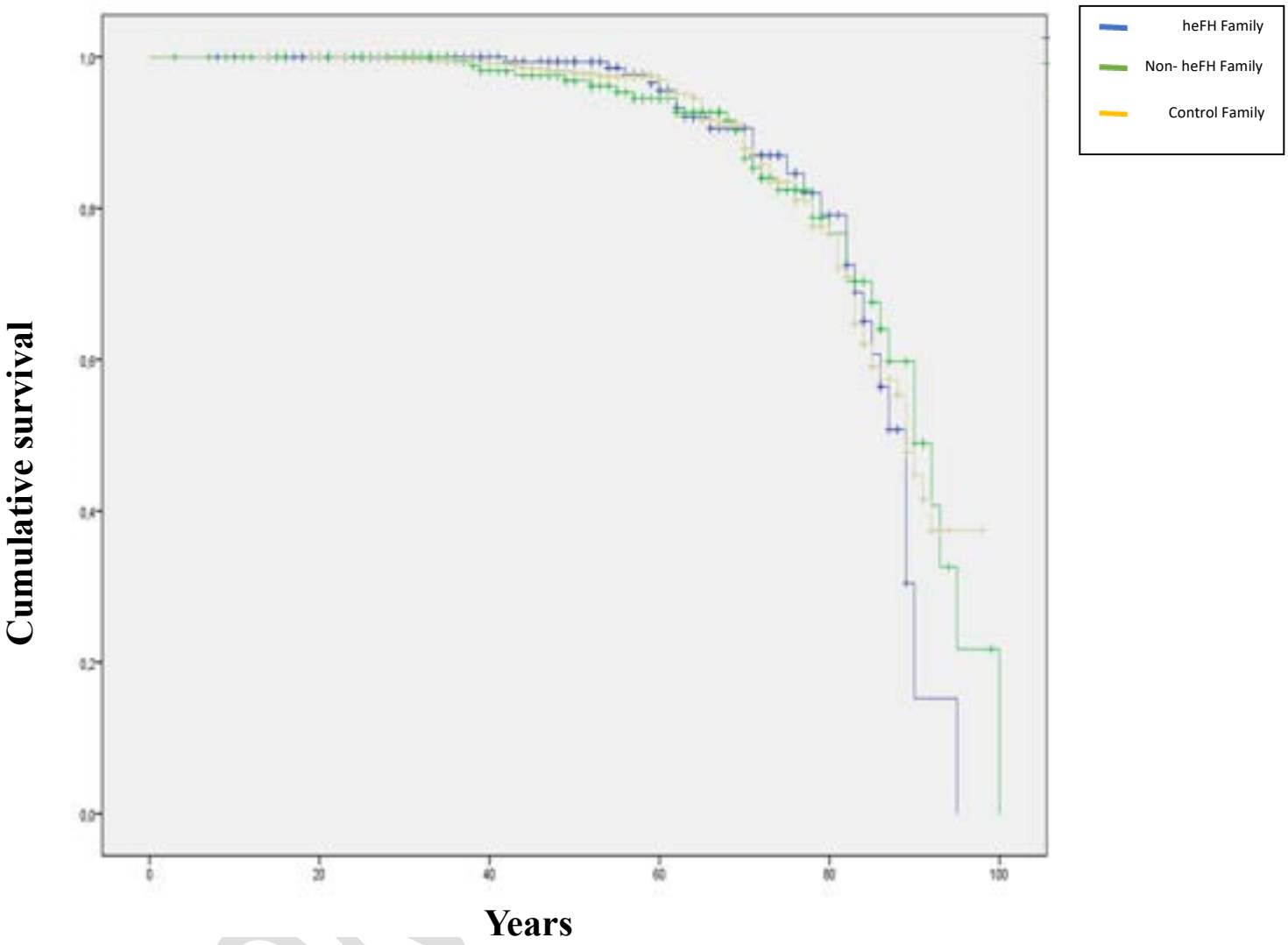
Figure 2.



	20	40	50	60	70	80	90	100
heFH family members, N=207	194	154	125	87	50	25	1	0
Nº cumulate death	0	0	5	15	23	30	37	37
Non-heFH family members, N =215	206	158	133	104	68	36	8	0
Nº cumulate death	0	0	1	3	4	11	17	20
Control family members, N=355	351	325	279	202	125	68	14	0
Nº cumulate death	0	0	1	5	13	24	33	37

Survival analysis free of cardiovascular death among groups.

Figure 3.



	20	40	50	60	70	80	90	100
heFH family members, N=207	194	154	125	87	50	25	1	0
Nº cumulate death	0	0	1	5	9	14	24	25
Non-heFH family members, N =215	206	158	133	104	68	36	29	0
Nº cumulate death	0	3	5	8	15	21	8	33
Control family members, N=355	351	325	279	202	125	68	14	0
Nº cumulate death	0	3	7	9	25	39	59	61

Survival analysis free of non-cardiovascular death among groups

Clinica e Investigacion en Arteriosclerosis 33 (2021) 10–17

Sociedad Española de Arteriosclerosis

CLÍNICA E INVESTIGACIÓN EN ARTERIOSCLEROSIS

www.elsevier.es/arterio

ORIGINAL

Carga de enfermedad. Cálculo del riesgo cardiovascular y objetivos terapéuticos

Victoria Marco-Benedí, Estíbaliz Jarauta, Martín Laclaustra y Fernando Civeira*

Unidad de Lípidos, Servicio de Medicina Interna, Hospital Universitario Miguel Servet, IIS Aragón, CIBERCV, Universidad de Zaragoza, Zaragoza, Aragón, España

Recibido el 2 de diciembre de 2020; aceptado el 14 de diciembre de 2020

PALABRAS CLAVE
Cálculo de riesgo cardiovascular;
Objetivos terapéuticos;
Prevención;
SCORE

Resumen La intervención terapéutica debe estar condicionada por el riesgo de aparición de enfermedad cardiovascular ateromatosa. Cuanto mayor es el riesgo, más intensa debe ser la acción. Por ello tenemos que estratificar el riesgo de los pacientes. En prevención primaria, las dos directrices principales: *American College of Cardiology* y *American Heart Association* (ACC/AHA) utilizan las «*pooled cohort equations*» (PCE) y las guías de las sociedades europeas, las tablas SCORE. Las PCE calculan riesgo de ECVA mortal y no mortal, y el SCORE calcula únicamente riesgo de ECVA mortal. En personas jóvenes es útil considerar el cálculo del riesgo a lo largo de la vida. La Sociedad Española de Arteriosclerosis (SEA) recomienda el sistema SCORE en nuestro país. SCORE y PCE calculan para personas hasta los 70 y 75 años. La predicción y los potenciales están disponibles para personas de 80 años o más, a partir de esa edad los datos disponibles son mucho más escasos. La estratificación del riesgo en prevención secundaria puede ser útil para identificar al subgrupo de pacientes que pueden beneficiarse de tratamientos más intensivos. Las pruebas de imagen, especialmente el calcio coronario y ecografía vascular, pueden ayudar a perfilar mejor el riesgo.

Las guías europeas señalan al colesterol LDL como objetivo terapéutico. Recomiendan iniciar tratamiento con estatinas y ascender en dosis y potencia hasta lograr los objetivos y luego el tratamiento con estatinas potentes a dosis máxima tolerada, y dar ezetimiba en caso de no alcanzar objetivos. Como tercer escalón indican los inhibidores de PCSK9 (iPCSK9). Establecen objetivos muy ambiciosos que llegan a 40 mg/dL, en aquellos sujetos con recurrencias antes de dos años de ECVA, a pesar de tratamiento con estatinas de alta intensidad e inferiores a 55 mg/dL para todos los sujetos de muy alto riesgo.

* Autor para correspondencia.
Correo electrónico: civeira@unizar.es (F. Civeira).

https://doi.org/10.1016/j.arteri.2020.12.006

KEYWORDS
Cardiovascular risk estimation;
Therapeutic goals;
Prevention;
SCORE

Burden of disease Calculation of cardiovascular risk and therapeutic objectives

Abstract Therapeutic intervention should be determined by the risk of developing atheromatous cardiovascular disease (CVD). The higher the risk, the more intense the action should be. This is the reason for the stratification of patient risk. In primary prevention, the two main guidelines used, the American Heart Association and the American College of Cardiology (ACC/AHA) use the Pooled cohort equations (PCE) and the guidelines of the European societies use the SCORE tables. The PCE calculates the risk of fatal and non-fatal CVD, and the SCORE calculates risk of fatal CVD only. In young people, it is useful to consider the lifetime risk calculation. The Spanish Society of Arteriosclerosis (SEA) recommends the SCORE system in Spain. SCORE and PCE calculate the risk for people up to 70 and 75 years of age. Prediction and potentials are available for 80 years of age and above, with the data available being much more scarce. Risk stratification in secondary prevention may be useful to identify the subgroup of patients who may benefit from more intensive treatment. Imaging tests, especially coronary calcium scans and vascular ultrasound, can help to better the profile risk.

European guidelines identify LDL cholesterol as a therapeutic target. They recommend initiating treatment with statins, and increasing dose and potency until targets are achieved, and then to treatment with potent statins at a maximum tolerated dose, and ezetimibe if targets are not achieved. As a third step, PCSK9 inhibitors are indicated. They set very ambitious targets, as low as 40 mg/dL in those subjects with recurrences before two years of CVD despite high-intensity statin therapy, and below 55 mg/dL for all very high-risk subjects.

Introducción

Riesgo cardiovascular

Todas las guías actuales para la prevención de las enfermedades cardiovasculares de origen en la arteriosclerosis (ECVA) recomiendan la evaluación del riesgo total de desarrollar la enfermedad en un plazo de tiempo determinado. La intervención terapéutica debe estar condicionada por el riesgo de aparición de la ECVA: cuanto mayor es el riesgo, más intensa debe ser la acción[1,2].

Dichas recomendaciones se fundamentan en que la indicación de cualquier tratamiento debe estar basada en tres factores: beneficio obtenido por la intervención, perjuicios atribuidos a la intervención y esfuerzo económico, social y personal de la intervención, que son criterios especialmente relevantes al tratar factores de riesgo prevalentes, orientados a la prevención en muchos casos en sujetos asintomáticos. De este modo, una intervención como prescribir estatinas de potencia intermedia de bajo precio, por ejemplo, la simvastatina, reúne todos los requisitos: es eficaz, con escasos efectos secundarios, es muy coste-efectiva por el bajo precio de la medicación, es fácil de implementar en la mayor parte de sistemas sanitarios con sus actuales estructuras sin costes adicionales y de fácil cumplimento por parte de una población bien informada. Pero no todos los tratamientos eficaces tienen este perfil. Por precio, por efectos secundarios o por un esfuerzo adicional no asumido por el sistema o por el individuo, en ocasiones un tratamiento eficaz, como puede ser un cambio de estilo de vida en el tratamiento de la obesidad y la dislipemia, tiene un éxito muy pobre para el esfuerzo terapéutico que requiere[3].

Por ello tenemos que estratificar las intervenciones de acuerdo con criterios mucho más pragmáticos, y movernos con dos conceptos clave: el NNT (número de pacientes necesarios a tratar para evitar un evento) y el ENA (esfuerzo necesario para aplicar la intervención).

Hay muchos sistemas de evaluación de riesgo disponibles. Idealmente, las tablas de riesgo deberían basarse en datos de cohortes específicos de cada país, pero no están disponibles para la mayoría de los países. Las dos directrices principales en el tratamiento de la hipercolesterolemia recomiendan ecuaciones diferentes. Las guías de las sociedades americanas: *American College of Cardiology* y *American Heart Association* (ACC/AHA)[2] utilizan las denominadas «*pooled cohort equations*» (PCE), creadas en 2003 con el objetivo de estimar el riesgo a 10 años de desarrollar un primer evento de ECVA[4]. Utilizaron los datos de cohortes americanas con criterios de valoración adjudicados para muerte por cardiopatía coronaria, infarto de miocardio no mortal y accidente cerebrovascular mortal o no mortal. Seleccionaron cohortes que incluían participantes afroamericanos o blancos con al menos 12 años de seguimiento: los estudios de riesgo de aterosclerosis en comunidades (ARIC), *Cardiovascular Health Study*, *Coronary Artery Risk Development in Young Adults* (CARDIA) y Framingham original y su descendencia (*Framingham Offspring Study*)[5,6]. La ecuación PCE estima el riesgo a 10 años en sujetos entre 40 y 75 años, sin evento ECVA previo. También puede estimar el riesgo a largo plazo a partir de los 21 años de edad.

Las recientes directrices de las sociedades europeas de cardiología y arteriosclerosis (ESC y EAS, respectivamente) sobre el tratamiento de hipercolesterolemia en la prevención de las ECVA recomiendan el uso de la *Systematic*

Coronary Risk Estimation (SCORE) porque se basa en conjuntos de datos de grandes estudios de cohortes representativas europeas y porque es relativamente sencillo recalibrarla para países individuales[1,7]. La ecuación SCORE estima el riesgo acumulado a 10 años de un primer evento aterosclerótico fatal en prevención primaria y excluye a las personas con diabetes, enfermedad renal crónica o niveles muy altos de un factor de riesgo concreto (como ocurre con la concentración de colesterol LDL en los sujetos con hipercolesterolemia familiar), ya que todas ellas ya se encuentran generalmente en niveles altos o muy altos de riesgo de ECVA. No se necesitan modelos de estimación de riesgos para estas personas; todos necesitan una gestión activa de todos los factores de riesgo[1]. Además, el sistema SCORE incluye cálculos diferentes para los países de Europa de alto riesgo y de bajo riesgo de ECVA. Si bien cualquier punto de corte es arbitrario, utilizando datos de la Organización Mundial de la Salud (OMS), los países se clasifican como de bajo riesgo si su tasa de mortalidad por ECVA de 2016 ajustada por edad fue de < 150/100.000[8]. España está incluida entre los países de riesgo bajo.

Las características principales de estos sistemas de evaluación de riesgo y sus diferencias más notables se describen en la tabla 1. Una de las diferencias principales entre los sistemas PCE y SCORE es que el primero, PCE, calcula riesgo de ECVA mortal y no mortal, y el segundo, SCORE, calcula únicamente riesgo de ECVA mortal. La razón para recomendar un sistema que estima solo eventos fatales en lugar de fatales y no fatales es que estos últimos dependen de su definición, de la realización de pruebas de diagnóstico no siempre estandarizadas y de los métodos de verificación de eventos, todos los cuales pueden variar entre estudios, lo que complica su utilización en la elaboración de la ecuación y su posterior interpretación. Para poder comparar ambos sistemas, se conoce que el riesgo de eventos totales de ECVA es aproximadamente tres veces mayor que el riesgo de ECVA fatal para los hombres, 3,5 veces mayor en mujeres y unas 2,5 veces mayor en personas mayores de 65 años. Es decir, en un varón de 55 años un riesgo de SCORE del 5% se traduce en un riesgo de ECVA mortal y no mortal del 15%[1].

Existen otras muchas ecuaciones de riesgo publicadas en los últimos años, que incorporan pequeñas variaciones en los factores de riesgo a utilizar. Muchas de ellas son específicas de un país determinado (tabla 2). En España ha sido muy utilizado el sistema de predicción basado en el estudio REGICOR, que calibra la ecuación de Framingham para nuestra población[9]. Sin embargo, en la actualidad tanto la Sociedad Española de Arteriosclerosis (SEA)[10] como la de Cardiología (SEC)[11] recomiendan el sistema SCORE para «países de bajo riesgo» en nuestro medio.

Otros factores que modifican el riesgo de ECVA

Las ecuaciones de riesgo de PCE y SCORE reconocen que con los factores de riesgo mayores que incluyen edad, sexo, colesterol total, colesterol HDL, tabaco y tensión arterial se identifican muy bien con sujetos de riesgo muy elevado, pero son un porcentaje pequeño de la población, especialmente en edades medias de la vida, y que la mayor parte de ECVA ocurre en personas de riesgo intermedio. Por ese motivo ambos sistemas recomiendan valorar otros factores de riesgo peor establecidos, pero que pueden ayudar a reclasificar a sujetos de riesgo intermedio/moderado. Este grupo de factores modificadores incluyen parámetros lipídicos como la lipoproteína(a), o la apolipoproteína B, marcadores inflamatorios como la proteína c reactiva, determinadas enfermedades como las enfermedades inflamatorias crónicas o la enfermedad renal crónica, o factores psicosociales como el estrés o la exclusión social[1,2]. La utilización en la práctica clínica de estos marcadores no es sencilla, ya que la definición y puntos de corte son totalmente arbitrarios en la mayor parte de los factores, por lo que su utilización se deja al criterio clínico del médico. Muchos otros biomarcadores también se asocian con un mayor riesgo de ECVA, aunque se ha demostrado que pocos de ellos se asocian con una reclasificación apreciable[13].

Riesgo absoluto, riesgo relativo y riesgo a lo largo de la vida

El riesgo absoluto corresponde a la predicción de ECVA para un período futuro determinado. Tanto en PCE como en SCORE se recomienda considerar el riesgo a 10 años. El riesgo relativo es la relación entre un determinado riesgo absoluto de ECVA y un riesgo determinado, habitualmente un riesgo absoluto bajo o bien el riesgo medio de una población determinada. El grupo de comparación de bajo riesgo se caracteriza comúnmente como el que corresponde con presión arterial menor de 120/80 mmHg, colesterol total entre 160 y 199 mg/dL, colesterol HDL $\geq$ 45 mg/dL para hombres y $\geq$ 55 mg/dL para mujeres, en personas no fumadoras y sin diabetes[14]. Prestar atención al riesgo relativo se aconseja en las guías de las sociedades europeas para las personas jóvenes. Un problema particular que tiene la prevención en los jóvenes con factores de riesgo extremos o múltiples factores es que tienen un riesgo absoluto a 10 años muy bajo, pero un riesgo relativo muy alto, por lo que el cálculo de este último nos ayuda a valorar mejor a eficacia de las medidas preventivas. El principal problema es que no existen recomendaciones basadas en la evidencia del beneficio de la intervención, según el riesgo relativo y, nuevamente, se deja al criterio clínico la actuación en aquellos casos de discrepancia entre el riesgo absoluto y relativo[1]. Una forma sencilla de solucionar la discordancia entre riesgo absoluto y relativo es la indicación de tratamiento a todas aquellas personas con un factor de riesgo extremo con independencia de su riesgo absoluto calculado con las ecuaciones e independientemente de su edad. Al igual que ocurre en el tratamiento de la hipertensión arterial, donde todas las recomendaciones están de acuerdo en iniciar tratamiento farmacológico en aquellos pacientes con hipertensión arterial grados 2 y 3 (> 159/99 mmHg)[15], las guías americanas recomiendan iniciar tratamiento hipolipemiante con estatinas de alta potencia en sujetos $\geq$ 20 años con cifras de colesterol LDL $\geq$ 190 mg/dL. Una indicación semejante se establece en las guías europeas. Otra forma de valorar el riesgo en personas jóvenes, es considerar el cálculo del riesgo total a lo largo de la vida[16]. El riesgo a 20 años se puede calcular duplicando la puntuación de riesgo de Framingham a 10 años. Pencina et al. han desarrollado un algoritmo de función para predecir el riesgo a 30 años,

Tabla 1 Estratificación del riesgo cardiovascular en las dos principales recomendaciones internaciones

	AHA/ACC	ESC/EAS
Prevención primaria		
Ecuación para el cálculo de riesgo	*Pooled cohort equations*	SCORE
Tiempo predicción	10 años	10 años
Eventos predichos	Infartos e ictus mortales y no mortales	Mortalidad cardiovascular ateromatosa
Factores de riesgo	Edad, raza, sexo, colesterol, HDLc, PAS, diabetes, tabaco y tratamiento HTA	Edad, sexo, colesterol, HDLc, PAS, tabaco
Grupos de riesgo	Alto $\geq$ 20% Intermedio $\geq$ 7,5-20% Límite $\geq$ 5-< 7,5% Bajo < 5%	Muy alto $\geq$ 10% Alto $\geq$ 5-< 10% Moderado $\geq$ 1-< 5 Bajo < 1%
Factores adicionales que modifican riesgo	Historia familiar LDLc 160-189 mg/dL Síndrome metabólico Enfermedad renal crónica Proteína C reactiva Inflamación crónica Lipoproteína(a) Apolipoproteína B Índice tobillo/brazo < 0,9 Menopausia precoz Preeclampsia	Historia familiar Marginación social Obesidad Sedentarismo Enfermedad renal crónica (FG < 30 mL/min) Estrés psicosocial Inflamación crónica Fibrilación auricular Sida Apnea obstructiva sueño Hipertrofia ventricular izquierda
Ateromatosis subclínica	Calcio coronario en riesgo intermedio	Calcio coronario y/o ecografía carotídea y femoral en riesgo bajo o moderado
Prevención secundaria		
Definición	Síndrome coronario agudo, IAM, angina estable o inestable o revascularización coronaria u otra arteria, ictus, ataque isquémico transitorio (AIT) o enfermedad arterial periférica), aneurisma aórtico, todos ateroscleróticos	Todo evento arterioscleroso documentado, bien sea clínico o inequívoco en imagen: placa significativa en la angiografía coronaria o TAC (enfermedad coronaria multivaso con dos arterias epicárdicas principales que tienen > 50% de estenosis), o en la ecografía carotídea.
Grupos de riesgo	Muy alto riesgo Alto riesgo	Todos considerados de muy alto riesgo
Factores adicionales que modifican riesgo	Muy alto riesgo si: Eventos cardiovasculares múltiples o Evento cardiovascular + múltiples condiciones de riesgo: edad $\geq$ 65 años, hipercolesterolemia familiar, historia previa de revascularización coronaria, diabetes, HTA, enfermedad renal crónica (FG 15-59 mL/min), tabaquismo, LDLc $\geq$ 100 mg/dL, a pesar de estatinas con ezetimiba, insuficiencia cardiaca	

AHA/ACC: *American Heart Association/American College of Cardiology*; ESC/EAS: *European Society of Cardiology/European Atherosclerosis Society*; HDLc: colesterol transportado en las lipoproteínas de alta densidad; PAS: presión arterial sistólica; LDLc: colesterol transportado en las lipoproteínas de baja densidad; HTA: hipertensión arterial; FG: filtrado glomerular; IAM: infarto agudo de miocardio.

de acuerdo con los factores de riesgo convencionales que siguen siendo los predictores más sólidos[17]. Tanto la AHA como la ACC tienen calculadoras de riesgo a lo largo de la vida[2].

Riesgo en ancianos

Las ecuaciones de SCORE y PCE están diseñadas para personas hasta los 70 y 75 años, respectivamente, y ambas tienden

Tabla 2 Otras ecuaciones utilizadas en la estimación de riesgo cardiovascular en sujetos sin enfermedad cardiovascular ateromatosa

Ecuación (región)	Tiempo predicción	Tipo enfermedad	Predictores
Framingham (EE. UU.)	10 años	Eventos coronarios	Sexo, edad, colesterol total, HDLc, PAS, tabaquismo, diabetes, tratamiento hipertensión
ASSIGN (Escocia)	10 años	Eventos cardiovasculares	Sexo, edad, colesterol total, HDLc, tabaquismo, diabetes, pobreza, historia familiar
QRISK2 (Inglaterra y Gales)	10 años	Eventos cardiovasculares	Sexo, edad, colesterol total/HDLc, tabaquismo, diabetes, pobreza, historia familiar, IMC, tratamiento antihipertensivo, etnia, artritis reumatoide, enfermedad renal crónica (FG < 30 mL/min), fibrilación auricular
PROCAM (Alemania)	10 años	Eventos coronarios e ictus isquémico	Sexo, edad, LDLc, HDLc, diabetes, tabaquismo, PAS
REYNOLS (EE. UU.)	10 años	Eventos cardiovasculares, incluyendo revascularización coronaria	Sexo, edad, PAS, tabaquismo, PCR, colesterol total, HDLc, antecedentes familiares de IAM (padres < 60 años), HbA1c
REGICOR (España)	10 años	Infarto miocardio mortal y no mortal, infarto silente, angina	Edad, sexo, tabaquismo, diabetes, colesterol total, HDLc, PAS, PAD
GLOBORISK (mundial)	10 años	Mortalidad cardiovascular	Sexo, edad, tabaco, PAS, diabetes, colesterol total

HDLc: colesterol transportado en las lipoproteínas de alta densidad; PAS: presión arterial sistólica; IMC: índice de masa corporal; FG: filtrado glomerular; LDLc: colesterol transportado en las lipoproteínas de baja densidad; PCR: proteína C reactiva; IAM: infarto agudo de miocardio; PAD: presión arterial diastólica.

a sobrevalorar el riesgo en los extremos de edad avanzada, como hemos señalado anteriormente. Los diferentes ensayos clínicos de forma relativamente consistente demuestran beneficio clínico en adultos hasta los 80 años[18], a partir de esa edad, los datos disponibles son mucho más escasos y las necesidades de tratamiento hipolipemiante más discutibles y, por tanto, la necesidad de una predicción de riesgo es menos importante. A medida que los adultos envejecen, son más susceptibles a los efectos secundarios y algunos riesgos pueden superar al beneficio esperado[19]. Lo razonable es mantener tratamientos instaurados previamente si la calidad y esperanza de vida del sujeto son buenos, y en caso de plantear el inicio de un tratamiento hipolipemiante a partir de los 80 años hacer un proceso de toma de decisiones compartido entre médicos y pacientes donde se valore comorbilidad, polifarmacia, esperanza de vida, así como las preocupaciones y expectativas del enfermo.

Riesgo en prevención secundaria

El documento de recomendaciones de las sociedades europeas califica de muy alto riesgo a un grupo importante de pacientes, entre los que incluye a toda la población con ECVA clínica, o subclínica, cualquier sujeto con hipercolesterolemia familiar junto con un factor de riesgo, sujetos con riesgo SCORE > 10% a 10 años y pacientes con filtrado glomerular < 30 mL/min[1]. Esta aproximación es discutible. Hay que considerar que el riesgo en prevención secundaria es extraordinariamente variable entre sujetos y que conocemos los factores asociados con recurrencias (tabla 3)[20]. La estratificación del riesgo en prevención secundaria puede ser útil para identificar al subgrupo de pacientes que pueden beneficiarse de tratamiento con iPCSK9, como recientemente ha recomendado la SEA y que incluye a sujetos con ECVA, pero con algún factor de riesgo adicional como diabetes, enfermedad polivascular, enfermedad renal crónica o lipoproteína (a) > 50 mg/dL[21]. También las recomendaciones de las sociedades ACC y AHA clasifican a los pacientes con ECVA en dos grupos: alto y muy alto riesgo. Muy alto riesgo en caso de eventos cardiovasculares múltiples, o bien evento cardiovascular asociado con múltiples condiciones de riesgo como edad $\geq$ 65 años, hipercolesterolemia familiar, historia previa de revascularización coronaria, diabetes, HTA, enfermedad renal crónica (filtrado glomerular 15-59 mL/min), tabaquismo, LDLc $\geq$ 100 mg/dL, a pesar de estatinas con ezetimiba e insuficiencia cardiaca (tabla 1).

Papel de las técnicas de imagen cardiovascular no invasivas en la evaluación del riesgo total de enfermedad cardiovascular

Las técnicas de imagen no invasivas pueden detectar la presencia, estimar la extensión y evaluar las consecuencias clínicas del daño vascular aterosclerótico. La detección de la calcificación de las arterias coronarias con tomografía computarizada (TC), sin contraste, proporciona una buena

Tabla 3 Factores de riesgo de recurrencia de enfermedad cardiovascular ateromatosa*

Factores de riesgo	Núm. de factores	Riesgo a 7 años (%)
- Insuficiencia cardiaca - Hipertensión - Edad > 75 años - Diabetes - Enfermedad polivascular - Antecedente de *bypass* coronario - Enfermedad renal crónica (FG < 60 mL/min) - Tabaquismo	0 1 2 3 4 ≥ 5	8,6 14,7 21,5 33,1 48,7 68,4

* Datos obtenidos de Bohula EA, et al. J Am Coll Cardiol 2017;69:911-21.

estimación de la carga aterosclerótica y está fuertemente asociada con el riesgo de desarrollar ECVA[22]. El calcio coronario, aunque no se dispone de estudios aleatorizados para conocer el verdadero valor de su uso clínico, mejora tanto la discriminación como la reclasificación. Por ello, tanto las recomendaciones europeas como las americanas señalan su valor en sujetos con riesgo intermedio/moderado, aunque sin hacer una recomendación explícita de su utilización en la práctica clínica. En general, se debe considerar la evaluación del calcio coronario con TC en individuos con riesgo moderado en quienes el objetivo de colesterol LDL no se logra con medidas higiénico-dietéticas, y la terapia farmacológica es una opción viable. La mayoría de los pacientes con puntuaciones de calcio coronario ≥ 100 unidades de Agatston tienen un riesgo suficientemente elevado a 10 años para empezar tratamiento hipolipemiante[23]. Las guías europeas también consideran la detección de placa por ultrasonidos en arterias carótidas y femorales en la estratificación del riesgo en dos aspectos: Al igual que el calcio coronario, la presencia de placa en cualquiera de los dos territorios estaría indicada en sujetos de riesgo moderado para establecer la indicación de estatinas; y en aquellos sujetos que la ecografía demuestre estenosis ateromatosa > 50% serían considerados directamente como sujetos de muy alto riesgo. Dada la alta prevalencia de placas en la población general, incluida la población española[24], muy superior a la prevalencia de calcio coronario > 100 unidades Agatston, el primer supuesto sería más discutible en ausencia de ensayos clínicos aleatorizados que avalen la modificación terapéutica con base en hallazgos de placa femoral o carotídea.

Tabla 4 Objetivos terapéuticos en las dos principales recomendaciones internaciones de tratamiento de la hipercolesterolemia

	AHA/ACC	ESC/EAS
Prevención primaria		
Grupos de riesgo		
LDLc > 190 mg/dL	Estatina alta potencia	
Diabetes 40-75 años	Estatina potencia intermedia	
Diabetes 40-75 años + factor de riesgo	Estatina alta potencia	
Diabetes 20-39 años + afectación órgano diana	Estatina potencia intermedia	
Riesgo muy alto		LDLc < 55 mg/dL + > 50% reducción
Riesgo alto	Estatina alta potencia	LDLc < 70 mg/dL + > 50% reducción
Riego intermedio	Estatina potencia intermedia	LDLc < 100 mg/dL
Riesgo bajo		LDLc < 116 mg/dL
Ezetimiba	Diabetes 40-75 años + riesgo > 20%, si reducción LDLc < 50% LDLc > 190 mg/dL si reducción LDLc < 50%	Fuera de objetivos con estatinas
Prevención secundaria		
Riesgo muy alto	> 50% reducción + LDLc < 70 mg/dL	LDLc < 55 mg/dL
Riesgo alto	> 50% reducción	
Fármacos no estatinas	Basado en el riesgo	Basado en la cifra de LDLc

* No establece criterio de edad.
AHA/ACC: American Heart Association/American College of Cardiology; ESC/EAS: European Society of Cardiology/European Atherosclerosis Society; LDLc: colesterol transportado en las lipoproteínas de baja densidad.

Objetivos terapéuticos

Una vez establecido el riesgo, los sujetos se clasifican en diferentes grupos de riesgo que son relativamente semejantes para las dos recomendaciones que venimos comentando (tabla 1). Si comparamos las guías americanas y europeas, estas últimas tienden a clasificar a mayor número de sujetos como de riesgo moderado, alto y muy alto, por lo que resultan más intervencionistas que las americanas.

Con respecto a los objetivos basados en el riesgo, ambas recomendaciones siguen patrones de intervención muy diferentes. Las guías europeas señalan al colesterol LDL como objetivo terapéutico (tabla 4). Señalan objetivos de reducción porcentual y objetivos de colesterol LDL a lograr en términos absolutos. Desde el punto de vista farmacológico, recomiendan iniciar tratamiento con estatinas y ascender en dosis y potencia hasta lograr objetivos y luego los tratamientos con estatinas potentes a dosis máxima tolerada, y dar ezetimiba en caso de no lograr objetivos. Como tercer escalón indican los iPCSK9 en todos los sujetos en prevención secundaria fuera de objetivos y en aquellos en prevención primaria de muy alto riesgo, especialmente si tienen hipercolesterolemia familiar. Establecen objetivos muy ambiciosos que llegan a 40 mg/dL en aquellos sujetos con recurrencias antes de dos años de ECVA, a pesar de tratamiento con estatinas de alta intensidad, e inferiores a 55 mg/dL para todos los sujetos de muy alto riesgo[1]. Estas indicaciones tan ambiciosas son un tema complicado porque no existe evidencia sólida de la eficiencia de dichas recomendaciones. Todos los ensayos clínicos aleatorizados con hipolipemiantes nos indican que cuanto más bajo el colesterol LDL, mayor beneficio[25], por lo que, a coste cero, todos los pacientes de alto riesgo deberían llevar triple terapia (estatina potente + ezetimiba + iPCSK9), sin importar sus cifras de colesterol LDL. Pero este no es el escenario real. Una mujer joven con hipercolesterolemia familiar, sin factores de riesgo mayores con una concentración de colesterol LDL > 200 mg/dL requería la triple terapia de por vida para llevarla a 70 mg/dL y esto supone un esfuerzo terapético no asumible.

A diferencia de las guías europeas, las guías de la ACC/AHA siguen fundamentándose en un tipo de fármaco, básicamente estatinas de potencia intermedia o alta, para cada nivel de riesgo[26]. Reservando la ezetimiba solamente en determinados sujetos con hipercolesterolemia familiar o diabetes, y en sujetos de muy alto riesgo, con indicaciones todavía más restrictivas para los iPCSK9 (tabla 4).

En resumen, el tratamiento hipolipemiante tiene como objetivo fundamental reducir el riesgo de ECVA y todas las intervenciones terapéuticas deben ajustarse al riesgo basal del sujeto a tratar. Esto nos obliga a una cuantificación aproximada del riesgo absoluto y relativo del paciente a tratar. La ecuación de riesgo SCORE para países de bajo riesgo es el sistema recomendado en nuestro medio para cuantificar el riesgo de ECVA a 10 años, y las intervenciones deben modularse con base en dicho riesgo calculado. Las pruebas de imagen, especialmente el calcio coronario y la ecografía vascular, pueden ayudar a perfilar mejor el riesgo y establecer un tratamiento más adecuado en sujetos con riesgo moderado. Los estudios de intervención nos indican que el colesterol LDL cuanto más bajo mejor, pero debemos conocer la eficiencia de cada intervención para seleccionar de forma correcta los pacientes a tratar[26].

Conflictos de interés

FC declara haber recibido compensaciones económicas por Advisory boards y/o conferencias de Daiichi Sankyo, Sanofi, Amgen y Ferrer, no relacionadas con la realización de este trabajo.

Nota al suplemento

Este artículo forma parte del suplemento «Lípidos y nuevos tratamientos en dislipemias», que cuenta con el patrocinio de Daiichi-Sankyo.

Bibliografía

1. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. ESC Scientific Document Group 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41:111–88.
2. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;139:e1082–143.
3. Kazi DS, Virani SS. Implications of cost-effectiveness analyses of lipid-lowering therapies: From the policy-maker's desk to the patient's bedside. Prog Cardiovasc Dis. 2019;62:406–13.
4. Goff DCJr, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, et al. American College of Cardiology/American Heart Association Task Force on Practice Guidelines 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129 25 Suppl 2:S49–73.
5. Budoff MJ, Young R, Burke G, Jeffrey Carr J, Detrano RC, Folsom AR, et al. Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events: the multi-ethnic study of atherosclerosis (MESA). Eur Heart J. 2018;39:2401–8.
6. Muntner P, Colantonio LD, Cushman M, Goff DCJr, Howard G, Howard VJ, et al. Validation of the atherosclerotic cardiovascular disease Pooled Cohort risk equations. JAMA. 2014;311:1406–15.
7. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Atherosclerosis. 2016;252:207–74.
8. Cooney MT, Selmer R, Lindman A, Tverdal A, Menotti A, Thomsen T, et al. SCORE and CONOR investigators Cardiovascular risk estimation in older persons: SCORE O.P. Eur J Prev Cardiol. 2016;23:1093–103.
9. Marrugat J, Vila J, Baena-Díez JM, Grau M, Sala J, Ramos R, et al. Validez relativa de la estimación del riesgo cardiovascular a 10 años en una cohorte poblacional del estudio REGICOR [Relative validity of the 10-year cardiovascular risk estimate in

a population cohort of the REGICOR study]. Rev Esp Cardiol. 2011;64:385–94.
10. Armario P, Brotons C, Elosua R, Alonso de Leciñana M, Castro A, Clarà A, et al. Comentario del CEIPV a la actualización de las Guías Europeas de Prevención Vascular en la Práctica Clínica [Statement of the Spanish Interdisciplinary Vascular Prevention Committee on the updated European Cardiovascular Prevention Guidelines]. Hipertens Riesgo Vasc. 2020;S1889-1837:30092–101, 14.
11. Escobar C, Anguita M, Arrarte V, Barrios V, Cequier Á, Cosín-Sales J, et al. Expert Reviewers Recommendations to improve lipid control. Consensus document of the Spanish Society of Cardiology. Rev Esp Cardiol (Engl Ed). 2020;73:161–7.
12. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med. 2002;347:1557–65.
13. Ruwanpathirana T, Owen A, Reid CM. Review on cardiovascular risk prediction. Cardiovasc Ther. 2015;33:62–70.
14. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. Authors/Task Force Members: 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. J Hypertens. 2018;36:1953–2041.
15. Jackson R. Lifetime risk: does it help to decide who gets statins and when? Curr Opin Lipidol. 2014;25:247–53.
16. Pencina MJ, D'Agostino RBSr, Larson MG, Massaro JM, Vasan RS. Predicting the 30-year risk of cardiovascular disease: The Framingham heart study. Circulation. 2009;119: 3078–84.
17. Ridker PM, Lonn E, Paynter NP, Glynn R, Yusuf S. Primary Prevention with Statin Therapy in the Elderly: New Meta-Analyses From the Contemporary JUPITER and HOPE-3 Randomized Trials. Circulation. 2017;135:1979–81.
18. Ho CK, Walker SW. Statins and their interactions with other lipid-modifying medications: safety issues in the elderly. Ther Adv Drug Saf. 2012;3:35–46.
19. Bohula EA, Morrow DA, Giugliano RP, Blazing MA, He P, Park JG, et al. Atherothrombotic Risk Stratification and Ezetimibe for Secondary Prevention. J Am Coll Cardiol. 2017;69:911–21.
20. Ascaso JF, Civeira F, Guijarro C, López Miranda J, Masana L, Mostaza JM, et al. Indications of PCSK9 inhibitors in clinical practice Recommendations of the Spanish Sociey of Arteriosclerosis (SEA), 2019. Clin Investig Arterioscler. 2019;31:128–39.
21. Budoff MJ, Young R, Burke G, Jeffrey Carr J, Detrano RC, Folsom AR, et al. Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events: the multi-ethnic study of atherosclerosis (MESA). Eur Heart J. 2018;39:2401–8.
22. Loria CM, Liu K, Lewis CE, Hulley SB, Sidney S, Schreiner PJ, et al. Early adult risk factor levels and subsequent coronary artery calcification: the CARDIA Study. J Am Coll Cardiol. 2007;49:2013–20.
23. Laclaustra M, Casasnovas JA, Fernández-Ortiz A, Fuster V, León-Latre M, Jiménez-Borreguero LJ, et al. Femoral and Carotid Subclinical Atherosclerosis Association With Risk Factors and Coronary Calcium: The AWHS Study. J Am Coll Cardiol. 2016;67:1263–74.
24. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38:2459–72.
25. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. American College of Cardiology/American Heart Association Task Force on Practice Guidelines 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129 25 Suppl 2:S1–45.
26. Navarese EP, Robinson JG, Kowalewski M, Kolodziejczak M, Andreotti F, Bliden K, et al. Association Between Baseline LDL-C Level and Total and Cardiovascular Mortality After LDL-C Lowering: A Systematic Review and Meta-analysis. JAMA. 2018;319:1566–79.

Nutrition, Metabolism & Cardiovascular Diseases (2021) **31**, 1594–1603

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Impact of statin therapy on LDL and non-HDL cholesterol levels in subjects with heterozygous familial hypercholesterolaemia

Elisenda Climent [a,b], Victoria Marco-Benedí [c], David Benaiges [a,b,d], Xavier Pintó [e], Manuel Suárez-Tembra [f], Núria Plana [g], Hannia Lafuente [e], Emilio Ortega-Martínez de Victoria [h], Ángel Brea-Hernando [i], Àlex Vila [j], Fernando Civeira [c], Juan Pedro-Botet [a,b,d,*]

[a] Endocrinology and Nutrition Department. Hospital Del Mar; Paseo Marítimo, 25-29; E-08003, Barcelona, Spain
[b] Department of Medicine, Universitat Autònoma de Barcelona. Campus Universitari Mar; Dr. Aiguader, 80; E-08003, Barcelona, Spain
[c] Lipid Unit, Hospital Universitario Miguel Servet, IIS Aragón, CIBERCV, Universidad de Zaragoza, Zaragoza, Spain
[d] Institut Hospital Del Mar D'Investigacions Mèdiques (IMIM), Dr. Aiguader, 80; E-08003, Barcelona, Spain
[e] Lipid and Vascular Risk Unit, Department of Internal Medicine, Hospital de Bellvitge, CIBEROBN, Hospitalet de Llobregat, Barcelona, Spain
[f] Lipid and Vascular Risk Unit, Hospital San Rafael, A Coruña, Spain
[g] Unitat de Medicina Vascular i Metabolisme, Hospital Universitari Sant Joan. IISPV, CIBERDEM, Universitat Rovira i Virgili, Reus, Spain
[h] Lipid Clinic, Hospital Clinic, CIBEROBN, 08036, Barcelona, Spain
[i] Lipid Unit, Department of Internal Medicine, Hospital San Pedro, Logroño, Spain
[j] Lipid Unit, Department of Internal Medicine, Hospital de Figueres, Figueres, Girona, Spain

Received 12 December 2020; received in revised form 13 January 2021; accepted 19 January 2021
Handling Editor: A.B. Cefalù
Available online 28 January 2021

KEYWORDS
Ezetimibe;
Familial hyper cholesterolaemia;
LDL cholesterol;
Lipid-lowering treatment;
Statins

Abstract *Background and aims:* Cardiovascular risk in heterozygous familial hypercholesterolaemia (HeFH) is driven by LDL cholesterol levels. Since lipid response to statin therapy presents individual variation, this study aimed to compare mean LDL and non-HDL cholesterol reductions and their variability achieved with different types and doses of the most frequently prescribed statins.

Methods and results: Among primary hypercholesterolaemia cases on the Spanish Arteriosclerosis Society registry, 2894 with probable/definite HeFH and complete information on drug therapy and lipid profile were included.

LDL cholesterol reduction ranged from 30.2 ± 17.0% with simvastatin 10 mg to 48.2 ± 14.7% with rosuvastatin 40 mg. After the addition of ezetimibe, an additional 26, 24, 21 and 24% reduction in LDL cholesterol levels was obtained for rosuvastatin, 5, 10, 20 and 40 mg, respectively. Subjects with definite HeFH and a confirmed genetic mutation had a more discrete LDL cholesterol reduction compared to definite HeFH subjects with no genetic mutation. A suboptimal response (<15% or <30% reduction in LDL cholesterol levels, respectively with low-/moderate-intensity and high-intensity statin therapy) was observed in 13.5% and, respectively, 20.3% of the subjects.

Conclusion: According to the LDL cholesterol reduction in HeFH patients, the ranking for more to less potent statins was rosuvastatin, atorvastatin and simvastatin; however, at maximum dosage, atorvastatin and rosuvastatin were nearly equivalent. HeFH subjects with positive genetic diagnosis had a lower lipid-lowering response. Approximately 1 in 5 patients on high-intensity statin therapy presented a suboptimal response.

* Corresponding author. Endocrinology and Nutrition Department, Hospital Universitario del Mar, Paseo Marítimo, 25-29; E-08003, Barcelona, Spain. Fax: +34 932483254.
E-mail address: 86620@parcdesalutmar.cat (J. Pedro-Botet).

https://doi.org/10.1016/j.numecd.2021.01.014
0939-4753/

Introduction

Heterozygous familial hypercholesterolaemia (HeFH), the most frequent monogenic disorder of human metabolism caused by mutations in the genes encoding for the low-density lipoprotein (LDL) receptor [1], apolipoprotein (Apo) B [2], proprotein convertase subtilisin/kexin-type 9 (PCSK9) [3] or apo E [4], entails high LDL cholesterol concentrations. Natural history studies in HeFH revealed that approximately 50% and 30% of men and women, respectively, would develop coronary heart disease by the age of 50 [5–7]. The introduction and widespread use of statins in recent years has markedly improved the prognosis for these patients [8–11]. However, the sad reality is that HeFH patients are undertreated and the achievement rate of therapeutic goals is unacceptably low [12–15]. Therefore, the 2019 *European Society of Cardiology* (ESC)/*European Atherosclerosis Society* [16] (EAS) guidelines for the management of dyslipidaemias advocate starting cholesterol-lowering treatment with high-intensity statins, in most cases combined with ezetimibe, as soon as possible after HeFH diagnosis and recommend a more aggressive LDL cholesterol therapeutic target in this specific population.

It has been assumed that the relative reduction in LDL cholesterol with statins in HeFH patients is roughly the same as in the general population regardless of the genetic defect [17,18], although absolute reductions often exceed those reported in the general population owing to higher baseline LDL cholesterol in HeFH patients. Similarly, the established dose-dependent LDL cholesterol reduction with statins and their different potency in terms of LDL-lowering effect also applies to HeFH. Furthermore, the response to the same statin dose revealed a considerable individual variation in patients without HeFH [19,20] and although the exact mechanism remains a matter of debate, high LDL cholesterol levels appear to play a role in this variability [21].

Cardiovascular risk in HeFH is largely driven by LDL cholesterol levels, with the efficacy of lipid-lowering therapy being based on mean LDL cholesterol reductions among randomised trials and among statins performing head-to-head. The present study aimed to compare mean LDL and non-high-density lipoprotein (HDL) cholesterol reductions and their variability achieved with different doses of the 3 most frequently prescribed statins in monotherapy or combined with ezetimibe, using individual data of clinically-defined HeFH subjects of the Spanish Atherosclerosis Society (SEA) Dyslipidaemia Registry. The percentage changes in LDL and non-HDL cholesterol with statins alone in HeFH subjects with a confirmed genetic mutation together with factors associated with a suboptimal response in LDL cholesterol levels were also evaluated.

Methods

Study protocol

The SEA Dyslipidaemia Registry was created on 2013 as an active on-line registry in which 65 certified lipid clinics across Spain report cases of various types of primary hyperlipidaemias [22]. Anonymous clinical data collection in this registry was approved by a central ethics committee (Comité Ético de Investigación Clínica de Aragón, Zaragoza, Spain) in accordance with the 1975 Declaration of Helsinki and participants gave their written informed consent. Minimum data for the inclusion of cases in the registry are: age, sex, smoking status, history of type 2 diabetes mellitus (T2DM), hypertension and cardiovascular disease (CVD) and age at diagnosis, body mass index, waist circumference, complete lipid profile including total cholesterol, LDL cholesterol, triglycerides and HDL cholesterol levels without lipid-lowering treatment at diagnosis.

The registry is designed so that at least once a year the data of the clinical evolution of the included patients is updated, with new anthropometric data, changes in risk factors or medications, and the appearance of new cardiovascular events. This provides an excellent framework to evaluate the impact of lipid-lowering therapies in a real clinical scenario.

CVD is defined as coronary heart disease (myocardial infarction, acute coronary syndrome with stenosis >50% of a main coronary artery and coronary revascularization), stroke (ischaemic and haemorrhagic), aortic aneurysm and lower limb ischaemia (intermittent claudication with ankle/brachial index < 0.9 or revascularization of lower limb arteries). Arterial hypertension is defined as systolic blood pressure $\geq$140 mm Hg or diastolic blood pressure $\geq$90 mm Hg or self-reported use of antihypertensive medication. T2DM is defined as fasting blood glucose >125 mg/dL, HbA1c $\geq$ 6.5% or taking blood glucose-lowering drug therapy. Current smoking is defined as current smoking or having smoked in the last year. Former smoker is defined as a subject having smoked at least 50 cigarettes in his lifetime, but not having smoked in the last year.

Finally, a suboptimal LDL cholesterol improvement is defined as a <15% reduction in LDL cholesterol levels compared to baseline values for patients on low-to moderate-intensity statin treatment (atorvastatin 10–20 mg, rosuvastatin 5–10 mg and simvastatin 10–40 mg). This definition is based on clinical experience and previous studies since no standard criteria have been established [23]. Moreover, based on the results of the VOYAGER meta-analysis [23], this cut-of level was upgraded to a <30% reduction for subjects receiving high-intensity statins (atorvastatin 40–80 mg and rosuvastatin 20–40 mg).

In the present study, all patients from the registry $\geq$18 years of age with probable or definite HeFH according to the Dutch Lipid Clinic Network criteria (DLCN) [7] with complete information on their lipid-lowering therapy and lipid profile before and after receiving specific treatment were included. The last lipid-lowering drug treatment followed by the patient for at least 3 months without changes was collected. Exclusion criteria were lack of data on lipid-lowering therapy or on complete lipid profile, DLCN <6 points and homozygous familial hypercholesterolaemia. Patients who had received statin therapy other than atorvastatin, rosuvastatin

or simvastatin were also excluded. Of the 5620 cases with primary hypercholesterolaemia listed in the SEA registry, 2894 with DLCN ≥6 points were finally included.

Statistical analysis

Data were expressed as mean ± standard deviation for continuous variables. Categorical variables were expressed as percentages and frequencies. The percentage change from baseline in LDL cholesterol and non-HDL was calculated for each patient regarding the different types of statin and dose. A multiple regression logistic model was applied and odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess factors related to a suboptimal LDL cholesterol improvement. A two-sided p value < 0.05 was considered statistically significant. Analyses were performed with SPSS (version 19.0 for Windows; SPSS, Chicago, IL).

Results

Of the 2894 included patients, 540 presented probable and 2354 definite HeFH (Fig. 1). The main clinical characteristics of the patients together with family and personal history and baseline lipid profile are described in Table 1: 1907 (65.9%) presented definite HeFH with a confirmed genetic mutation, 447 (15.4%) definite HeFH with no genetic mutation and 540 (18.7%) probable HeFH. Mean follow-up time of the subjects was 5.2 ± 3.1 years.

LDL cholesterol change

Statins in monotherapy

Atorvastatin was the most frequently prescribed treatment in the 1155 HeFH patients who received statin alone (39.9% of the total included patients), with 40 mg being the most used dosage. The lowest mean percentage LDL cholesterol reduction was observed with 10 mg of simvastatin (30.2 ± 17.0%) while the highest was obtained with rosuvastatin 40 mg (48.2 ± 14.7%). Atorvastatin 10–80 mg reduced LDL cholesterol levels by a mean of 32.5 ± 23.3%, to 48.1 ± 21.8%. As for rosuvastatin, doses of 5–40 mg lowered LDL cholesterol levels by a mean of 35.5 ± 19.6% to 48.2 ± 14.7%. Finally, simvastatin 10–40 mg lowered LDL cholesterol levels by a mean of 30.2 ± 17.0% to 36.1 ± 21.8% (Fig. 2).

Statins combined with ezetimibe

One thousand, seven hundred and thirty-nine HeFH patients were treated with a statin plus ezetimibe (60% of the total included patients), with atorvastatin being the most frequently combined statin; regarding dosage, 40 mg was the most used. Again, the lowest and highest mean percentage LDL cholesterol reduction was obtained with ezetimibe plus simvastatin 10 mg (41.6 ± 22.6%) and plus rosuvastatin 40 mg (59.7 ± 15.5%), respectively. Atorvastatin 10–80 mg with ezetimibe lowered LDL cholesterol by a mean of 45.6 ± 17.2% to 54.9 ± 16.5%. As for rosuvastatin, doses 5–40 mg combined with ezetimibe reduced LDL cholesterol levels by a mean of 44.7 ± 23.4% to

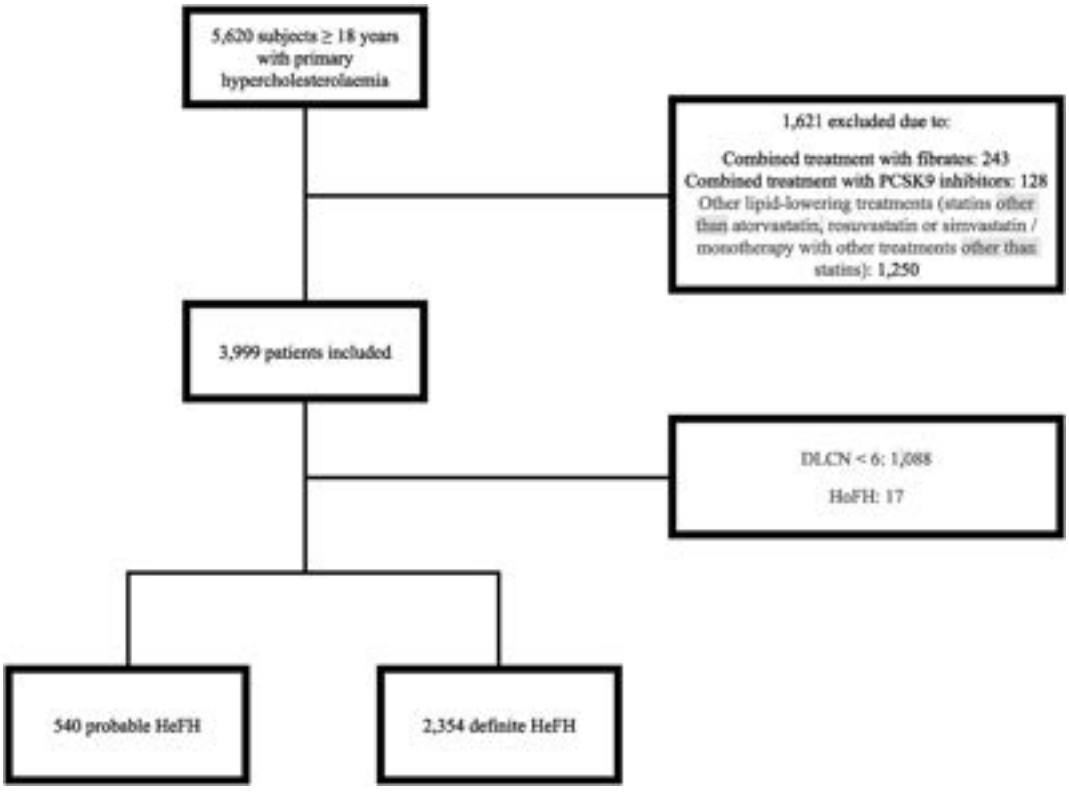


Figure 1 Study flow diagram of the patients included in the database. DLCN: Dutch Lipid Clinic Network; HeFH: heterozygous familial hypercholesterolaemia; HoFH: homozygous familial hypercholesterolaemia; PCSK9: proprotein convertase subtilisin/kexin type 9.

Table 1 Baseline characteristics of the 2894 patients with heterozygous familial hypercholesterolaemia.

VARIABLE	Definite HeFH with mutation	Definite HeFH without mutation	Probable HeFH
DEMOGRAPHIC CHARACTERISTICS			
Total subjects, n (%)	1907 (65.9)	447 (15.4)	540 (18.7)
Male sex, n (%)	903 (47.4)	209 (46.8)	289 (53.5)
Age (years), mean ± SD	49.4 ± 17.2	56.1 ± 12.7	54.8 ± 12.7
BMI (kg/m^2), mean ± SD	25.5 ± 4.7	27.4 ± 4.7	26.8 ± 4.3
GENETIC INFORMATION			
Affected gene, n (%)	*LDLR: 1808 (94.8) APOB/APOE: 82 (4.3) PCSK9: 17 (0.9)*		
Phenotype, n (%)	Simple heterozygous: 1770 (92.8) Combined heterozygous: 82 (4.3)		
FAMILY HISTORY			
Paternal hypercholesterolaemia, n (%)	780 (40.9)	196 (43.8)	193 (35.7)
Maternal hypercholesterolaemia, n (%)	868 (45.5)	185 (41.4)	204 (37.8)
Paternal CVD, n (%)	313 (16.4)	117 (26.2)	137 (25.4)
Maternal CVD, n (%)	153 (8.0)	51 (11.4)	41 (7.6)
Premature CVD in a first-degree relative, n (%)	583 (30.6)	189 (42.3)	172 (31.9)
PERSONAL HISTORY			
CVD, n (%)	265 (13.9)	70 (15.7)	137 (25.4)
T2DM, n (%)	101 (5.3)	35 (7.8)	62 (11.4)
Hypertension, n (%)	302 (15.8)	113 (25.3)	146 (27.0)
Systolic BP (mmHg), mean ± SD	120.8 ± 35.1	125.5 ± 29.2	125.7 ± 28.6
Diastolic BP (mmHg), mean ± SD	74.3 ± 39.4	76.3 ± 17.8	76.0 ± 18.0
Tendinous xanthomata, n (%)	514 (27.0)	289 (64.7)	85 (15.7)
Arcus cornealis before age 45 years, n (%)	494 (25.9)	207 (46.3)	210 (38.9)
Smoking, n (%)	390 (20.5)	121 (27.1)	146 (27.0)
LIPID PROFILE			
Total cholesterol (mg/dL), mean ± SD	347.1 ± 87.3	375.5 ± 81.3	342.1 ± 84.8
HDL cholesterol (mg/dL), mean ± SD	55.0 ± 15.8	55.8 ± 14.6	54.0 ± 14.7
Non-HDL cholesterol (mg/dL), mean ± SD	292.0 ± 88.4	319.7 ± 82.5	288.1 ± 85.3
LDL cholesterol (mg/dL), mean ± SD	268.8 ± 85.6	290.7 ± 81.2	253.2 ± 74.4
Triglycerides (mg/dL), mean ± SD	117.5 ± 82.6	145.1 ± 73.8	163.8 ± 142.9

BMI: body mass index; BP: blood pressure; CVD: cardiovascular disease; HDL: high-density lipoprotein; LDL: low-density lipoprotein; non-HDL: non-high-density lipoprotein; SD: standard deviation; T2DM: type 2 diabetes mellitus.

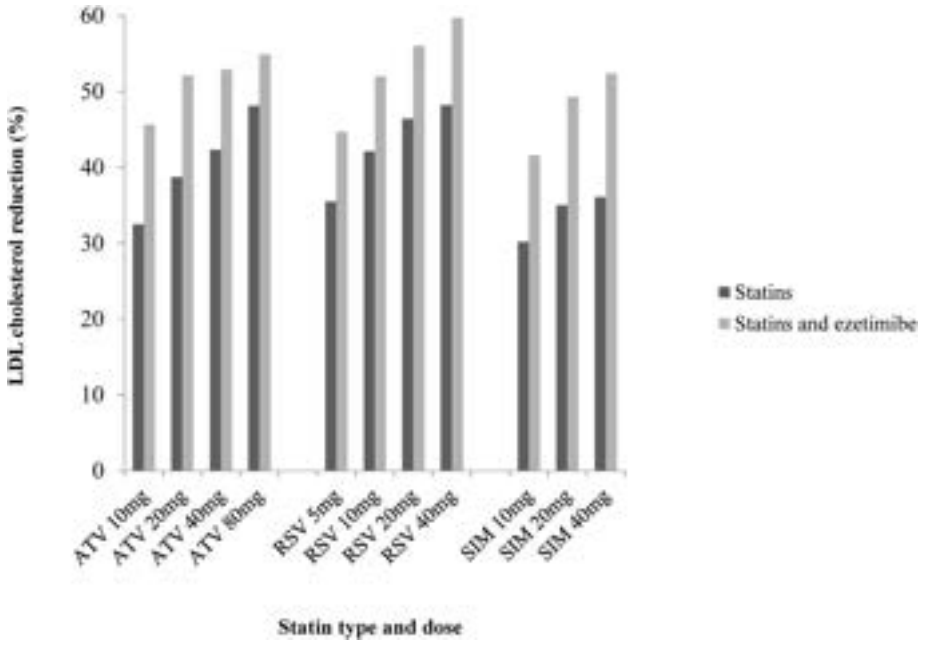


Figure 2 Mean percentage change in LDL cholesterol with statin treatment alone and combined with ezetimibe. ATV: atorvastatin; LDL: low-density lipoprotein; RSV: rosuvastatin; SIM: simvastatin.

59.7 ± 15.5%, and simvastatin 10–40 mg with ezetimibe by a mean of 41.6 ± 22.6% to 52.4 ± 15.1% (Fig. 2).

One hundred and sixty-seven patients (6% of the subjects with monotherapy) achieved an LDL cholesterol level < 100 mg/dL with statins alone (27.5% on atorvastatin 80 mg and 16.8% on rosuvastatin 40 mg) and 329 (28.1%) with combined treatment (24.3% with atorvastatin 80 mg and 21.3% with rosuvastatin 40 mg).

Regarding LDL cholesterol <70 mg/dL, 31 (2.8% of subjects with monotherapy; 25.8% on atorvastatin 80 mg and 25.8% on rosuvastatin 40 mg) and 56 (4.8% of subjects with combined treatment; 21.4% with atorvastatin 80 mg and 21.4% with rosuvastatin 40 mg) reached this specific LDL goal with statin monotherapy and combined treatment, respectively.

Non-HDL cholesterol change

Statins in monotherapy

The lowest mean percentage of non-HDL cholesterol reduction with statins in monotherapy was observed with simvastatin 10 mg (27.9 ± 16.7%) while the highest was attained with atorvastatin 80 mg (46.4 ± 19.5%). Atorvastatin 10–80 mg reduced non-HDL cholesterol levels by a mean of 30.5 ± 22.3% to 46.4 ± 19.5%. As for rosuvastatin, doses 5–40 mg reduced non-HDL cholesterol levels by a mean of 32.6 ± 18.8% to 45.5 ± 14.2%. Finally, simvastatin 10–40 mg obtained a reduction in non-HDL cholesterol levels by a mean of 27.9 ± 16.7% to 35.3 ± 21.7% (Fig. 3).

Statins combined with ezetimibe

The lowest mean percentage change in non-HDL cholesterol was achieved with simvastatin 10 mg and ezetimibe (38.8 ± 22.0%) while the greatest mean percentage reduction was observed with rosuvastatin 40 mg and ezetimibe (56.2 ± 15.3%). Atorvastatin 10–80 mg plus ezetimibe reduced non-HDL cholesterol by a mean of 43.5 ± 17.5% to 51.3 ± 18.5%. Rosuvastatin 5–40 mg with ezetimibe lowered non-HDL cholesterol levels by a mean of 41.4 ± 23.5% to 56.2 ± 15.3%. Finally, simvastatin 10–40 mg and ezetimibe produced a drop in non-HDL cholesterol levels by a mean of 38.8 ± 22.0% to 49.7 ± 15.2%. All these results are summarised in Fig. 3.

LDL and non-HDL cholesterol percentage change in HeFH patients with and without a confirmed genetic mutation

After the effect of statins in monotherapy on LDL and non-HDL cholesterol concentrations had been evaluated, subjects were stratifyied the in 3 groups (definite HeFH with a confirmed genetic mutation, definite HeFH with no genetic mutation, and probable HeFH). In the first group, rosuvastatin 40 mg was superior to the two other statin types and doses regarding LDL cholesterol percentage reduction (47.8 ± 3.9%). With respect to non-HDL cholesterol, atorvastatin 80 mg was better than the other treatment types (45.4 ± 28.1%).

In subjects with DLCN score >8 points but with no genetic mutation, rosuvastatin 40 mg was superior to the

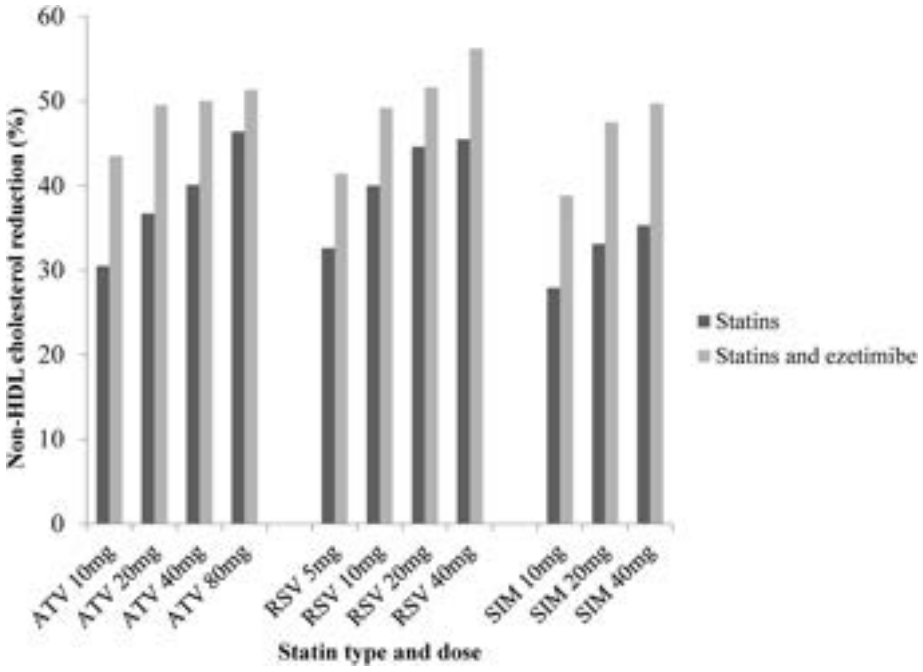


Figure 3 Mean percentage change in non-HDL cholesterol with statin treatment alone and combined with ezetimibe. ATV: atorvastatin; non-HDL: non-high-density lipoprotein; RSV: rosuvastatin; SIM: simvastatin.

Table 2 Mean percentage change in LDL and non-HDL cholesterol with statin treatment in HeFH subjects, stratified as definite HeFH with mutation, definite HeFH without mutation, and probable HeFH.

Statin type and dose	Definite HeFH with mutation			Definite HeFH without mutation			Probable HeFH		
	N (%)	LDL cholesterol reduction (%) (mean ± SD)	Non-HDL cholesterol reduction (%) (mean ± SD)	N (%)	LDL cholesterol reduction (%) (mean ± SD)	Non-HDL cholesterol reduction (%) (mean ± SD)	N (%)	LDL cholesterol reduction (%) (mean ± SD)	Non-HDL cholesterol reduction (%) (mean ± SD)
General results	1907 (65.9)	43.1 ± 25.1	41.0 ± 24.3	447 (15.4)	48.2 ± 26.4	45.6 ± 23.6	540 (18.7)	43.6 ± 27.3	41.0 ± 25.7
Atorvastatin									
10 mg	198 (10.4)	33.6 ± 23.1	28.4 ± 11.1	25 (5.6)	36.1 ± 15.4	33.6 ± 19.2	52 (9.6)	34.4 ± 15.4	29.1 ± 16.6
20 mg	262 (13.7)	37.2 ± 25.3	34.7 ± 20.1	42 (9.4)	39.1 ± 21.3	37.5 ± 17.1	60 (11.1)	37.6 ± 17.1	36.2 ± 15.2
40 mg	282 (14.8)	41.4 ± 21.0	38.1 ± 19.2	49 (11.0)	41.2 ± 31.6	39.9 ± 24.1	82 (15.2)	40.2 ± 25.5	38.5 ± 31.1
80 mg	123 (6.4)	45.2 ± 23.2	45.4 ± 28.1	30 (6.7)	49.4 ± 25.1	45.9 ± 11.0	46 (8.5)	47.3 ± 11.1	46.5 ± 19.0
Rosuvastatin									
5 mg	84 (4.4)	37.1 ± 19.1	34.8 ± 24.7	32 (7.2)	38.3 ± 19.1	35.2 ± 22.2	62 (11.5)	38.8 ± 9.23	35.6 ± 16.6
10 mg	144 (7.6)	43.2 ± 20.0	39.0 ± 18.5	51 (11.4)	43.5 ± 23.7	39.5 ± 41.8	48 (8.9)	39.7 ± 15.1	37.3 ± 23.7
20 mg	178 (9.3)	46.3 ± 16.8	43.2 ± 21.1	48 (10.7)	47.6 ± 25.3	45.7 ± 20.3	32 (5.9)	45.2 ± 25.3	42.3 ± 15.3
40 mg	78 (4.1)	47.8 ± 3.93	44.4 ± 12.1	47 (10.5)	51.0 ± 21.3	48.6 ± 19.3	36 (6.7)	49.2 ± 13.8	45.5 ± 45.7
Simvastatin									
10 mg	91 (4.8)	31.1 ± 19.9	27.5 ± 13.1	43 (9.6)	32.0 ± 30.1	28.1 ± 15.4	43 (8.0)	31.7 ± 17.2	25.3 ± 12.2
20 mg	312 (16.4)	32.8 ± 43.4	30.3 ± 35.9	35 (7.8)	35.5 ± 21.3	30.3 ± 17.2	41 (7.6)	34.2 ± 20.0	27.3 ± 14.8
40 mg	155 (8.1)	35.7 ± 13.8	32.7 ± 21.2	45 (10.1)	39.0 ± 19.2	34.5 ± 5.46	38 (7.0)	38.9 ± 16.5	31.8 ± 54.4

HeFH: heterozygous familial hypercholesterolaemia; LDL: low-density lipoprotein; non-HDL: non-high-density lipoprotein; SD: standard deviation.

other statins both for LDL (51.0 ± 21.3%) and non-HDL cholesterol (48.6 ± 19.3%) normalisation.

Finally, in subjects with probable HeFH, rosuvastatin 40 mg was more effective than the other lipid-lowering treatments for reducing LDL cholesterol (49.2 ± 13.8%), whereas atorvastatin 80 mg was superior for lowering non-HDL cholesterol (46.5 ± 19.0%). These results are further detailed in Table 2.

Factors associated with suboptimal LDL cholesterol level improvement

Finally, 18.1% (atorvastatin 10–20 mg), 13.5% (rosuvastatin 5–10 mg) and 20% (simvastatin 10–40 mg) of subjects had suboptimal response, defined as a <15% reduction in LDL cholesterol levels. As for high-intensity statin therapy, 20.3% and 17.4% of patients receiving atorvastatin 40–80 mg and rosuvastatin 20–40 mg, respectively, presented a <30% reduction in LDL cholesterol levels.

A multiple logistic regression model to assess factors related to a suboptimal response in LDL cholesterol to statin therapy was applied. In this respect, when suboptimal response was considered to be a <15% reduction in LDL cholesterol levels, male sex was independently associated with a greater probability of being a hypo-responder. Moreover, as age or baseline LDL cholesterol levels were higher, the possibility of presenting a poor response to statins was lower. When suboptimal response as a <30% reduction in LDL cholesterol levels (patients being treated with atorvastatin 40–80 mg or rosuvastatin 20–40 mg) was taken into account, only low baseline LDL cholesterol concentrations were found to be an associated factor. A positive genetic diagnosis was not associated with a suboptimal response when both <15% and <30% reduction criteria were considered (Table 3).

Table 3 Logistic regression analysis evaluating factors associated with suboptimal response in LDL cholesterol improvement.

	Odds ratio	95% CI	*p*
Suboptimal response (< 15% reduction in LDL cholesterol levels)[a]			
Sex (male)	1.488	1.177–1.881	**0.001**
Age[b]	0.978	0.970–0.987	**< 0.001**
BMI	1.008	0.925–1.099	0.851
CVD	0.716	0.141–3.647	0.688
T2DM	1.057	0.548–2.039	0.935
Hypertension	1.153	0.762–1.747	0.202
Baseline LDL cholesterol[c]	0.980	0.974–0.987	**< 0.001**
Genetic mutation	1.949	0.967–3.929	0.062
Suboptimal response (< 30% reduction in LDL cholesterol levels)[d]			
Sex (male)	1.362	0.828–2.242	0.224
Age[b]	0.996	0.969–1.023	0.789
BMI	0.969	0.913–1.028	0.296
CVD	2.317	0.845–6.353	0.102
T2DM	2.370	0.457–12.302	0.305
Hypertension	0.752	0.341–1.660	0.481
Baseline LDL cholesterol[c]	0.981	0.976–0.986	**< 0.001**
Genetic mutation	1.610	0.347–1.075	0.08

BMI: body mass index; CI: confidence interval; CVD: cardiovascular disease; LDL: low-density lipoprotein; T2DM: type 2 diabetes mellitus.

Values in bold indicate results with statistical significance.

[a] Only in heterozygous familial hypercholesterolaemia patients treated with atorvastatin 10–20 mg, rosuvastatin 5–10 mg and simvastatin 10–40 mg.

[b] For every 5-year increase in age.

[c] For every 10 mg/dL increase in LDL cholesterol.

[d] Only in heterozygous familial hypercholesterolaemia patients treated with atorvastatin 40–80 mg or rosuvastatin 20–40 mg.

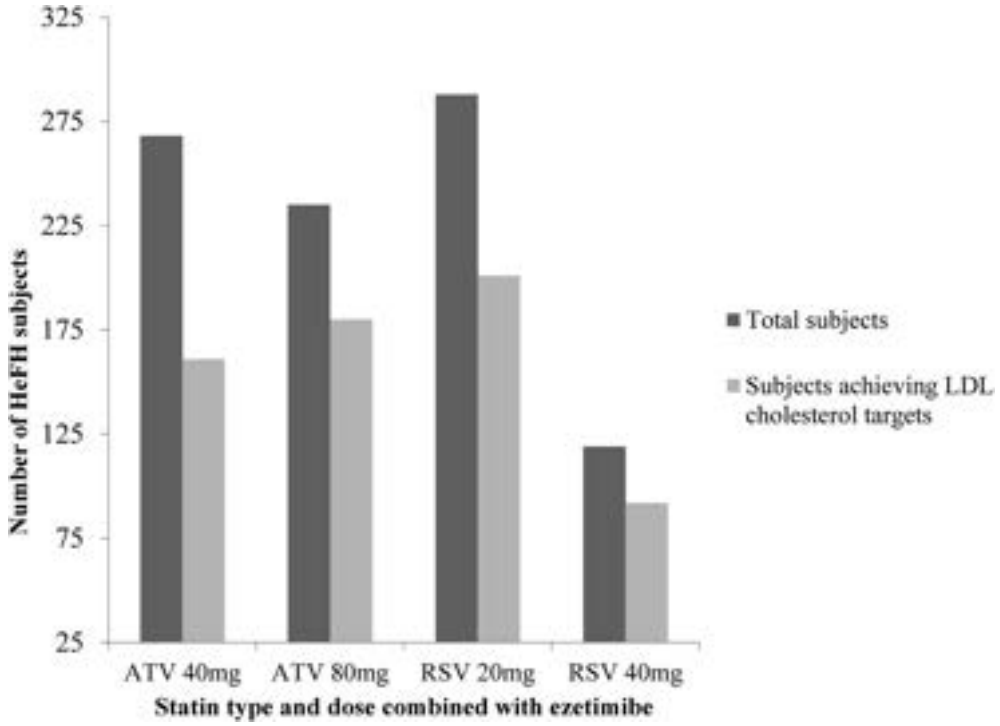


Figure 4 Patients receiving high-intensity statin treatment combined with ezetimibe and achieving LDL cholesterol targets. ATV: atorvastatin; HeFH: heterozygous familial hypercholesterolaemia; LDL: low-density lipoprotein; RSV: rosuvastatin.

Percentage of patients achieving LDL cholesterol targets with high-intensity combined treatment

High-intensity combined treatment includes atorvastatin 40–80 mg or rosuvastatin 20–40 mg, both combined with ezetimibe 10 mg. The numbers of subjects receiving either combination and those achieving LDL cholesterol targets are shown in Fig. 4. The combined treatment with atorvastatin 20 mg yielded the lowest percentage of subjects reaching LDL cholesterol targets (60.1%), while the rosuvastatin 40 mg combination had more favourable results regarding the number of subjects achieving LDL cholesterol goals (77.3%).

Discussion

The present study evaluated LDL and non-HDL reductions in HeFH patients with different types and doses of statins alone or combined with ezetimibe in a real clinical setting. Rosuvastatin 40 mg and atorvastatin 80 mg in monotherapy were superior to the other statins type and doses regarding LDL and non-HDL cholesterol level improvement. As to combined treatment, rosuvastatin 40 mg was superior to the other types of statin regarding LDL and non-HDL cholesterol normalisation, and was also superior in HeFH patients with a positive genetic mutation. A suboptimal response was observed in 13.5–20.3% of subjects, with male sex, younger age and low baseline LDL cholesterol levels being the factors associated with a suboptimal response.

The CURVES study [24], the first to compare the lipid-lowering efficacy of diverse HMG-CoA reductase inhibitors, reported that atorvastatin 10, 20 and 40 mg produced greater LDL cholesterol reductions than the milligram equivalent doses of simvastatin, pravastatin, lovastatin and fluvastatin (rosuvastatin was not available at that time). Furthermore, atorvastatin 10–80 mg showed a mean percentage reduction in LDL cholesterol levels from 38 ± 10% to 54 ± 9%, which was slightly greater than the results obtained in the present study (32.5 ± 23.3% to 48.1 ± 21.8%), and probably due to the present study having focused on the HeFH population.

Furthermore, it has been reported that doubling the statin dose generates on average a further 6% reduction in LDL cholesterol levels [25]. In the present study, however, a higher percentage was achieved, with an extra 15, 18 and 19% decline in LDL cholesterol levels being observed when the lower dose of each statin was doubled. By contrast, doubling the higher doses of atorvastatin, rosuvastatin and simvastatin resulted in more modest results, achieving an extra reduction of 3–13% in LDL cholesterol levels.

The inter-individual variability in the response to statin therapy was also evaluated in the VOYAGER meta-analysis [23]. That study included 32,258 subjects where the mean LDL cholesterol reduction ranged from 28.4 to 55.5% after lipid-lowering treatment with atorvastatin 10–80 mg, rosuvastatin 5–40 mg or simvastatin 10–80 mg, with rosuvastatin being superior to other statins. Hence, as described in previous publications [23,24], in the present study which focused on a real clinical setting with HeFH patients, mg to mg, rosuvastatin was more potent than

atorvastatin and the latter more than simvastatin. However, the maximum doses of atorvastatin and rosuvastatin, 80 and 40 mg, respectively, were nearly equivalent regarding lipid profile normalisation.

Different algorithms for improving LDL cholesterol goals in different clinical settings have been reported [26,27]. However, the variability in statin response in HeFH patients should be taken into account, since it may translate into a significant number of patients never achieving LDL cholesterol therapeutic targets even though receiving high-intensity statins. In this respect, Karlson et al. [23] observed that 2.7–12.7% of subjects experienced a suboptimal response (<15% reduction in LDL cholesterol levels). In the present study, 18.1% (atorvastatin 10–20 mg), 13.5% (rosuvastatin 5–10 mg) and 20% (simvastatin 10–40 mg) of the subjects had a suboptimal response, defined as a <15% reduction in LDL cholesterol levels. This percentage was higher than those reported in previously mentioned studies, suggesting that the LDL-cholesterol-lowering effect of statins is lesser in HeFH subjects than in those with non-FH [28]. These results are in accordance with the significantly lower LDL cholesterol percentage decrease for the HeFH population with a confirmed genetic mutation observed in the present study, in comparison to the rest of the cohort.

Since high-intensity statins are recommended in HeFH subjects owing to their superior efficacy, a suboptimal response was considered in these cases when a <30% reduction in LDL cholesterol concentration from baseline was achieved. Using this cut-off, nearly 1 in 5 patients on high-intensity statin therapy had a suboptimal response, which may indicate the need to initiate combined treatment before changing the statin type or doubling the statin dosage.

Regarding on combined statin plus ezetimibe treatment in the present study, the percentage decline in LDL cholesterol levels was almost 24% higher when ezetimibe was combined with rosuvastatin 40 mg than when the statin was used alone, with a significant increase also being observed after ezetimibe was added to atorvastatin 80 mg and simvastatin 40 mg, respectively. On the same lines, a recent meta-analysis [29] including 12 studies found the addition of ezetimibe to statin therapy to produce a greater absolute LDL cholesterol reduction than statin monotherapy (mean difference: 21.86 mg/dL; 95% CI 26.56 to 17.17; $p < 0.0001$) after 6 months of treatment. These results, concurring with those of the present study, were consistent with the 19–23% LDL cholesterol reduction previously described for ezetimibe when added to statin therapy [30,31]. Moreover, the addition of ezetimibe to statin treatment appears to minimise the variability in LDL cholesterol response, rendering the results more homogeneous. Regarding non-HDL cholesterol, although the mean percentage decline was more discrete than for LDL cholesterol, 39–56% reductions were observed, with these results being superior to those observed with statins in monotherapy.

It would be useful in clinical practice to understand the factors involved in individual variability in statin treatment response and thus identify patients with a greater likelihood of never achieving therapeutic goals. This variability seems to be due to genetic and non-genetic factors. In the present study, male sex, younger age and low baseline LDL cholesterol levels were associated with a suboptimal LDL cholesterol improvement when a <15% reduction was considered. When a <30% reduction was considered a poor response, only baseline LDL cholesterol concentration was found to be an associated factor. On the same lines, the VOYAGER database [23] also found low baseline LDL cholesterol and younger age to be strong predictive factors. Furthermore, Masson et al. [32] described male sex, younger age and low baseline LDL cholesterol values as predictive factors of a suboptimal LDL cholesterol response. Similarly, a Cochrane systematic review [29] in a subgroup analysis found the LDL cholesterol-lowering effect of atorvastatin to be greater in females than in males, and lesser in subjects with FH than in non-FH. A significantly lower percentage decrease in LDL and non-HDL cholesterol was observed in the present cohort in patients with a confirmed genetic mutation. However, a pathogenic mutation did not modify the lipid-lowering response, even though the patients were receiving similar lipid-lowering treatment. When LDL cholesterol targets are not achieved with high-intensity statins combined with ezetimibe in clinical practice, PCSK9 inhibitors are the next therapeutic option. In this regard, recent reports [33,34] obtained favourable results in HeFH subjects, besides LDL cholesterol reduction, with this new lipid-lowering therapy. In this respect, Mandraffino et al. [33] compared the addition of ezetimibe or PCSK9 inhibitors in HeFH subjects who had failed to meet LDL cholesterol targets despite high-intensity statin treatment. The PCSK9 inhibitor group achieved both greater LDL cholesterol and pulse wave velocity reductions than the ezetimibe group (−51% vs −22.8%, $p < 0.001$ and −15% vs −8.5%, $p < 0.01$, respectively).

The present study had some limitations. First, it was an observational study and lipid-lowering treatment was assigned to each patient following clinical criteria. Furthermore, all subjects included were extracted from the SEA Dyslipidaemia Registry, and thus the findings cannot be generalised. Genetic analysis had not been performed in most of the subjects in the group “with no genetic mutation”, and only 9% had had a genetic test that did not reveal a genetic mutation. As the study was conducted in the real world, lipid profile determination was not performed at a centralised laboratory. This also explains the lack of availability of lipoprotein (a) concentrations in all cases, which could account for the lack of therapeutic efficacy of statins in some HeFH subjects. This study focused on most frequently used statins and did not include results on others.

Conclusions

In our real clinical setting, in HeFH patients, rosuvastatin mg to mg was more potent than atorvastatin and this in turn more than simvastatin. However, the maximum doses

of atorvastatin and rosuvastatin, 80 and 40 mg, respectively, were nearly equivalent regarding lipid profile normalisation. HeFH subjects with a confirmed genetic mutation appeared to have a slightly lower lipid-lowering response than other subjects. This suggests that the LDL cholesterol-lowering effect of statins is lesser in individuals with HeFH than in the non-FH population. A great variability was observed in the response to statins and seemed to be minimised when statins were combined with ezetimibe. We believe this updated information will be useful for making clinical decisions to select the most appropriate lipid-lowering therapy for each HeFH patient.

Authors' contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by all authors. Data analysis was performed, and the first draft of the manuscript was written by EC, DB and J.P–B, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgement of grant support

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Declaration of competing interest

Dr. Climent has nothing to disclose. Dr. Marco-Benedí has nothing to disclose. Dr. Benaiges has nothing to disclose. Dr. Pintó reports personal consulting fees from Amgen and Sanofy, and payment for lectures from Astra-Zeneca, Esteve, Ferrer and Mylan. Dr. Manuel Suárez Tembra payment for lectures from Mylan and Sanofi, outside the submitted work. Dr. Plana has nothing to disclose. Dr. Lafuente reports payment for lectures from Amgen, Ferrer, Mylan and Sanofi, outside the submitted work. Dr. Ortega-Martínez de Victoria reports personal fees from Amgen, Esteve, MSD, Sanofi, NovoNordisk, Lilly-Boehringer, non-financial support from Amgen, MSD, Sanofi, NovoNordisk, grants from Amgen, outside the submitted work. Dr. Brea–Hernando payment for lectures from Mylan and Sanofi, outside the submitted work. Dr. Vila has nothing to disclose. Dr. Civeira reports personal fees from Amgen, Daiichi Sankyo, Ferrer, MSD Spain and Sanofy, outside the submitted work. Dr. Pedro-Botet reports consulting fees from Amgen, Mylan and Sanofi, and payment for lectures from Amgen, Astra-Zeneca, Esteve, Ferrer, MSD, Mylan and Sanofi, outside the submitted work.

Acknowledgements

We thank the personnel of Spanish Lipid Clinics for inclusion of cases in the Dyslipidaemia Registry of the Spanish Arteriosclerosis Society, and Miss Christine O'Hara for review of the English version of the manuscript. Sanofi provided a research grant to support the Dyslipemia Registry of the Spanish Atherosclerosis Society, but had no role in the study design, data collection, analysis, and interpretation, report writing, or decision to submit for publication.

References

[1] Brown MS, Goldstein JL. Familial hypercholesterolemia: a genetic defect in the low-density lipoprotein receptor. N Engl J Med 1976;294:1386–90.
[2] Innerarity TL, Mahley RW, Weisgraber KH, Bersot TP, Krauss RM, Vega GL, et al. Familial defective apolipoprotein B-100: a mutation of apolipoprotein B that causes hypercholesterolemia. J Lipid Res 1990;31:1337–49.
[3] Abifadel M, Rabès J-P, Devillers M, Munnich A, Erlich D, Junien C, et al. Mutations and polymorphisms in the proprotein convertase subtilisin/kexin 9 (PCSK9) gene in cholesterol metabolism and disease. Hum Mutat 2009;30:520–9.
[4] Cenarro A, Etxebarria A, de Castro-Orós I, Stef M, Bea AM, Palacios L, et al. The p.Leu167del mutation in APOE gene causes autosomal dominant hypercholesterolemia by down-regulation of LDL receptor expression in hepatocytes. J Clin Endocrinol Metab 2016;101:2113–21.
[5] Stone NJ, Levy RI, Fredrickson DS, Verter J. Coronary artery disease in 116 kindred with familial type II hyperlipoproteinemia. Circulation 1974;49:476–88.
[6] Civeira F. International panel on management of familial hypercholesterolemia guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. Atherosclerosis 2004;173:55–68.
[7] Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. Eur Heart J 2013;34:3478–3490a.
[8] Besseling J, Hovingh GK, Huijgen R, Kastelein JJP, Hutten BA. Statins in familial hypercholesterolemia: consequences for coronary artery disease and all-cause mortality. J Am Coll Cardiol 2016;68:252–60.
[9] Humphries SE, Cooper JA, Seed M, Capps N, Durrington PN, Jones B, et al., Simon Broome Familial Hyperlipidaemia Register Group. Coronary heart disease mortality in treated familial hypercholesterolaemia: update of the UK Simon Broome FH register. Atherosclerosis 2018;274:41–6.
[10] Perez-Calahorra S, Laclaustra M, Marco-Benedí V, Lamiquiz-Moneo I, Pedro-Botet J, Plana N, et al., Dyslipemia Registry of Spanish Arteriosclerosis Society. Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia. Atherosclerosis 2019;284:245–52.
[11] Perez de Isla L, Arroyo-Oliveras R, Alonso R, Muñiz-Grijalvo O, Díaz-Díaz JL, Zambón D, et al. SAFEHEART Investigators. Incidence of cardiovascular events and changes in the estimated risk and treatment of familial hypercholesterolemia: the SAFEHEART registry. Rev Esp Cardiol 2020;73:828–34.
[12] Pijlman AH, Huijgen R, Verhagen SN, Imholz BPM, Liem AH, Kastelein JJP, et al. Evaluation of cholesterol lowering treatment of patients with familial hypercholesterolemia: a large cross-sectional study in The Netherlands. Atherosclerosis 2010;209:189–94.
[13] Beliard S, Carreau V, Carrié A, Giral P, Duchêne E, Farnier M, et al. Improvement in LDL-cholesterol levels of patients with familial hypercholesterolemia: can we do better? Analysis of results obtained during the past two decades in 1669 French subjects. Atherosclerosis 2014;234:136–41.
[14] Lahoz C, Mostaza JM, Pintó X, de la Cruz JJ, Banegas JR, Pedro-Botet J, grupo de investigadores EDICONDIS-ULISEA. LDL-cholesterol control in patients with genetic dyslipidemia followed up by

lipid and vascular risk units of the spanish society of arteriosclerosis. Clín Invest Arterioscler 2015;27:1–8.
[15] Masana L, Plana N, Pérez-Calahorra S, Ibarretxe D, Lamiquiz-Moneo I, Pedro-Botet J, et al. Dyslipidemia Registry of the Spanish Arteriosclerosis Society. How many familial hypercholesterolemia patients are eligible for PCSK9 inhibition? Atherosclerosis 2017; 262:107–12.
[16] Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimón L, et al. ESC Scientific Document Group. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2020;41:111–88.
[17] Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJ, Stalenhoef AF. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolaemia (ASAP): a prospective, randomised, double-blind trial. Lancet 2001;357:577–81.
[18] Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 2005;366:1267–78.
[19] Pedro-Botet J, Schaefer EJ, Bakker-Arkema RG, Black DM, Stein EM, Corella D, et al. Apolipoprotein E genotype affects plasma lipid response to atorvastatin in a gender specific manner. Atherosclerosis 2001;158:183–93.
[20] Boekholdt SM, Hovingh GK, Mora S, Arsenault BJ, Amarenco P, Pedersen TR, et al. Very low levels of atherogenic lipoproteins and the risk for cardiovascular events: a meta-analysis of statin trials. J Am Coll Cardiol 2014;64:485–94.
[21] Ridker PM, Mora S, Rose L, JUPITER Trial Study Group. Percent reduction in LDL cholesterol following high-intensity statin therapy: potential implications for guidelines and for the prescription of emerging lipid-lowering agents. Eur Heart J 2016;37:1373–9.
[22] Pérez-Calahorra S, Sánchez-Hernández RM, Plana N, Valdivielso P, Civeira F. National dyslipidemia registry of the Spanish Arteriosclerosis Society: current status. Clín Invest Arterioscler 2017;29: 248–53.
[23] Karlson BW, Wiklund O, Palmer MK, Nicholls SJ, Lundman P, Barter PJ. Variability of low-density lipoprotein cholesterol response with different doses of atorvastatin, rosuvastatin, and simvastatin: results from VOYAGER. Eur Heart J Cardiovasc Pharmacother 2016;2:212–7.
[24] Jones P, Kafonek S, Laurora I, Hunninghake D. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). Am J Cardiol 1998;81:582–7.
[25] Nicholls SJ, Brandrup-Wognsen G, Palmer M, Barter PJ. Meta-analysis of comparative efficacy of increasing dose of atorvastatin versus rosuvastatin versus simvastatin on lowering levels of atherogenic lipids (from VOYAGER). Am J Cardiol 2010;105:69–76.
[26] Escobar C, Anguita M, Arrarte V, Barrios V, Cequier A, Cosín-Sales J, et al. Recomendaciones para mejorar el control lipídico. Documento de consenso de la Sociedad Española de Cardiología. Rev Esp Cardiol 2020;73:161–7.
[27] Pedro-Botet J, Climent E. Colesterol LDL en un paso. Med Clin 2020;155:316–7.
[28] Adams SP, Tsang M, Wright JM. Lipid-lowering efficacy of atorvastatin. Cochrane Database Syst Rev 2015;2015(3):CD008226.
[29] Shaya FT, Sing K, Milam R, Husain F, Del Aguila MA, Patel MY. Lipid-lowering efficacy of ezetimibe in patients with atherosclerotic cardiovascular disease: a systematic review and meta-analyses. Am J Cardiovasc Drugs 2020;20:239–48.
[30] Morrone D, Weintraub WS, Toth PP, Hanson ME, Lowe RS, Lin J, et al. Lipid-altering efficacy of ezetimibe plus statin and statin monotherapy and identification of factors associated with treatment response: a pooled analysis of over 21,000 subjects from 27 clinical trials. Atherosclerosis 2012;223:251–61.
[31] Catapano A, Toth PP, Tomassini JE, Tershakovec AM. The efficacy and safety of ezetimibe coadministered with statin therapy in various patient groups. Clin Lipidol 2013;8:13–41.
[32] Masson M, Lobo M, Maenent D, Viatgliano L, Rostan M, Siniawski D, et al. Response to statins in cardiovascular prevention: hypo-responders' evaluation. Rev Argent Cardiol 2014;82: 34–41.
[33] Mandraffino G, Scicali R, Rodríguez-Carrio J, Savarino F, Mamone F, Scuruchi M, et al. Arterial stiffness improvement after adding on PCSK9 inhibitors or ezetimibe to high-intensity statins in patients with familial hypercholesterolemia: a two–lipid center real-world experience. J Clin Lipidol 2020;14:231–40.
[34] Scicali R, Russo GI, Di Mauro M, Manuele F, Di Marco G, Di Pino A, et al. Analysis of arterial stiffness and sexual function after adding on PCSK9 inhibitor treatment in male patients with familial hypercholesterolemia: a single lipid center real-world experience. J Clin Med 2020;9(11):3597.

scientific reports

Check for updates

OPEN

ANGPTL3 gene variants in subjects with familial combined hyperlipidemia

A. M. Bea[1], E. Franco-Marín[1], V. Marco-Benedí[1,2], E. Jarauta[1,2], I. Gracia-Rubio[1], A. Cenarro[1,3]✉, F. Civeira[1,2] & I. Lamiquiz-Moneo[1,2]

Angiopoietin-like 3 (ANGPTL3) plays an important role in lipid metabolism in humans. Loss-of-function variants in *ANGPTL3* cause a monogenic disease named familial combined hypolipidemia. However, the potential contribution of *ANGPTL3* gene in subjects with familial combined hyperlipidemia (FCHL) has not been studied. For that reason, the aim of this work was to investigate the potential contribution of ANGPTL3 in the aetiology of FCHL by identifying gain-of-function (GOF) genetic variants in the *ANGPTL3* gene in FCHL subjects. *ANGPTL3* gene was sequenced in 162 unrelated subjects with severe FCHL and 165 normolipemic controls. Pathogenicity of genetic variants was predicted with PredictSNP2 and FruitFly. Frequency of identified variants in FCHL was compared with that of normolipemic controls and that described in the 1000 Genomes Project. No GOF mutations in *ANGPTL3* were present in subjects with FCHL. Four variants were identified in FCHL subjects, showing a different frequency from that observed in normolipemic controls: c.607-109T>C, c.607-47_607-46delGT, c.835+41C>A and c.*52_*60del. This last variant, c.*52_*60del, is a microRNA associated sequence in the 3′UTR of *ANGPTL3*, and it was present 2.7 times more frequently in normolipemic controls than in FCHL subjects. Our research shows that no GOF mutations in *ANGPTL3* were found in a large group of unrelated subjects with FCHL.

Angiopoietin-like 3 (ANGPTL3) is a 70 kDa-secreted (54 kDa before glycosylation) protein, mainly expressed in the liver, discovered by Conklin et al. in 1999[1]. ANGPTL3 is an endogenous inhibitor of lipoprotein lipase (LPL) and endothelial lipase (EL)[2,3]. Different studies in families with hypolipemia and in general population have reported that loss-of-function (LOF) variants in *ANGPTL3* gene are associated with decreased plasma levels of triglycerides (TG), low-density lipoprotein cholesterol (LDLc) and high-density lipoprotein cholesterol (HDLc)[4]. The N-terminal domain of ANGPTL3 containing residues from 17 to 207 is responsible for the increased plasma TG levels in mice. Loss of this region prevents the inhibition of LPL[5] and EL[3] by ANGPTL3. Recently, the inhibition of ANGPTL3 with a human monoclonal antibody against ANGPTL3 (evinacumab) in dyslipidemic mice and in healthy volunteers caused a dose-dependent placebo-adjusted reduction in fasting TG levels of up to 76% and LDLc levels of up to 23%[4]. Therefore, ANGPTL3 has been considered a potent modulator of TG[2] and supports an important role of ANGPTL3 in lipid metabolism in humans.

In addition, new evidence sustains a possible role of ANGPTL3 in the progression of atherosclerosis through a lipid-independent mechanism[6]. Carriers of LOF mutations in *ANGPTL3* associated a 34% decrease in cardiovascular events[7] and ANGPTL3 plasma concentration was associated with arterial wall thickness in humans[8]. Moreover, a decreased expression of ANGPTL3 in apolipoprotein E null (apoE-/-) mice was protective in the development of atherosclerosis[9].

Familial combined hyperlipidemia (FCHL) is a common and complex inherited disorder of lipid metabolism with important environmental influences[10]. FCHL is characterized by elevated very low-density lipoprotein (VLDL) and/or LDL concentrations, low HDLc levels[11], and frequently, reduced LPL activity[12]. The FCHL genetic background is mostly polygenic and associated with the variation in at least 35 different genes, including genes related to metabolic disorders such as obesity, peripheral insulin resistance, type 2 diabetes, hypertension and metabolic syndrome[13,14]. However, FCHL is a genetically heterogeneous syndrome and monogenic and oligogenic cases have been also described[15–17]. Subjects with FCHL have high predisposition to develop premature cardiovascular disease (CVD). Actually, FCHL is the most common genetic lipid abnormality found in subjects with premature coronary heart disease[18]. The FCHL phenotype is quite similar to that observed after

[1]Unidad de Lípidos, IIS Aragón, CIBERCV, Hospital Universitario Miguel Servet, Avda. Isabel La Católica 1-3, 50009 Zaragoza, Spain. [2]Universidad de Zaragoza, Zaragoza, Spain. [3]Instituto Aragonés de Ciencias de la Salud (IACS), Zaragoza, Spain. ✉email: ana.cenarro@gmail.com

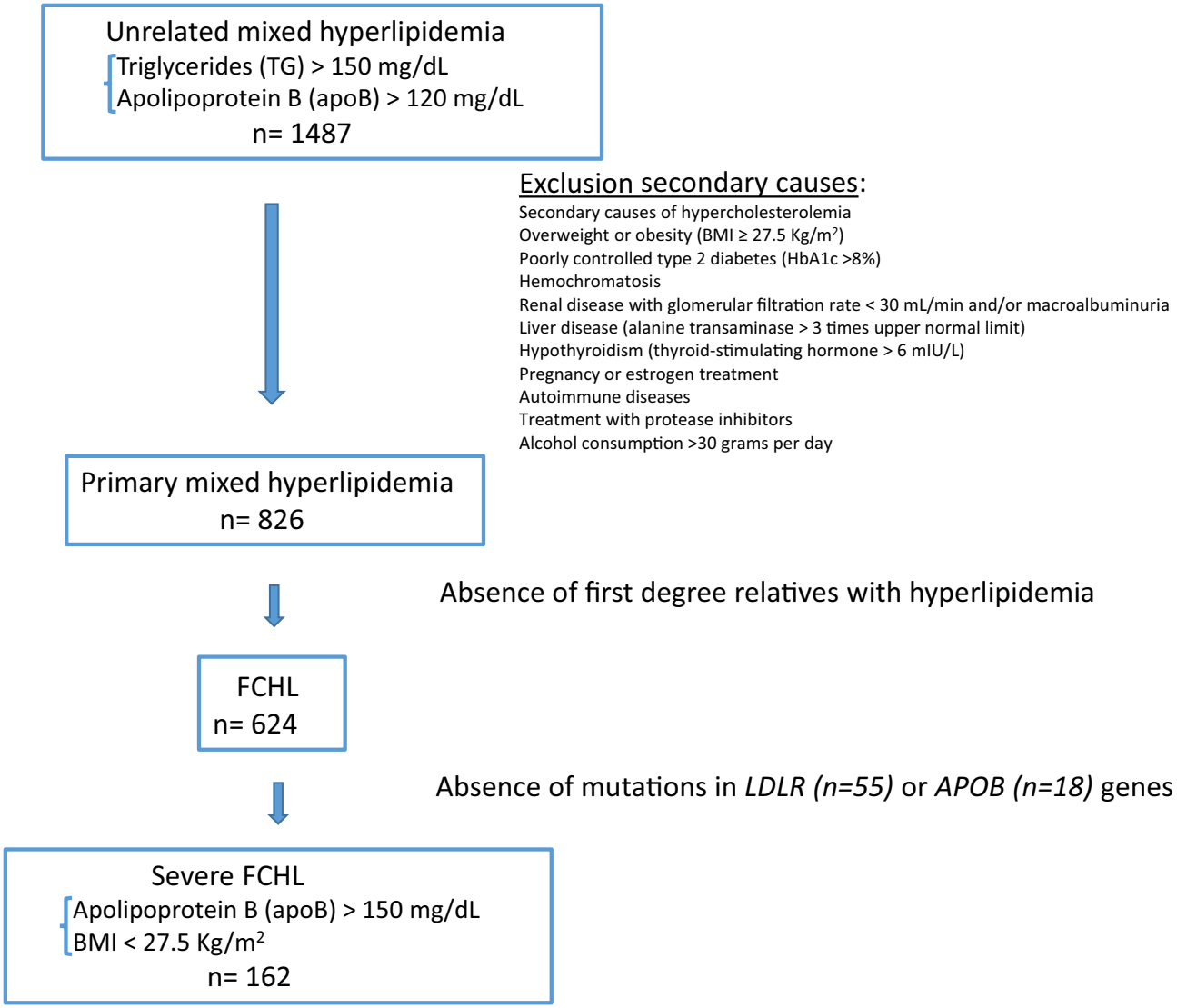


Figure 1. Flow chart of subject selection process.

ANGPTL3 administration in mice. However, the potential involvement of the *ANGPTL3* gene in FCHL has not been previously analysed in contrast with the major role of a loss-of-function mutation in *ANGPTL3* in the opposite situation, familial combined hypolipidemia[19,20]. Therefore, the aim of this study was to identify gain-of-function (GOF) genetic variants in *ANGPTL3* gene in FCHL subjects and to establish the potential contribution of ANGPTL3 in the aetiology of FCHL.

Material and methods

Subjects. *Cases.* A total of 162 unrelated subjects, aged 23 to 82, with the clinical diagnosis of severe FCHL from Lipid Unit at Hospital Universitario Miguel Servet, Zaragoza, Spain, were selected for this study. Severe FCHL included: LDLc and TG > 90th percentile adjusted for age and sex, apolipoprotein B (apoB) > 150 mg/dL, body mass index (BMI) < 27.5 kg/m^2 and at least one first-degree family member with mixed hyperlipidemia. Clinical exclusion criteria were: secondary causes of hypercholesterolemia including significant overweight or obesity (BMI ≥ 27.5 kg/m^2), poorly controlled type 2 diabetes (HbA1c > 8%), hemochromatosis, renal disease with glomerular filtration rate < 30 mL/min and/or macroalbuminuria, liver disease (alanine transaminase > 3 times upper normal limit), hypothyroidism (thyroid-stimulating hormone > 6 mIU/L), pregnancy or estrogen treatment, autoimmune diseases, treatment with protease inhibitors and alcohol consumption > 30 g per day (Fig. 1).

Most of the subjects included in this work had been studied previously to discard severe genetic defects in the genes regulating the LPL pathway[21]. Subjects with *LDLR, APOB* or *PCSK9* functional mutations causing familial hypercholesterolemia (FH) and subjects with dysbetalipoproteinemia and the *APOE*2/2 genotype were excluded from the study. The lipid phenotype of FH and dysbetalipoproteinemia may overlap with FCHL and with this approach both genetic hyperlipidemias were ruled out to avoid confusion with FCHL.

ANGPTL3	Primer sequence 5′→3′	Annealing temperature (°C)	Product size (bp)
Fragment 1	F: CCTTACCTTTTCTGGGCAA	51.5	821
	R: AAATGCAAATTTTCAGTGTTTTCA		
Fragment 2	F: GCTGGGCTTTTTCTTTTAATTG	51	496
	R: CTTCAGAGCCTGCAATTTT		
Fragment 3	F: CCGACCAATGTCTGCTTTTT	51	555
	R: TCAAGTCCATATTTGTATTTCTCTG		
Fragment 4	F: TCCAGACTGGTGATAGAACAAG	53.5	597
	R: GGCAATTAATGAATTTTGGCATAGT		
Fragment 5	F: TCTCCTTTTCCTCTAAAATAATCTGAA	52.5	596
	R: TGATCATTGTAAGCCGTGG		
Fragment 6	F: ATGCATTATAGAAAGGATAATCAGACT	52.5	700
	R: GAGGAAGATTAGAGGTAAAATACCTG		
Fragment 7	F: ACCTCTAATCTTCCTCAGATTTTC	51	599
	R: TTTTGATTGAGAAATGTAAACGGTA		

Table 1. Primers and conditions used for *ANGPTL3* amplification and sequencing. Each amplified fragment comprises the corresponding exon and its 5′ and 3′ flanking sequences, including intron–exon boundaries. *F* forward, *R* reverse.

Controls. We selected 165 consecutive normolipemic, unrelated subjects, aged 20–79, who underwent a medical visit at our hospital as control group. Exclusion criteria for control subjects were personal or parental history of premature cardiovascular disease (before 55 years in men and 65 years in women) or personal or parental dyslipidaemia, current acute illness, or use of drugs that might influence glucose or lipid metabolism.

In all subjects, clinical and analytical variables were registered, including personal and familial risk factors, history of cardiovascular disease and intake of drugs affecting intestinal or lipid metabolism.

All experimental protocols were approved by our local ethical committee (Comité Ético de Investigación Clínica de Aragón, CEICA, Zaragoza, Spain). Informed consent was obtained from all subjects before participating in the protocol. Samples from patients included in this study were provided by the Biobank of the Aragon Health System (PT17/0015/0039), integrated in the Spanish National Biobanks Network, and they were processed following standard operating procedures with the appropriate approval of the Ethics and Scientific Committees.

Biochemical analysis. Ethylenediaminetetraacetic acid (EDTA) plasma and serum samples were collected from all participants after at least 10 h fasting, without lipid-lowering drugs for > 5 weeks, to obtain baseline biochemical characteristics. Total cholesterol (TC) and TG measurements were performed with commercially available diagnostic kits (Boehringer Mannheim, Germany), in a laboratory participating in a lipid standardisation programme. HDLc was measured directly by an enzymatic reaction using cholesterol oxidase (UniCel DxC 800; Beckman Coulter Inc., Brea, California, USA). ApoA1, apoB and lipoprotein(a)[22] were determined by IMMAGE kinetic immunonephelometry (Beckman Coulter Inc., Brea, California, USA). LDLc was calculated using the Friedewald's formula[23]. All methods were carried out in accordance with guidelines and regulations of Spanish Society of Clinical Biochemistry.

Genetic analysis. DNA was isolated from EDTA blood samples using the KingFisher Duo Prime System (Thermo Fisher Scientific). A previously described protocol for sequencing the exon 4 of *APOE* gene[24] was used for disclosing *APOE*2/2 genotype or functional mutations in exon 4 of the *APOE* gene in order to rule out carrier subjects. Moreover, *LDLR*, *APOB* and *PCSK9* genes were analysed for functional mutations with Lipochip platform (Progenika Grifols, Spain)[25] in order to rule out subjects with any pathogenic mutation in these genes.

ANGPTL3 gene (NM_014495.4) was amplified in 7 fragments by polymerase chain reaction with primers showed in Table 1. Each amplified fragment comprised the corresponding exon and its 5′ and 3′ flanking sequences, including intron–exon boundaries. After purification with ExoSap-IT (USB), amplified fragments were sequenced by the Sanger method[26] using the BigDye 3.1 sequencing kit (Applied Biosystems) in an automated ABI 3500xL sequencer (Applied Biosystems). DNA sequences were analysed using Variant Reporter software (Applied Biosystems).

To evaluate the pathogenicity of new identified genetic variants, we used PredictSNP2[27]. The effect of variants in potential splicing sites was predicted with FruitFly[28]. To compare the frequency of identified variants with that of the general population, we compiled the allele frequencies of identified variants from the 1000 Genomes Project[29] and genome aggregation data base (gnomAD)[30]. ClinVar database was used for additional information about genomic variation and its relationship to human health[31]. Finally, information about microRNAs was obtained from PolymiRTS Database 3.0[32]. All methods were carried out in accordance with guidelines and regulations of Spanish Society of Human Genetics.

Statistical analysis. Analyses were performed using statistical computing software R version 3.5.0[33]. The level of significance was set at $P < 0.05$. The distribution of the variables was analysed by the Shapiro test. Quan-

	FCHL subjects n = 162	Normolipemic controls n = 165	*p*
Men, n (%)	98 (60.5)	78 (47.3)	0.022
Age (years)	50.4 ± 11.4	38.5 ± 14.7	< 0.001
Body Mass Index (kg/m^2)	25.6 (24.2–26.5)	23.6 (21.4–26.6)	< 0.001
Total Cholesterol (mg/dL)	312 ± 36.1	170 ± 21.0	< 0.001
Triglycerides (mg/dL)	277 (232–373)	64.0 (49.0–93.0)	< 0.001
LDL cholesterol (mg/dL)	204 (183–230)	108 (91.8–117)	< 0.001
HDL cholesterol (mg/dL)	48.5 ± 12.0	55.7 ± 11.4	0.015
Apolipoprotein A1 (mg/dL)	147 ± 25.0	147 ± 27.4	0.930
Apolipoprotein B (mg/dL)	167 (165–190)	83.0 (72.0–91.0	< 0.001
Lipoprotein(a), (mg/dL)	39.1 (10.3–80.8)	16.2 (7.79–44.5)	0.003
Glucose (mg/dL)	93.0 (86.0–103)	85.0 (80.0–92.0)	< 0.001
HbA1c (%)	5.50 (5.30–5.80)	5.20 (5.00–5.40)	< 0.001
Type 2 diabetes, n (%)	13 (8.02)	2 (1.21)	0.009
Hypertension, n (%)	30 (18.5)	10 (6.06)	0.001
Cardiovascular disease, n (%)	7 (4.32)	0	0.016
Tobacco, n (%)			
Non smoker	51 (31.5)	96 (58.1)	< 0.001
Smoker	70 (43.2)	31 (18.8)	
Former smoker	40 (24.7)	28 (16.7)	
Apolipoprotein E genotype, n (%)			
E3/3	113 (69.8)	109 (66.1)	0.035
E3/2	9 (5.56)	25 (15.2)	
E2/2	0	0	
E3/4	31 (19.1)	25 (15.2)	
E4/4	6 (3.70)	2 (1.21)	
E2/4	3	4	

Table 2. Clinical and biochemical characteristics in FCHL subjects and normolipemic controls. Quantitative continuous variables are expressed as mean ± standard deviation or median [percentile 25–75]. Student's t or Mann–Whitney tests were used to assess differences between two groups. Quantitative categorical variables are expressed as n (%) and statistical differences were assessed by Chi-squared.

titative variables with a normal distribution were expressed as mean ± standard deviation and were analysed by the Student *t* test. Variables with a skewed distribution were expressed as medians and interquartile ranges and were analysed with the Mann–Whitney *U* test. Qualitative variables were expressed as percentages and were analysed by the Chi squared test.

Results

Study subjects. The main clinical and biochemical characteristics of both studied groups (162 FCHL subjects and 165 normolipemic controls) are presented in Table 2. FCHL subjects showed higher predominance of males (60.5%) and were significantly older than normolipemic subjects ($P = 0.022$ and $P < 0.001$, respectively). Compared with normolipemic controls, FCHL subjects had significantly higher values of BMI, TC, TG, LDLc, apoB and lipoprotein(a) ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ and $P = 0.003$, respectively). FCHL subjects presented higher prevalence of hypertension, type 2 diabetes and CVD than normolipemic subjects ($P = 0.009$, $P = 0.001$ and $P = 0.016$, respectively). The *APOE* genotype distribution was homogenous between both cohorts, being E3/3 genotype the most frequent in both groups, although E3/2 genotype had a lower frequency in FCHL subjects (5.56%) in contrast to normolipemic subjects (15.2%).

***ANGPTL3* genetic variants.** Table 3 shows all variants in the *ANGPTL3* gene identified in both groups. A total of 16 genetic variants, four of them not previously described, were identified by sequencing analysis. Only four of them (c.607-109T>C, c.607-47_607-46delGT, c.835+41C>A and c.*52_*60del) presented significantly different allele frequency in normolipemic group than in FCHL subjects ($P = 0.020$, $P = 0.031$, $P = 0.043$ and $P < 0.001$, respectively). Out of the 16 variants, seven variants were located in the coding region (c.379C>T, c.565T>C, c.961T>A, c.1003T>C, c.1028A>G, c.1089T>G and c.1122G>A), and three of them were missense variants: p. (Leu127Phe), p.(Tyr321Asn) and p.(His343Arg), but only p.(Leu127Phe) was described as deleterious by bioinformatics analysis. The other four variants located in the coding region, p.(Leu189Leu), p.(Leu335Leu), p.(Val363Val) and p.(Pro374Pro) were synonymous variants. Seven variants were located in the intronic region, c.496-88T>G, c.607-120A>G, c.607-109T>C, c.607-47_607-46delGT, c.835+41C>A, c.1198+111G>A and c.1198+140T>C. All of them were described as benign or not splicing change affected by the bioinformatics analysis. Nevertheless, three of them, c.607-109T>C, c.607-47_607-46delGT and c.835+41C>A, presented sig-

Variant	Location	Nucleotide change	Protein change	Bioinformatics analysis		Allele frequency in the general population		Allele frequency in our study			ACMG classification[e]	MicroRNAs[f]
				PredictSNP2[a] (probability)	FruitFly[b]	GnomAD[c]	1000 Genomes Project[d]	Normolipemic subjects	FCHL subjects	*p*		
rs72649573	Exon 1	c.379C>T	p.(Leu127Phe)	Deleterious (82%)	NA	0.00711	0.0020	0.000	0.003	0.313	Benign[g]	NR
–	Intron 1	c.496-88T>G	NA	Neutral (88%)	Not splicing change	–	–	0.000	0.003	0.313	–	–
rs111414963	Exon 2	c.565T>C	p.(Leu189Leu)	Neutral (88%)	Not splicing change	0.00025	0.0008	0.003	0.000	0.313	Likely benign	NR
rs531071581	Intron 2	c.607-120A>G	NA	Neutral (88%)	Not splicing change	0.00013	0.0006	0.000	0.003	0.313	–	NR
rs72649576	Intron 2	c.607-109T>C	NA	Neutral (88%)	Not splicing change	0.01079	0.0042	0.024	0.003	0.020	–	NR
rs72649577	Intron 2	c.607-47_607-46delGT	NA	Neutral (88%)	NA	0.02222	0.0136	0.022	0.003	0.031	–	NR
rs185472483	Intron 3	c.835+41C>A	NA	–	Not splicing change	0.00032	0.0006	0.000	0.012	0.043	–	NR
rs747725081	Exon 6	c.961T>A	p.(Tyr321Asn)	Neutral (88%)	NA	NR	NR	0.000	0.003	0.313	–	NR
rs12563308	Exon 6	c.1003T>C	p.(Leu335Leu)	Neutral (88%)	NA	0.03550	0.0559	0.003	0.003	0.989	VUS[g]	NR
rs199555921	Exon 6	c.1028A>G	p.(His343Arg)	Neutral (89%)	NA	0.00016	NR	0.003	0.000	0.321	–	NR
rs763259225	Exon 6	c.1089T>G	p.(Val363Val)	Neutral (96%)	NA	NR	NR	0.003	0.000	0.321	–	NR
rs145086916	Exon 6	c.1122G>A	p.(Pro-374Pro)	Neutral (96%)	NA	0.00077	0.0006	0.003	0.000	0.321	–	NR
rs72651034	Intron 6	c.1198+111G>A	NA	–	Not splicing change	NR	NR	0.003	0.003	0.989	–	NR
rs908541128	Intron 6	c.1198+140T>C	NA	–	Not splicing change	0.000	0.000	0.003	0.000	0.321	–	NR
rs34483103	3′UTR	c.*52_*60del	NA	–	NA	0.33531	0.3484	0.276	0.102	<0.001	–	hsa-miR-151a-3p hsa-miR-7702
–	3′UTR	c.*76T>G	NA	–	NA	–	–	0.000	0.003	0.313	–	–

Table 3. Frequency and bioinformatics analysis of identified variants in *ANGPTL3* in FCHL cases and controls. *NR* not reported, *NA* not applicable, *VUS* variant of uncertain significance. [a]PredictSNP2 uses CADD, DANN, FATHMM and Funseq2 as predictors. [b]FruitFly. New prediction score 0.87 (wild type score 0.89). [c]GnomAD. https://gnomad.broadinstitute.org/ [d]1000 Genomes Project Consortium, Abecasis GR, Auton A, Books LD et al. An integrated map of genetic variation from 1092 human genomes. Nature 2012;491:56–65. [e]Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405–24. https://doi.org/10.1038/gim.2015.30. Epub 2015 Mar 5. PMID: 25741868; PMCID: PMC4544753. [f]PolymiRTS Database 3.0: http://compbio.uthsc.edu/miRSNP/ [g]Tikka A, Metso J, Jauhiainen M. ANGPTL3 serum concentration and rare genetic variants in Finnish population. Scand J Clin Lab Invest. 2017;77:601–609.

nificantly higher allele frequency in FCHL subjects than in the normolipemic group. Finally, two variants were located in the 3′UTR, c.*52_*60del and c.*76T>G. One of them, c.*52_*60del, showed significantly higher allele frequency in the normolipemic group than in FCHL subjects.

Discussion

We have studied the possible contribution of the gene encoding ANGPTL3 in the aetiology of FCHL. Our hypothesis was that some rare gain-of-function variants could have a major effect on the disease or, on the contrary, that common variants with minor effect on ANGPTL3 function could be in different frequency with respect to the general population. The results of our study do not support the first possibility, since the identified variants are not predictive of relevant functional changes in the protein. There are no previous *ANGPTL3* sequencing studies looking for GOF mutations in subjects with FCHL. At least 5 different *loci* have been associated with rare cases of monogenic FCHL: *LDLR*[16,17], *LPL*[15], *APOE*[34], *PCSK9*[35] and *APOA5*[36,37], but *ANGPTL3*

does not appear to be associated with this form of FCHL nor familial hypercholesterolemia (FH). Although FH and FCHL are different phenotypes, there is some degree of overlap between the two entities since they share many clinical aspects. Studies in subjects with genetic hypercholesterolemia of unknown origin suggestive of FH have also failed to detect causal mutations in *ANGPTL3*. We have not found any severe mutation neither in cases nor in controls in the total of 654 alleles investigated. This leads us to think about how well preserved is this gene probably related to the importance of this gene in human metabolism.

These results contrast with the role of ANGPTL3 in the lipid phenotype called familial combined hypolipidemia (FHBL2, OMIM #605019)[20], in which LOF mutations in *ANGPTL3* are responsible of reduced plasma levels of TC, TG, VLDL cholesterol, LDLc, apoB, and free fatty acids, just the opposite lipid profile found in FCHL. Furthermore, FCHL and familial combined hypolipidemia share abnormal hepatic VLDL secretion rates as the main mechanism of the lipid abnormalities, being increased in FCHL[38,39] and decreased in familial combined hypolipidemia[40].

Most cases of FCHL are considered as a complex disease with interaction of polygenes or multiple allele relationships with effect on TC, TG and environmental factors, mainly obesity and diets rich in saturated fat. *ANGPTL3* genetic variation has not been associated with FCHL or mixed hyperlipidemia in genome-wide association studies (GWAS)[13,41]. Similar conclusions can be drawn from large-scale deep-coverage whole-genome sequencing[42]. Our study cannot rule out that the genetic variation in *ANGPTL3* participates in the final phenotype of polygenic forms of FCHL. We found four variants with different allele frequency in FCHL subjects and in normolipemic controls: c.607-109T>C, c.607-47_607-46delGT, c.835+41C>A and c.*52_*60del. The first three are located in intron regions and the in silico analysis does not predict any splicing change with clinical significance, so their contribution to FCHL seems unlikely. The variant c.*52_*60del, located in 3′UTR, presented statistically significant differences in allelic frequencies between FCHL subjects and normolipemic controls: 0.276 and 0.102, respectively ($P < 0.001$). This variant has been previously associated with two microRNAs, hsa-miR-151a-3p and hsa-miR-7702, modulators of gene expression[32]. However, this is a very frequent genetic variant in the general population and this variation has not been previously associated with cholesterol and triglyceride concentrations[43,44], so its implication in the FCHL pathogenesis is unlikely, although it should be confirmed in future studies.

In summary, no GOF mutations in *ANGPTL3* were present in a large group of unrelated subjects with FCHL. Our results do not support a substantial role of *ANGPTL3* in FCHL.

Received: 22 December 2020; Accepted: 9 March 2021
Published online: 26 March 2021

References

1. Conklin, D. *et al.* Identification of a mammalian angiopoietin-related protein expressed specifically in liver. *Genomics* **62**, 477–482 (1999).
2. Shimizugawa, T. *et al.* ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. *J. Biol. Chem.* **277**, 33742–33748 (2002).
3. Shimamura, M. *et al.* Angiopoietin-like protein3 regulates plasma HDL cholesterol through suppression of endothelial lipase. *Arterioscler. Thromb. Vasc. Biol.* **27**, 366–372 (2007).
4. Dewey, F. E. *et al.* Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. *N. Engl. J. Med.* **377**, 211–221 (2017).
5. Ono, M. *et al.* Protein region important for regulation of lipid metabolism in angiopoietin-like 3 (ANGPTL3): ANGPTL3 is cleaved and activated in vivo. *J. Biol. Chem.* **278**, 41804–41809 (2003).
6. Korstanje, R. *et al.* Locating Ath8, a locus for murine atherosclerosis susceptibility and testing several of its candidate genes in mice and humans. *Atherosclerosis* **177**, 443–450 (2004).
7. Stitziel, N. O. *et al.* ANGPTL3 deficiency and protection against coronary artery disease. *J. Am. Coll. Cardiol.* **69**, 2054–2063 (2017).
8. Hatsuda, S. *et al.* Association between plasma angiopoietin-like protein 3 and arterial wall thickness in healthy subjects. *J. Vasc. Res.* **44**, 61–66 (2007).
9. Ando, Y. *et al.* A decreased expression of angiopoietin-like 3 is protective against atherosclerosis in apoE-deficient mice. *J. Lipid Res.* **44**, 1216–1223 (2003).
10. Goldstein, J. L., Schrott, H. G., Hazzard, W. R., Bierman, E. L. & Motulsky, A. G. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* **52**, 1544–1568 (1973).
11. Gaddi, A., Galetti, C., Pauciullo, P. & Arca, M. Familial combined hyperlipoproteinemia: experts panel position on diagnostic criteria for clinical practice. Committee of experts of the Atherosclerosis and Dysmetabolic Disorders Study Group. *Nutr. Metab. Cardiovasc. Dis. NMCD* **9**, 304–311 (1999).
12. Babirak, S. P., Brown, B. G. & Brunzell, J. D. Familial combined hyperlipidemia and abnormal lipoprotein lipase. *Arterioscler. Thromb.* **12**(10), 1176–1183. https://doi.org/10.1161/01.atv.12.10.1176 (1992).
13. Brouwers, M. C. G. J., van Greevenbroek, M. M. J., Stehouwer, C. D. A., de Graaf, J. & Stalenhoef, A. F. H. The genetics of familial combined hyperlipidaemia. *Nat. Rev. Endocrinol.* **8**, 352–362 (2012).
14. Veerkamp, M. J., de Graaf, J. & Stalenhoef, A. F. H. Role of insulin resistance in familial combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1026–1031 (2005).
15. Yang, W. S., Nevin, D. N., Peng, R., Brunzell, J. D. & Deeb, S. S. A mutation in the promoter of the lipoprotein lipase (LPL) gene in a patient with familial combined hyperlipidemia and low LPL activity. *Proc. Natl. Acad. Sci.* **92**, 4462–4466 (1995).
16. Civeira, F. *et al.* Frequency of low-density lipoprotein receptor gene mutations in patients with a clinical diagnosis of familial combined hyperlipidemia in a clinical setting. *J. Am. Coll. Cardiol.* **52**, 1546–1553 (2008).
17. Minicocci, I. *et al.* Contribution of mutations in low density lipoprotein receptor (LDLR) and lipoprotein lipase (LPL) genes to familial combined hyperlipidemia (FCHL): A reappraisal by using a resequencing approach. *Atherosclerosis* **242**, 618–624 (2015).
18. Austin, M. A. *et al.* Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. *Circulation* **101**, 2777–2782 (2000).
19. Di Costanzo, A. *et al.* Clinical and biochemical characteristics of individuals with low cholesterol syndromes: A comparison between familial hypobetalipoproteinemia and familial combined hypolipidemia. *J. Clin. Lipidol.* **11**, 1234–1242 (2017).

20. Arca, M., D'Erasmo, L. & Minicocci, I. Familial combined hypolipidemia: Angiopoietin-like protein-3 deficiency. *Curr. Opin. Lipidol.* **31**, 41–48 (2020).
21. De Castro-Orós, I. *et al.* Common genetic variants contribute to primary hypertriglyceridemia without differences between familial combined hyperlipidemia and isolated hypertriglyceridemia. *Circ. Cardiovasc. Genet.* **7**, 814–821 (2014).
22. Marcovina, S. M. *et al.* Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein(a). *Clin. Chem.* **46**, 1956–1967 (2000).
23. Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18**, 499–502 (1972).
24. Bea, A. M. *et al.* Lipid-lowering response in subjects with the p.(Leu167del) mutation in the APOE gene. *Atherosclerosis* **282**, 143–147 (2019).
25. Palacios, L. *et al.* Molecular characterization of familial hypercholesterolemia in Spain. *Atherosclerosis* **221**, 137–142 (2012).
26. Sanger, F., Nicklen, S. & Coulson, A. R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* **74**, 5463–5467 (1977).
27. Bendl, J. *et al.* PredictSNP2: A unified platform for accurately evaluating SNP effects by exploiting the different characteristics of variants in distinct genomic regions. *PLoS Comput. Biol.* **12**, e1004962 (2016).
28. Reese, M. G., Eeckman, F. H., Kulp, D. & Haussler, D. Improved splice site detection in Genie. *J. Comput. Biol. J. Comput. Mol. Cell Biol.* **4**, 311–323 (1997).
29. 1000 Genomes Project Consortium *et al.* An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65 (2012).
30. Karczewski, K. J. *et al.* Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. http://biorxiv.org/lookup/. https://doi.org/10.1101/531210 (2019).
31. ClinVar. https://www.ncbi.nlm.nih.gov/clinvar/.
32. Bhattacharya, A., Ziebarth, J. D. & Cui, Y. PolymiRTS Database 3.0: linking polymorphisms in microRNAs and their target sites with human diseases and biological pathways. *Nucleic Acids Res.* **42**, D86–D91 (2014).
33. Team, R. C. R Foundation for Statistical Computing; Vienna, Austria: 2014. *R Lang. Environ. Stat. Comput.* 2013 (2018).
34. Solanas-Barca, M. *et al.* Apolipoprotein E gene mutations in subjects with mixed hyperlipidemia and a clinical diagnosis of familial combined hyperlipidemia. *Atherosclerosis* **222**, 449–455 (2012).
35. Abifadel, M. *et al.* A PCSK9 variant and familial combined hyperlipidaemia. *J. Med. Genet.* **45**, 780–786 (2008).
36. van der Vleuten, G. M. *et al.* Haplotype analyses of the APOA5 gene in patients with familial combined hyperlipidemia. *Biochim. Biophys. Acta* **1772**, 81–88 (2007).
37. Di Taranto, M. D. *et al.* Association of USF1 and APOA5 polymorphisms with familial combined hyperlipidemia in an Italian population. *Mol. Cell. Probes* **29**, 19–24 (2015).
38. Baila-Rueda, L. *et al.* Cholesterol oversynthesis markers define familial combined hyperlipidemia versus other genetic hypercholesterolemias independently of body weight. *J. Nutr. Biochem.* **53**, 48–57 (2018).
39. van Himbergen, T. M. *et al.* Familial combined hyperlipidemia is associated with alterations in the cholesterol synthesis pathway. *Arterioscler. Thromb. Vasc. Biol.* **30**, 113–120 (2010).
40. Hess, A. L. *et al.* Analysis of circulating angiopoietin-like protein 3 and genetic variants in lipid metabolism and liver health: The DiOGenes study. *Genes Nutr.* **13**, 7 (2018).
41. Ripatti, P. *et al.* The contribution of GWAS Loci in Familial Dyslipidemias. *PLOS Genet.* **12**, e1006078 (2016).
42. Natarajan, P. *et al.* Deep-coverage whole genome sequences and blood lipids among 16,324 individuals. *Nat. Commun.* **9**, 3391 (2018).
43. Surakka, I. *et al.* The impact of low-frequency and rare variants on lipid levels. *Nat. Genet.* **47**, 589–597 (2015).
44. UK10K consortium. The UK10K project identifies rare variants in health and disease. *Nature* **526**, 82–90 (2015).

Acknowledgements

This work was supported by Grants from Gobierno de Aragon, B14-7R, Spain; Spanish Ministry of Economy and Competitiveness PI15/01983, PI18/01777 and CIBERCV. We want to particularly acknowledge the patients and the Biobank of the Aragon Health System (PT17/0015/0039) integrated in the Spanish National Biobanks Network for their collaboration.

Author contributions

A.M.B.: Data acquisition, methodology, writing—original draft. E.F.-M.: Data acquisition. V.M.-B., E.J. and I.G.-R.: Data acquisition and methodology. A.C.: Conceptualization, Formal analysis, Writing—original draft, Supervision. F.C.: Conceptualization, Funding acquisition, Project administration, writing—original draft, Supervision. I.L.-M.: Data acquisition and methodology, formal analysis and writing—original draft. All authors have reviewed and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to A.C.

Clinical Nutrition xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized Control Trials

High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early-onset diabetic adults with overweight or obesity

Victoria Marco-Benedí [a], Sofía Pérez-Calahorra [a], Ana M. Bea [a], Itziar Lamiquiz-Moneo [a], Lucía Baila-Rueda [a], Ana Cenarro [a], Fernando Civeira [a, b], Rocío Mateo-Gallego [a, b, *]

[a] Hospital Universitario Miguel Servet, Instituto de Investigación Sanitaria Aragón (IIS Aragón), CIBERCV, Zaragoza, Spain
[b] Universidad de Zaragoza, Zaragoza, Spain

ARTICLE INFO

Article history:
Received 19 November 2018
Accepted 7 June 2019

Keywords:
Diabetes
High protein diet
Weight loss
Adipokines
Insulin resistance
Obesity

SUMMARY

Background & aims: It has not been elucidated if an energy-restricted diet with high protein content could induce a benefit in insulin resistance in subjects with type 2 diabetes (T2DM); and if an adipose tissue functionality improvement could mediate this effect. We aimed to assess the effect of energy-restricted diets with standard (18% from total calories; SP) vs high (35%) protein (HP), mainly coming from lean animal source, composition on glucose metabolism and adipokine concentration in overweight and obese subjects with T2DM. HOMA-IR change was the primary outcome.

Methods: Six-month weight-loss intervention including 73 subjects (43.8% men, 55.6 ± 8.37 aged and 32.8 ± 3.67 of BMI) with T2DM that were randomized to follow one of two calorie-restricted diets with the following distribution of calories: 18% (0.75 [95%CI: 0.71−0.78] g/kg/day) protein, 52% carbohydrates and 30% fat, or 35% (1.34 [95%CI: 1.27−1.41] g/kg/day) protein, 35% carbohydrates, and 30% fat. Anthropometric, clinical, biochemical (involving leptin, RBP4 and adiponectin) and lifestyle assessments were performed.

Results: Sixty-seven participants completed the study. Weight loss homogenously decreased among diets. HOMA-IR in HP diminished 2-fold than in SP diet ($P = 0.023$ and $P = 0.004$ at 3 and 6-months between diets). Participants following HP diet showed higher decrease in insulin, in glucose at 6-months ($P = 0.004$) and in HbA1c at 3-months ($P = 0.003$). RBP4 and leptin significantly decreased in both diets although no differences were found between diets. Adiponectin increased by 6.05% and 29.9% at 3-months in SP and HP diets, respectively ($P = 0.167$), and 23.7% and 53.5% at 6-months in SP and HP diets ($P = 0.219$). Adiponectin variation was inversely correlated with HbA1c, insulin and HOMA-IR changes at 6-months.

Conclusions: An energy-restricted diet containing 35% of total calories coming from protein lead to a greater improvement in glucose homeostasis, indicated by HOMA-IR and fasting plasma insulin concentrations, irrespective of weight loss in subjects with prediabetes or early stages of T2DM. This effect cannot be explained by changes in plasma concentration of adipokines.

Clinical trial registration: The clinical trial has been registered in ClinicalTrials.gov (Identifier: NCT02559479).

Introduction

Weight loss via lifestyle changes is the first-line therapy for type 2 diabetes mellitus (T2DM) [1,2]. A weight reduction achieving greater loss of adipose tissue has been shown to be more efficient in improving peripheral resistance to insulin, one of the fundamental

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; GGT, gamma glutamil transferase; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; HP, high-protein; IFG, impaired fasting glucose; RBP4, retinol Binding Protein 4; T2DM, type 2 diabetes mellitus.

* Corresponding author. Unidad Clínica y de Investigación en Lípidos y Aterosclerosis, Hospital Universitario Miguel Servet, Paseo Isabel La Católica, 1-3, 50009, Zaragoza, Spain. Fax: +34 976765500.
E-mail address: rmateo@unizar.es (R. Mateo-Gallego).

https://doi.org/10.1016/j.clnu.2019.06.005
0261-5614/

components of T2DM [3]. Weight loss–induced improvements in glucose metabolism are most likely to occur early in the natural history of T2DM when obesity associated insulin resistance has produced reversible β-cell dysfunction, but insulin secretory capacity remains fairly preserved [4]. In fact, studies of weight loss in subjects with long-standing diabetes have not demonstrated long-term clinical benefits in morbidity and mortality [5].

In recent years, energy-restricted protein-rich diets resulted in greater weight loss than more conventional high-carbohydrate, low-fat diets [6,7]. Beyond the weight-loss enhancing ability, it has been demonstrated that high-protein (HP) diets improve cardiometabolic parameters regardless of weight loss [8,9]. Individuals on a HP diet demonstrated lower insulin resistance and triglyceride levels than those on a diet with a standard protein (SP) content [10]. The American Diabetes Association has recently encouraged increased protein consumption as part of a healthy lifestyle intervention [11]. However, research on the ideal amount of dietary protein to optimize glycemic control or cardiovascular risk is inconclusive, so further research is encouraged to fully establish dietary goals.

Evidence of the potential benefit comes from very different studies regarding the design, percentage of proteins in diet, duration of diabetes, concomitant treatments, and their duration. A meta-analysis including nine trials exploring HP diet's effect on cardiometabolic parameters in subjects with T2DM, pointed out a benefit in glycated hemoglobin (HbA1c) but glucose findings were inconclusive [12]. Heterogeneous results could be due to differences in quantity and protein sources. Thus, the purpose of this study was to demonstrate the effect of a energy-restricted diet with 35% of total calories coming from protein (considered as HP diet) on glucose metabolism in subjects with early-diagnosed impaired fasting glucose (IFG) or T2DM and overweight or obesity, comparing to a diet with an 18% of total energy coming from protein diet (considered as SP diet). We have previously shown that a diet with 35% of total calories from protein is well-tolerated and is associated with a better metabolic profile than other HP diets [10]. Primary outcome of this study was HOMA-IR while secondary outcomes included insulin, glucose and HbA1c.

Subjects and methods

Subjects

Eligible volunteers were women and men aged 18–70, with a body mass index (BMI) ranging from 27.5 to 40 kg/m^2 and steady weight (±4 Kg) in the previous 2 months. We included those subjects: a) with the diagnosis of IFG or T2DM according to international guidelines by including fasting glucose concentration over 100 mg/dL and/or HbA1c over 5.7% [13], and not taking antidiabetic drugs; b) with the diagnosis of IGF or T2DM as previously defined and taking a stable dose of metformin for 2 months, regardless of glucose and/or HbA1c levels. We excluded those subjects with HbA1c concentration over 7% at baseline. Exclusion criteria involved: lipid-lowering drugs and/or sterols supplements, omega-3 fatty acids, weight loss medications, kidney disease (glomerular filtration rate < 45 mL/min), active liver disease, uncontrolled hypothyroidism and any other disease or condition that could limit the study's compliance. Volunteers were recruited by public advertisements on local television and newspapers and were invited to an informative session where objectives, and inclusion and exclusion criteria were explained in detail. Participants willing to participate completed a questionnaire that included: body weight, height, medical history, common medications and availability to participate. Those volunteers who completed the study questionnaire and were eligible according to the inclusion and exclusion criteria were scheduled for a pre-screening visit. Informed consent was obtained at the pre-screening visit along with clinical and biochemical parameters, to confirm eligibility, before proceeding to randomization if applicable.

The study protocol was approved by the local ethical committee institution (Comité de Ética e Investigación Clínica de Aragón); all procedures were in accordance with the ethical standards of that committee. This clinical trial was registered in ClinicalTrials.gov under identifier NCT02559479.

Study design

This study consisted of a 6-month weight-loss intervention and was carried out between September 2015 and May 2017. Those individuals selected at the screening visit, were randomized 1:1 to a diet containing 18% of daily calories from protein (SP diet group) or to a diet with 35% of daily calories from protein (HP diet group). The two prescribed diets had the following distribution of calories: 18% protein, 52% carbohydrates, 30% fat; and 35% protein, 35% carbohydrates, 30% fat. Estimated quantity of protein that was prescribed was 0.75 (95% CI: 0.71–0.78) g/kg/day in SP diet group and 1.34 (95% CI: 1.27–1.41) g/kg/day in HP group. A SP diet was considered when 18% of calories coming from protein (around 0.80 g/kg/day within an energy-restricted diet) in agreement with WHO recommendations [14]. The rationale that a HP diet involved a 35% of energy intake from protein was based on our previous findings that revealed that this amount lead to greater cardiometabolic improvement (including glucose homeostasis) when compared to other HP quantity [10]. Randomization was performed by using an online software and participants and the staff, except for the nutritionists, were blinded to the type of diet individuals were assigned. The total number of calories was calculated using the Harris–Benedict equation by applying an activity factor (energy expenditure for various activities established by the WHO [14]) according to personal physical activity habits and a daily 600 kcal (2510 kJ)-deficit. In general, the prescribed energy intake was 1200–2000 kcal (5020–8370 kJ)/day. Diets included a wide variety of foods typical of the Mediterranean diet and participants were provided with daily menus (Supplemental Table 1). Around 80% of total proteins came from lean animal sources like lean meat (leg and shoulder of *rasa aragonesa* lamb, chicken or turkey), low-fat dairy or fish. Dieticians performed individual consultations every 2 weeks to reinforce the intervention and to motivate weight loss. If the subject had achieved a significant weight loss, a 100–200 kcal further restriction was added to the prescribed diet to compensate basal metabolic rate change. We did not prescribe diets containing less than 1200 kcal (5020 kJ)/day since nutritional requirements could be not reached.

Despite dietary intervention was the main target of the intervention, all participants were provided with general physical-activity advice that was in accordance with their physical status. Patients were counseled to increase exercise in each monitoring visit based on the training reported in each visit to promote weight loss. Physical activity advice was quite heterogeneous due to the different fitness conditions of subjects (i.e.: walk 1 h a day or running 30 min three times a week).

Main study outcomes were assessed at 3-time points: baseline, after 3 and 6 months of dietary intervention. They included anthropometric, clinical and biochemical parameters and dietary and exercise evaluation. Participants were asked to complete a 3-day weighed food record before each visit to focus their dietary intervention, to monitor dietary changes, and to check compliance with the diet during the study. Dietary analysis was performed by EasyDiet® (Biocentury, S.L.U, Barcelona, Spain) which is based on Spanish food-composition tables [15]. The International Physical

Please cite this article as: Marco-Benedí V et al., High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early-onset diabetic adults with overweight or obesity, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2019.06.005

Activity Questionnaire (IPAQ) – a brief validated exercise questionnaire – was administered at baseline and after 3 and 6 months to monitor activity changes [16].

Anthropometric and clinical parameters

Body weight was measured in subjects without shoes to the nearest 0.1 kg with a calibrated scale (Seca 813, Seca Deutschland®, Hamburg, Deutschland). Height was assessed to the nearest 0.1 cm with a wall-mounted stadiometer (Seca 217, Seca Deutschland®, Hamburg, Deutschland). BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured with anthropometric tape midway between the lowest rib and the iliac crest. Body composition was assessed via bioelectrical impedance through the bipolar foot-to-foot technique (Tanita TBF 410 GS, Omron Corporation®, Tokyo, Japan) [17]. Visceral fat depots were analyzed by means of bioelectrical impedance ("Tanita ViScan" AB-140, Omron Corporation®, Tokyo, Japan). As established by the manufacturer, visceral measurement is expressed on a scale of 1–35 levels and the interpretation of the results would be: a) average (when visceral fat level ranges from 1.0 to 12.5); b) high (when visceral level ranges from 13.0 to 17.5); c) very high (when visceral fat level is over 18.0). Based on manufacturer's validation studies, a level of 13.0 generally correlates to a visceral fat area of 130 cm^2 as measured by computerized tomography and X-ray. All measurements were taken in accordance with the recommended guidelines: no food or drink 3 h prior to measurements, no exhausting exercise 12 h prior to measurements, and no alcohol or caffeine consumption 24 h prior to measurements. Blood pressure was measured in triplicate with a validated semiautomatic oscillometer (Omron M3, Omron Cop; Hoofddorp, the Netherlands).

Biochemical parameters

Blood samples were drawn by venipuncture after 12 h fasting. The levels of total cholesterol, triglycerides, and HDL cholesterol, uric acid, gamma-glutamyl transpeptidase (GGT) and glutamic-pyruvic transaminase (GPT) were measured in serum with standard enzymatic methods, all of them on a Beckman Coulter AU analyser (Beckman Coulter, USA). Total cholesterol was quantified by the esterase-oxidase-4-aminoantipyrine method. Triglycerides were determined by the lipase-peroxidase method. HDL cholesterol was determined by direct method (non-apoB lipoproteins). Uric acid was quantified by the uricase method.The kinetic determination of gamma-glutamyl transpeptidase activity was measured by the change in absorbance at 410/480 nm, according to the methodology recommended by the International Federation of Clinical Chemistry (IFCC). The kinetic determination of glutamic pyruvic transaminase was measured by the decrease in absorbance due to the consumption of NADH at 340 nm, according to IFCC. LDL cholesterol levels were estimated with the Friedewald formula when serum triglycerides were <400 mg/dL. The levels of non-HDL cholesterol were calculated as the levels of total cholesterol minus the levels of HDL cholesterol. Blood glucose levels were measured in serum with the glucose hexokinase G-6-PDH method on a Beckman Coulter AU analyser (Beckman Coulter, USA). Insulin levels were determined in serum by a chemiluminescent microparticle immunoassay (CMIA, Abbott Architect, USA). We used homeostasis model assessment of insulin resistance (HOMA-IR) as a marker for insulin resistance. HOMA-IR was estimated as fasting glucose (mg/dL) × insulin (μU/mL)/405. HbA1c levels were determined in plasma by high-performance liquid chromatography (HPLC). C-reactive protein (CRP) was determined in serum by nephelometry using IMMAGE-Immunochemistry System (Beckman Coulter, USA). Subjects also collected a spot urine sample in which urea nitrogen concentrations were determined by the urease-GLDH method on a Beckman Coulter AU analyser (Beckman Coulter, USA). This parameter was used as surrogate marker of protein intake to assess dietary compliance [18].

We determined leptin, adiponectin and Retinol Binding Protein 4 (RBP4) as relevant adipokines related to weight loss according to previous studies [19,20]. Adipokine profiles were determined in plasma using the Human Adipokine Magnetic Bead Panel 1 (Adiponectin), Human Adipokine Magnetic Bead Panel 2 (Leptin) and Human Kidney Injury Magnetic Bead Panel 6 (RBP4) protocols from the MILLIPLEX® MAP Kits (Cat. #. HADK1MAG-61K, HADK2MAG-61K, HKI6MAG-99K, Millipore) according to manufacturer's instructions. Analyses were performed by duplicate and plasma sample dilutions were done according to the detection range of each panel. Assay sensitivities were 0.013 ng/mL for RBP4, 19 pg/mL for leptin and 11 pg/mL for adiponectin. Intra-assay precision (mean of % CV) was <10% for RBP4, 5 for leptin and 4 for adiponectin while inter-assay precision (mean of % CV) was <10% for RBP4, 13 for leptin and 10 for adiponectin. Accuracy was 104% of recovery in plasma samples for RBP4, 96% of recovery in plasma samples for leptin and 89% of recovery in plasma samples for adiponectin.

Statistical analyses

HOMA-IR was established as the main outcome and its variability was estimated as 2 units. We expected a difference of HOMA-IR change after dietary intervention of 25% among diet groups according to previous findings [10]. A total sample size of 41 subjects per group was obtained by considering 80% power ($Z\beta$ unilateral = 0.842) to detect a difference between treatment groups and a confidence interval ($1 - \alpha$) of 90% ($Z\alpha$ unilateral = 1.645). All subjects who completed the study were included in the data analysis, independent of reported dietary compliance, as indicated by food records, or weight loss according to intention-to-treat analysis. Thus, we included all subjects who attended to 3 and 6-month visits regardless of study intervention compliance. Continuous variables are expressed as mean ± SD or mean (95% confidence interval) when normally distributed or as median [25th percentile–75th percentile] or mean ± interquartile range otherwise. Categorical variables are reported as percentages. Differences in continuous variables were calculated by *t*-test or Mann–Whitney test, as appropriate, while categorical variables were compared using the chi-square test. Pearson or Spearman tests were used to analyze correlation between changes in adipokines concentration and other clinical and biochemical variables. Differences across dietary intervention within diet group were calculated by repeated measures ANOVA or Friedman tests as applicable. We used multiple linear regressions to evaluate the impact of: a) the type of diet on glucose metabolism parameters and adipokine concentration by adjusting weight loss and other confounding factors (gender, baseline visceral fat, weight loss, metformin use (yes/no), baseline concentrations of glucose and HbA1c in fully-adjusted model); b) weight loss and type of diet on the relation between adipokine and cardiometabolic changes. All statistical analyses were performed with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and significance was set at $P < 0.05$.

Results

Participants

Among 100 subjects that performed randomization visit, 80 were finally randomized to one of two reduced-calorie diets of whom 67 completed the whole study intervention. Seven

Please cite this article as: Marco-Benedí V et al., High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early-onset diabetic adults with overweight or obesity, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2019.06.005

participants (8.75% of all participants, 5 and 2 from SP and HP groups respectively) withdrew from the study during the first 3 months and six subjects (7.50% of all participants, 3 from each diet group) withdrew during the next three months. Withdrawal reasons included: personal issues (N = 6), change of place of residence (N = 1) and unknown reasons (N = 6). Subjects who withdrew from the study did not differ from the remaining participants in terms of any clinical characteristics at baseline according to sensitivity analysis. The complete study flow chart is shown in Fig. 1.

Both diet groups did not differ in terms of clinical and biochemical characteristics ($P \geq 0.05$ for all variables among diet groups) except for visceral fat. Those subjects randomized to the HP group had higher visceral fat level than those following the SP diet. Distribution of gender was homogeneous ($P = 0.185$) by including 43 women (58.9%) and 30 men (41.1%). Participants were mostly middle-aged (55.6 ± 8.37) with a mean BMI of 32.8 kg/m^2 ($P = 0.288$ between groups) who showed high fat mass, visceral fat and other clinical and biochemical characteristics expected according to inclusion and exclusion criteria. Metformin use did not differ between groups at the beginning of the study and it was maintained stable across the study. Baseline characteristics are included in Table 1.

Weight loss and body composition

Mean weight loss was −6.81 ± 3.82% at 3-month visit and −8.79 ± 5.15% at 6-month visit by including statistically significant differences across the study in both diets. However, there was not significant difference between diets. Differences in weight loss between diets were similar than those reported in previous studies comparing SP and HP diets. Lack of statistical significance could be due to the small sample size that was calculated to explore changes in glucose homeostasis not in body weight variation. Similar trends were observed in fat-free mass and fat mass change after dietary intervention between diet groups. Visceral fat showed a greater decrease in subjects following HP diet comparing to those randomized to HP diet which was especially relevant in 3-month visit ($P = 0.069$). These differences remained after adjusting by baseline visceral fat levels.

Glucose metabolism parameters

Glycemic control showed a greater improvement in subjects consuming a HP diet which was especially remarkable in insulin, HOMA-IR index and HbA1c (Table 2 and Supplemental Table 2). The decrease in fasting plasma glucose concentration was markedly greater in those subjects following HP diet than in those following SP diet although it was just statistically significant after 6-months of dietary intervention (weight loss adjusted-$P = 0.013$, Fig. 2). Statistical significance disappeared after adjusting by metformin treatment, gender, baseline visceral fat, baseline concentration of glucose, baseline concentration of HbA1c, and weight loss (full model adjustment $P = 0.053$). Those participants following HP diet showed higher insulin decrease that those in SP group both at 3 and 6-months (weight loss adjusted-$P = 0.014$ and $P = 0.007$ respectively). Statistical difference kept after fully-adjusted at 6-month visit (B [95% CI]: 19.4 [6.21−32.5], $P = 0.005$, corrected $R^2 = 0.38$) but disappeared at 3-month visit ($P = 0.054$). Participants following HP diet had higher reduction of HOMA-IR than those following SP diet after 3-month intervention (weight loss adjusted-$P = 0.016$) and after 6-month intervention (weight loss adjusted-$P = 0.001$). Fully-adjusted model showed statistical impact of diet on HOMA-IR variation after 6 months (B [95% CI]: 21.6 [7.82−35.4], $P = 0.003$, corrected $R^2 = 0.40$) but not at 3-month visit ($P = 0.099$). HbA1c diminished more in those subjects following HP diet after 3-months of weight loss intervention while statistical differences disappeared at 6-months visit. However, statistical differences disappeared after adjusting by body weight reduction ($P = 0.051$) and in fully-adjusted model ($P = 0.115$). The effect of type of diet on glucose metabolism did not significantly differ according to baseline concentration of each parameter (glucose, HbA1c, insulin and HOMA-IR) and the use of metformin (data not shown).

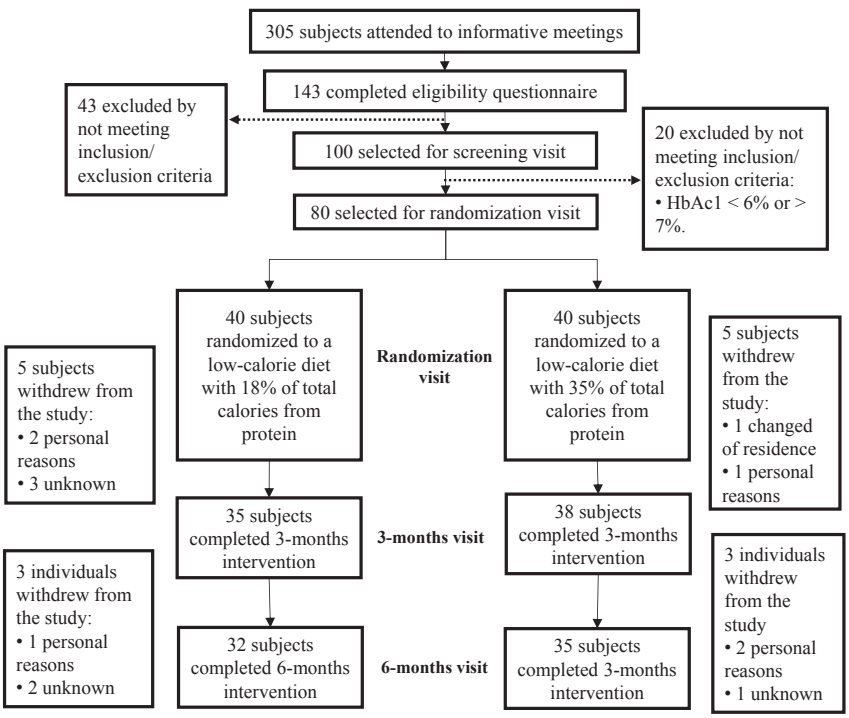


Fig. 1. Schematic representation of randomization and study course. BMI denotes body mass index and HbA1c denotes glycated hemoglobin.

Please cite this article as: Marco-Benedí V et al., High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early-onset diabetic adults with overweight or obesity, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2019.06.005

Table 1
Baseline clinical and biochemical characteristics according to diet group.[a]

	SP diet $n = 35$	HP diet $n = 38$	P[b]
Age, years	54.6 ± 8.11	56.5 ± 8.59	0.322
Gender (men), n (%)	12 (34.3)	20 (47.4)	0.185
Weight, kg	86.3 ± 11.8	91.4 ± 12.7	0.082
BMI, kg/m^2	32.3 ± 3.70	33.2 ± 3.63	0.288
Waist circumference, cm	109 ± 8.98	110 ± 9.37	0.565
Fat mass, kg	32.6 ± 7.39	35.9 ± 9.32	0.097
Fat free mass, kg	51.2 ± 15.7	53.0 ± 15.2	0.415
Visceral fat, level	12.2 ± 3.49	14.9 ± 5.23	0.019
Systolic blood pressure, mmHg	134 ± 13.9	134 ± 14.5	0.984
Diastolic blood pressure, mmHg	82.9 ± 6.97	86.9 ± 9.86	0.069
Metformin, n (%)	12 (36.4)	13 (35.1)	0.915
Total cholesterol, mg/dL	215 ± 38.8	224 ± 47.4	0.382
HDL cholesterol, mg/dL	50.7 ± 9.81	54.3 ± 11.9	0.168
Triglycerides, mg/dL	184 ± 88	164 ± 108	0.899
LDL cholesterol, mg/dL	132 ± 29.4	136 ± 37.9	0.635
Apolipoprotein B, mg/dL	115 ± 28.9	124 ± 38.6	0.261
Glucose, mg/dL	113 ± 18.0	116 ± 17.1	0.409
Insulin, μUI/mL	14.9 ± 11	15.6 ± 10	0.851
HOMA-IR	4.37 ± 3.47	4.52 ± 2.82	0.622
HbA1c, %	6.11 ± 1.00	6.38 ± 1.00	0.319
GGT, U/L	41.9 ± 33.0	44.0 ± 36.0	0.592
ALT, U/L	29.9 ± 18.0	32.5 ± 18.0	0.404
Uric acid, mg/dL	6.05 ± 1.43	6.07 ± 1.22	0.956
Blood urea, mg/dL	37.9 ± 16.0	35.7 ± 13.0	0.436
Urine urea, g/L	24.0 ± 20.9	17.3 ± 9.08	0.105
Urine albumin-to-creatinine ratio, mg/g creatinine	6.53 (4.46–8.60)	6.10 (4.92–12.3)	0.070
Glomerular filtration rate, mL/min/1.73 m^2	92.0 ± 15.6	93.2 ± 18.3	0.773
CRP, g/L	4.04 ± 3.00	5.75 ± 5.00	0.962
RBP4, mg/L	25.9 ± 7.94	25.6 ± 10.1	0.885
Adiponectin, pg/μL	13,094 ± 7,086	13,059 ± 9,198	0.554
Leptin, pg/μL	23.1 ± 21.1	27.8 ± 24.8	0.404
Physical activity level, METs/min	712 ± 1040	1112 ± 1856	0.046

[a] Values are mean ± SD or median (25th percentile–75th percentile) as applicable. ALT, alanine aminotransferase; BMI; Body mass index; CRP; C-reactive protein; GGT, gamma glutamil transferase; HbA1c, glycated hemoglobin; HOMA-IR, Homeostasis model assessment of insulin resistance; HP, High protein; RBP4, Retinol Binding Protein 4; SP, Standard protein. All biochemical variables refer to fasting serum concentrations except for adipokines, which were determined in plasma, and those specifically indicated that were measured in urine.
[b] P refers to differences calculated by t-test, U-Mann–Whitney or chi-squared test, as appropiate.

In a sensitive analysis, we assessed by multiple regression analysis the effect of total protein consumption reported by participants (mean across intervention) on glucose metabolism parameters in weight loss and fully-adjusted models. We found that total protein consumption (mean of protein reported along the study, expressed as g/day) directly and significantly influenced HOMA-IR variation both at 3 and 6-months ($P = 0.036$ and $P = 0.019$, respectively, in the fully adjusted model) and insulin variation both at 3 and 6-months ($P = 0.029$ and $P = 0.017$, respectively, in the fully adjusted model). We did not observe significant association of carbohydrates consumption along the study and variation in any parameter of glucose homeostasis.

Other cardiometabolic parameters

The lipid profile homogenously improved in both diet conditions, mainly due to an improvement in the triglyceride levels. Subjects consuming HP diet showed a higher decrease in apolipoprotein B concentration after 3-months of weight loss intervention ($P = 0.047$ after adjusting by weight loss). CRP and liver enzymes significantly decreased in both diets although we did not find statistical differences between diets.

Systolic and diastolic blood pressure significantly decreased across intervention only in subjects in HP diet, although we just observed statistical differences between diets for diastolic blood pressure at 3-months assessment ($P = 0.049$ after adjusting by weight loss).

Adipokine concentration

RBP4 significantly decreased after dietary intervention both in SP and HP groups although no significant differences between diet groups were found (Table 3). Adiponectin increased more than double in HP group with respect to SP group although no statistically significant differences were denoted between diets. We neither found statistical differences after adjusting by weight loss. Leptin homogeneously decreased in both diets ($P < 0.001$ in both diet groups across intervention).

Leptin reduction showed the highest correlation with weight loss (r = 0.59 and r = 0.76 at 3 and 6-months) (Supplemental Table 3). Adiponectin inversely and moderately correlated with weight loss, while RBP4 only positively correlated with body weight change after 6-months of dietary intervention. Quite similar results were observed for body composition change correlation with adipokine concentrations change. Correlations between adipokines concentration and cardiometabolic and other biochemical parameters (although some of them did not significantly change across the study) are also shown in Supplemental Table 3. Adiponectin change inversely correlated with glucose, HbA1c and HOMA-IR changes while leptin reduction directly correlated with all glucose metabolism parameter reductions. RBP4 reduction directly correlated with total cholesterol and LDL cholesterol changes. Adiponectin concentration change was directly correlated with HDL cholesterol and CRP after 6-months of intervention although it was not observed at 3-month visit. Leptin was positively associated to triglycerides, apolipoprotein B, GGT and CRP changes

Please cite this article as: Marco-Benedí V et al., High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early-onset diabetic adults with overweight or obesity, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2019.06.005

Table 2
Changes in clinical and biochemical characteristics after 3 and 6 months of dietary intervention according to diet group.[a]

	SP diet			HP diet			*P* Δ% SP vs HP diets at 3 months[c]	*P* Δ% SP vs HP diets at 6 months[d]
	% Change from randomization to 3 months $n = 35$	% Change from randomization to 6 months $n = 32$	*P*-trend across dietary intervention[b]	% Change from randomization to 3 months $n = 38$	% Change from randomization to 6 months $n = 35$	*P*-trend across dietary intervention[b]		
Weight	−6.48 ± 3.93	−8.36 ± 5.03	<0.001	−7.12 ± 3.75	−9.20 ± 5.31	<0.001	0.485	0.519
BMI	−6.14 ± 4.36	−7.95 ± 5.32	<0.001	−7.08 ± 3.77	−9.15 ± 5.32	<0.001	0.327	0.362
Waist circumference	−4.96 ± 3.98	−7.06 ± 5.58	<0.001	−4.95 ± 4.72	−6.28 ± 5.28	<0.001	0.996	0.581
Fat mass	−10.1 ± 13.5	−13.4 ± 28.3	0.010	−14.5 ± 14.2	−16.9 ± 18.9	<0.001	0.217	0.559
Fat free mass	−3.11 ± 3.58	−3.92 ± 4.82	<0.001	−3.37 ± 3.69	−2.90 ± 4.07	<0.001	0.788	0.383
Visceral fat	−8.71 ± 12.5	−11.1 ± 21.1	<0.001	−12.5 ± 10.6	−12.5 ± 13.6	<0.001	0.069	0.885
Systolic blood pressure	−2.95 ± 11.3	−4.22 ± 11.6	0.099	−4.94 ± 10.2	−5.71 ± 9.06	0.005	0.380	0.597
Diastolic blood pressure	−1.64 ± 11.6	−2.54 ± 12.1	0.488	−7.57 ± 10.6	−6.62 ± 10.5	<0.001	0.043	0.187
Total cholesterol	−4.32 ± 10.6	−1.23 ± 14.8	0.010	−7.39 ± 10.6	−3.35 ± 10.8	<0.001	0.227	0.488
HDL cholesterol	−1.30 ± 11.9	4.23 ± 14.4	0.066	−1.20 ± 11.0	3.89 ± 14.1	0.104	0.971	0.924
Triglycerides	−18.9 ± 37.1	−15.0 ± 42.7	0.001	−21.8 ± 26.3	−19.0 ± 30.0	0.001	0.705	0.668
LDL cholesterol	0.56 ± 13.6	2.68 ± 20.9	0.927	−4.31 ± 11.1	−0.84 ± 15.2	0.081	0.123	0.462
Apolipoprotein B	−6.56 ± 15.3	−13.6 ± 26.3	<0.001	−14.2 ± 12.2	−13.8 ± 13.3	<0.001	0.025	0.966
Glucose	−6.45 ± 12.6	−1.24 ± 19.8	0.021	−10.6 ± 12.0	−12.9 ± 11.0	<0.001	0.159	0.004
Insulin	−23.4 ± 27.7	−20.6 ± 34.6	<0.001	−37.7 ± 21.3	−41.7 ± 18.1	<0.001	0.021	0.004
HOMA-IR	−27.1 ± 31.9	−20.5 ± 43.8	<0.001	−43.5 ± 24.8	−49.5 ± 16.9	<0.001	0.023	0.001
HbA1c	−3.20 ± 5.86	−4.88 ± 5.60	<0.001	−5.50 ± 6.02	−6.25 ± 8.46	<0.001	0.003	0.235
GGT	−13.1 ± 42.2	−14.5 ± 44.8	<0.001	−22.9 ± 22.1	−18.7 ± 29.9	<0.001	0.220	0.658
ALT	−12.3 ± 38.8	−15.0 ± 38.1	0.001	−22.5 ± 27.6	−21.6 ± 31.5	<0.001	0.203	0.443
Uric acid	−2.01 ± 12.0	−4.47 ± 17.0	0.015	−4.02 ± 10.4	−6.07 ± 10.9	0.001	0.453	0.651
Blood urea	0.04 ± 15.1	4.67 ± 23.0	0.631	14.8 ± 27.6	17.9 ± 24.7	<0.001	0.007	0.029
Urine urea	−25.7 ± 90.7	−12.3 ± 61.8	0.496	6.09 ± 93.0	14.6 ± 63.5	0.714	0.294	0.147
Urine albumin-to-creatinine ratio creatinine	−28.2 ± 93.1	−7.74 ± 67.4	0.056	−42.3 ± 84.2	−17.5 ± 95.4	0.120	0.510	0.993
Glomerular filtration rate	4.83 ± 7.58	3.23 ± 10.5	0.051	1.52 ± 15.3	0.98 ± 12.5	0.891	0.279	0.448
CRP	−20.8 ± 50.3	−43.5 ± 88.8	0.004	−15.0 ± 34.1	−28.6 ± 40.7	<0.001	0.348	0.304
Physical activity level	40.4 ± 127	77.3 ± 191	0.001	14.1 ± 103	40.0 ± 152	0.010	0.636	0.383

[a] Values are mean ± SD or median ± interquartile range as applicable. ALT, alanine aminotransferase; CRP; C-reactive protein; GGT, gamma glutamil transferase HbA1c, glycated hemoglobin; HOMA-IR, Homeostasis model assessment of insulin resistance; HP, High protein; SP, Standard protein. All biochemical variables refer to fasting serum concentrations except for those specifically indicated that were measured in urine.
[b] *P* refers to differences across dietary intervention within diet group what was calculated by repeated measures ANOVA or Friedman tests as applicable.
[c] *P* refers to differences (% change with respect to baseline) between diet groups what was calculated by *t*-test or U-Mann–Whitney test as applicable.
[d] *P* refers to differences (% change with respect to baseline) between diet groups what was calculated by *t*-test or U-Mann–Whitney test as applicable.

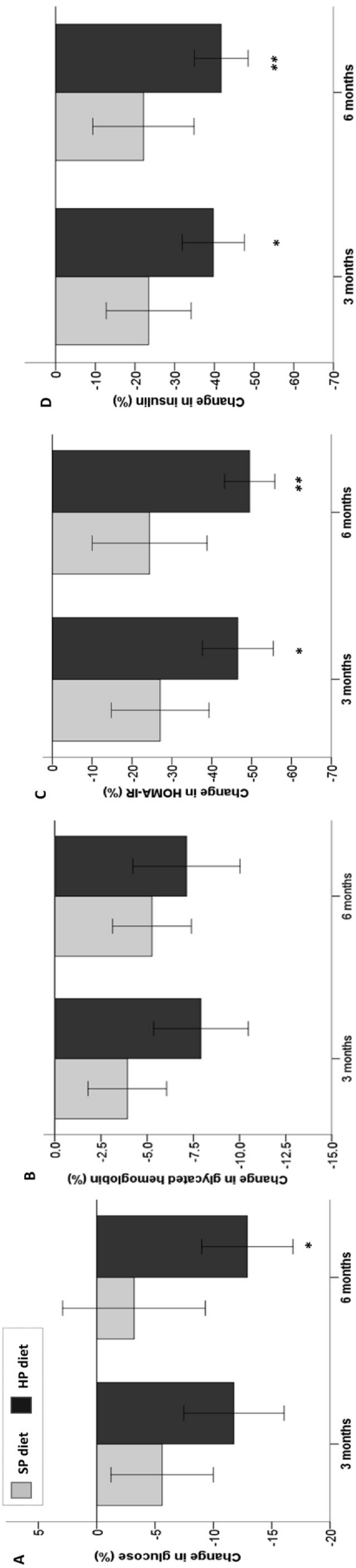


Fig. 2. Change in glucose metabolism parameters after 3 and 6 months of dietary intervention according to type of diet. Change in A) glucose; B) HbA1c; C) HOMA-IR; D) insulin concentration after 3 and 6 months of dietary intervention according to type of diet. HP, High protein; SP, Standard protein. $^{*}P < 0.05$ in weight-loss adjusted model comparing both diets at that visit and $^{**}P < 0.05$ in weight-loss adjusted model and in fully adjusted model (weight loss, baseline visceral fat, metformin treatment (yes/no) and baseline concentrations of glucose and HbA1c) comparing both diets at that visit.

Please cite this article as: Marco-Benedí V et al., High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early-onset diabetic adults with overweight or obesity, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2019.06.005

Table 3
Plasma adipokines concentration change after 3 and 6 months of dietary intervention according to diet group.[a]

		SP diet			HP diet				
		3 months $n = 35$	6 months $n = 32$		3 months $n = 38$		6 months $n = 35$		
		Mean (CI)	Mean (CI)	*P*-trend across dietary intervention[b]	Mean (CI)	*P*[c]	Mean (CI)	*P*[c]	*P*-trend across dietary intervention[b]
RBP4	Average change from baseline	−8.32 (−17.0, 0.35)	−3.06 (−14.7, 8.56)	0.031	−8.89 (−16.8, 0.35)	–	0.75 (−9.10, 10.6)	–	0.024
	Model 1: raw	Dif. vs SP	Ref.	Ref.	–	−3.40 (13.9, 7.12)	0.521	0.591	0.591	–
	Model 2: adjusted for weight loss	Dif. vs SP	Ref.	Ref.	–	−3.20 (13.8, 7.44)	0.550	0.285	0.285	–
Adiponectin	Average change from baseline	6.05 (−4.01, 16.1)	23.7 (10.6, 36.7)	<0.001	29.9 (0.07, 58.1)	–	53.5 (19.1, 88.0)	–	<0.001
	Model 1: raw	Dif. vs SP	Ref.	Ref.	–	19.8 (−8.50, 48.0)	0.167	0.114	0.114	–
	Model 2: adjusted for weight loss	Dif. vs SP	Ref.	Ref.	–	17.5 (−10.6, 45.6)	0.219	0.184	0.184	–
Leptin	Average change from baseline	−37.6 (−45.6, −29.6)	−33.5 (−42.7, −24.3)	<0.001	−36.0 (−45.3, −26.8)	–	−33.1 (−43.5, −22.6)	–	<0.001
	Model 1: raw	Dif. vs SP	Ref.	Ref.	–	0.99 (−11.2, 13.2)	0.872	0.927	0.927	–
	Model 2: adjusted for weight loss	Dif. vs SP	Ref.	Ref.	–	4.19 (−5.51, 13.9)	0.392	0.136	0.136	–

[a] Values are percentage with respect to baseline and are expressed as means (95% confidence interval). Dif. denotes difference and refers to the difference HP vs SP. HP, High protein; RBP4, Retinol Binding Protein 4; SP, Standard protein.
[b] *P* refers to significance of adipokine change across intervention within dietary group. It is calculated by repeated measures ANOVA or Friedman tests as appropriate.
[c] *P* refers to significance of adipokine change comparing to SP diet which was calculated by multiple linear regression.

although these relationships disappeared after adjusting by weight loss. Linear regression models showed that: a) all these significant associations disappeared after adjusting by weight loss except for the relation between adiponectin with HbA1c changes; b) the type of diet did not show any significant impact on the association between adipokine and cardiometabolic changes.

Study intervention adherence

Urine urea concentration decreased during intervention in SP diet while it increased in those subjects randomized to HP diet (Table 2). We calculated a urine urea/weight (kg) ratio to normalize this variable for body weight. A decrease in the urine urea/body weight ratio (−23.6 ± 104% and −6.99 ± 66.7% after 3 and 6-months respectively) was observed in SP group while it increased (10.2 ± 99.6% and 33.3 ± 71.9% after 3 and 6-months respectively) in HP group ($P = 0.327$ and $P = 0.090$ comparing SP and HP diets at 3 and 6-month visits). Blood urea increased more across intervention in subjects in HP group ($P = 0.007$ and $P = 0.029$ at 3 and 6-months). These data showed a quite different consumption of protein in both groups by suggesting a correct adherence to prescribed diets.

Physical activity significantly increased during intervention in both diet groups. Exercise homogenously increased across the study without showing statistically significance between groups.

Discussion

The main finding of this study is that an energy-restricted diet containing 35% of total calories coming from protein, mainly from animal source, leads to a greater glucose metabolism improvement, especially observed in HOMA-IR, irrespective of weight loss in subjects with prediabetes or early stages or T2DM comparing to a diet with an 18% of total energy from protein energy-restricted diet. Adipokine concentration significantly and homogeneously decreased after dietary intervention in both diets, so the effect of the energy-restricted HP diet probably cannot be explained by changes in the adipose tissue functionality.

The results of our study are in agreement with a meta-analysis aimed to explore the effect of a HP diet (25–32% of total calories coming from protein) or a SP diet (15–20% of total calories coming from protein) on glucose and HbA1c in subjects with T2DM [12]. Results showed that HP diets resulted in more HbA1c decrease (−0.52%; 95% CI: −0.90, −0.14) although they did not find statistical significance in fasting blood glucose levels. We also observed higher HbA1c concentration decreases in those participants following HP diet (−6.25% vs −4.88% in HP and SP diets respectively) although we neither found statistical differences between diets after weight loss adjustment. Our results did show a glucose reduction enhance-ability of a HP diet compared to an SP diet even after adjusting by weight loss. Some important factors differ our study from previous studies included in the meta-analysis which could determine our findings. First, HP diet in our study involved a 35%-energy coming from protein while HP diets included in previous studies were up to 32% of total calories coming from protein. Higher protein consumption could lead to superior effects since a dose-dependent metabolic effect of proteins has previously been described [6,21,22]. Second, the majority of the studies included in the meta-analysis had a short duration which could be insufficient to detect significant differences in glucose metabolism outcomes. Third, participants from our study had prediabetes or new-onset diabetes, which are early stages of T2DM with certain insulin secretory capacity preservation. It has been established that those patients with short duration of T2DM may benefit from more aggressive targets and better glycemic control lead to higher rates

Please cite this article as: Marco-Benedí V et al., High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early-onset diabetic adults with overweight or obesity, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2019.06.005

of diabetes remission and/or lower risk of recidivism within these subjects. Several studies have demonstrated that weight loss improves cardiometabolic parameters especially in early stages of the disease [5]. Our findings confirm that HP diets lead to significant benefit on glucose metabolism in prediabetic and early diagnosed T2DM subjects who would benefit from a high intensity target. However, further research is needed to confirm it glycemic control enhance ability of HP diets would also be observed in subjects with T2DM of long duration.

The mechanism responsible for glucose metabolism-induced improvements of HP diets is not yet known. Our findings show that a HP diet lead to the largest reductions in HOMA-IR and insulin concentrations by pointing out the improvement in insulin resistance as a key factor in physiological effects of protein intake. Few other small-sample studies have explored the effect of energy-restricted HP diets on these outcomes in T2DM with divergent results [23–26]. Gannon et al. compared the effect of a HP diet (30% of total calories coming from protein) with a SP diet (15% of total calories coming from protein) for 5 weeks [23]. Authors observed significant differences between both diets in HbA1c concentrations but not in HOMA-IR. However, another study found a 75%-insulin sensitivity improvement in 10 obese subjects with TD2M following a 14-day diet with a low percentage of carbohydrates but HP content [24]. Other studies carried out in non-diabetic subjects also reported opposing findings on the effect of HP diets on insulin and HOMA-IR [7,10,27].

Elucidating the mechanisms mediating insulin resistance improvement deserves special attention. Many studies have focused on the effect of HP diets on incretins secretion, like GLP-1 or GIP, which have an important impact in glucose metabolism regulation and satiety [28]. Recently, some studies have proposed reductions of pro-inflammatory adipokine concentrations while increments in anti-inflammatory adipokine concentrations after weight loss through HP diets [19]. Thus, HP content diets could lead to an adipose tissue functionality improvement, which may mediate the cardiometabolic profile improvement. Our study is the first one to compare the effect of different protein content in an energy-restricted diet on adipokine concentration in subjects with IFG or T2DM. The results showed that both HP and SP diets homogenously improved adipokine concentration which was directly related to weight loss. Despite we found no statistically significant differences between diets, we observed a tendency in adiponectin variation after weight loss since those subjects consuming the HP diet had more than double increase in adiponectin concentration than those randomized to SP diet. Adiponectin exhibits anti-atherogenic, insulin-sensitizing and anti-inflammatory properties [29,30]. Some authors have described an increase in its concentration after weight loss in non-diabetic subjects while others have not found any significant change [19]. Our data do not support adipokine profile change as the key mechanism mediating glucose metabolism-enhance ability of HP diets. However, further research is needed to confirm adiponectin findings and other less common adipokines by taking other possible confounder factors like study duration or protein sources.

Total calories of a diet directly impact the absolute protein amount of protein that is consumed [31]. Thus, a 35%-protein within a 1800 kcal (7530 kJ) diet involves 158 g of protein per day while the same diet would imply 105 g of protein per day in a 1200 kcal (5020 kJ) diet. Thus, it is essential to state the absolute protein quantity regardless of calorie content of diet and protein sources determining its quality. Protein source could also play an essential role in its physiological effect. When consumed in excess of postprandial protein synthesis, amino acids can readily be used as substrate for oxidation [31]. If protein oxidation provides more ATP than the liver could use, amino acids could lead to hepatic gluconeogenesis or they can be converted into ketone bodies through ketogenesis. Threonine or isoleucine can be converted into either glucose or ketone bodies, whereas lysine and leucine are strictly ketogenic and therefore not used as a substrate for gluconeogenesis. Thus, differential impact on glucose metabolism of different protein foods is clear. Few studies have described the differential effect of vegetal and animal proteins in metabolic effect of proteins and it has not been previously explored in subjects with IFG or T2DM [32,33]. Prescribed diets in our study include >150 g/day of protein coming mainly from animal sources which could be a key element in the insulin resistance improvement we found in those subjects consuming the HP diet.

Considering that the diets of this trial had the same amount of fat and they just differed in protein and carbohydrates, the impact of each macronutrient in the results deserves special attention. If the low content in carbohydrate in the diet has a role in the cardiometabolic beneficial effect within HP diets cannot be discarded. However, the studies exploring the potential benefits of low-carbohydrate diets on glucose metabolism in absence of HP do not shown consistent results [34,35], in contrast with HP trials [7,9,12]. Furthermore, in our study the protein content in the diet was an independent factor associated to insulin resistance improvement but carbohydrate content was not. Further long-term studies exploring the independent effect of each macronutrient should be carried out to elucidate this issue.

Our study has some limitations worth mentioning. The mid-term intervention design could have influenced findings, although most interventional studies that have explored the effect of HP diets on glucose metabolism in diabetic subjects have a shorter time frame. The relatively small sample size could have limited the significance of the effect of HP in some outcomes. We have not assessed incretins and other glucose-related metabolites which are also proposed as potential mediators for glucose metabolism improvement effect of protein consumption. We have studied a limited number of adipokines, though we selected those accumulating more evidence on glucose metabolism. Diet compliance and physical activity were assessed by self-reported questionnaires which is a limitation. We assessed urine nitrogen in a spot sample which is established as a good surrogate biomarker of protein consumption [18]; however, the determination of 24 h urinary nitrogen would have provided a more reliable measurement of protein intake and patient's nitrogen balance. Accelerometry would have also provided a more accurate physical activity assessment. It is also difficult to discern if our findings could be entirely due to high protein consumption or lower carbohydrates intake which is inherent due to the stable fat content. Finally, to analyze the total years of prediabetes or T2DM duration would have been useful to explore if this factor, determining the pancreatic insulin reserve, could directly impact the effect of HP diets effect on glucose metabolism, as previously stated.

In conclusion, an energy-restricted diet containing 35% of total calories coming from protein (mean of 1.34 g/kg/day), mainly from animal source, and low in carbohydrates, leads to a greater glucose metabolism improvement, especially observed in HOMA-IR, irrespective of weight loss in subjects with IFG or early stages of T2DM comparing to a diet with an 18% of total energy from protein (mean of 0.75 g/kg/day) energy-restricted diet. This effect was not explained by changes in plasma concentrations of adipokines. Further research is crucial to confirm the mechanisms responsible for the beneficial effect of HP diets on glucose metabolism by elucidating the role of protein source and the exact and absolute protein amount leading to this improvement.

Please cite this article as: Marco-Benedí V et al., High-protein energy-restricted diets induce greater improvement in glucose homeostasis but not in adipokines comparing to standard-protein diets in early-onset diabetic adults with overweight or obesity, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2019.06.005

Sources of support

This study was supported in part by three grants from the Carlos III Research Institute: CIBERCV (co-supported by the European Regional Development Fund (ERDF) which is allocated by the European Union; IIS16/0114), PI13/02507 and PI15/01983; and a grant from INTEROVIC. Funding sources were not involved in study design, collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Conflict of interest

Authors have no relevant conflict of interest to disclose.

Acknowledgments

Author contributions were as follows: V.M.B conducted research and analyzed data; S.P.C conducted research and analyzed data; A.M.B conducted research; I.L.M conducted research; L.B.R conducted research; A.C conducted research, F.C designed the research, analyzed data, wrote the manuscript and had primary responsibility for final content; R.M.G designed the research, analyzed data, wrote the manuscript and had primary responsibility for final content. All authors have read and approved the final manuscript.

The authors thank Antonio Oliván and Patricio Pérez from Pastores (Teruel, Spain) and I.G.P. Ternasco de Aragón (Zaragoza, Spain), respectively, for help with study logistics, and Jacob Momberger for his English language assistance. Rocío Mateo-Gallego research activity is funded by Instituto Aragonés de Ciencias de la Salud.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2019.06.005.

References

[1] Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393–403.
[2] Association AD. Obesity management for the treatment of type 2 diabetes. Diabetes Care 2017;40:S57–63.
[3] de Souza RJ, Bray GA, Carey VJ, Hall KD, LeBoff MS, Loria CM, et al. Effects of 4 weight-loss diets differing in fat, protein, and carbohydrate on fat mass, lean mass, visceral adipose tissue, and hepatic fat: results from the POUNDS LOST trial. Am J Clin Nutr 2012;95:614–25.
[4] Pastors JG, Warshaw H, Daly A, Franz M, Kulkarni K. The evidence for the effectiveness of medical nutrition therapy in diabetes management. Diabetes Care 2002;25:608–13.
[5] Steven S, Hollingsworth KG, Al-Mrabeh A, Avery L, Aribisala B, Caslake M, et al. Very low-calorie diet and 6 months of weight stability in type 2 diabetes: pathophysiological changes in responders and nonresponders. Diabetes Care 2016;39:808–15.
[6] Leidy HJ, Clifton PM, Astrup A, Wycherley TP, Westerterp-Plantenga MS, Luscombe-Marsh ND, et al. The role of protein in weight loss and maintenance [Internet] Am J Clin Nutr 2015. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25926512.
[7] Wycherley TP, Moran LJ, Clifton PM, Noakes M, Brinkworth GD. Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2012;96:1281–98.
[8] Appel LJ, Sacks FM, Carey VJ, Obarzanek E, Swain JF, Miller ER, et al. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. J Am Med Assoc 2005;294:2455–64.
[9] Larsen TM, Dalskov SM, van Baak M, Jebb SA, Papadaki A, Pfeiffer AF, et al. Diets with high or low protein content and glycemic index for weight-loss maintenance. N Engl J Med 2010;363:2102–13.
[10] Mateo-Gallego R, Marco-Benedí V, Perez-Calahorra S, Bea AM, Baila-Rueda L, Lamiquiz-Moneo I, et al. Energy-restricted, high-protein diets more effectively impact cardiometabolic profile in overweight and obese women than lower-protein diets. Clin Nutr 2017;36:371–9.
[11] Association AD. 4. Lifestyle management. Diabetes Care 2017;40:S33–43.
[12] Dong JY, Zhang ZL, Wang PY, Qin LQ. Effects of high-protein diets on body weight, glycaemic control, blood lipids and blood pressure in type 2 diabetes: meta-analysis of randomised controlled trials. Br J Nutr 2013;110:781–9.
[13] American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. Diabetes Care 2018;41:S13–27.
[14] FAO/WHO/UNU. Energy and protein requirements: report of a joint FAO/WHO/UNU expert consultation. Geneva, Switzerland: FAO/WHO/UNU; 1985. Report No.: WHO Technical Report Series No. 724.
[15] Mataix Verdu J, Manas Almendros M, Llopis Gonzalez J, Martinez de Victoria Munoz E. Tabla de composicion de alimentos espanoles. 1995 [cited 2018 Feb 1]; Available from: http://agris.fao.org/agris-search/search.do?recordID=XF2015033769.
[16] Hagströmer M, Oja P, Sjöström M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. Public Health Nutr 2006;9:755–62.
[17] Linares CL, Ciangura C, Bouillot J-L, Coupaye M, Declèves X, Poitou C, et al. Validity of leg-to-leg bioelectrical impedance analysis to estimate body fat in obesity. Obes Surg 2011;21:917–23.
[18] Umesawa M, Yamagishi K, Sawachi S, Ikeda A, Noda H, Ikehara S, et al. Urea nitrogen concentrations in spot urine, estimated protein intake and blood pressure levels in a Japanese general population. Am J Hypertens 2010;23:852–8.
[19] Klempel MC, Varady KA. Reliability of leptin, but not adiponectin, as a biomarker for diet-induced weight loss in humans. Nutr Rev 2011;69:145–54.
[20] Mateo-Gallego R, Lamiquiz-Moneo I, Perez-Calahorra S, Marco-Benedí V, Bea AM, Baila-Rueda L, et al. Different protein composition of low-calorie diet differently impacts adipokine profile irrespective of weight loss in overweight and obese women. Nutr Metab Cardiovasc Dis NMCD; 2017.
[21] Belza A, Ritz C, Sørensen MQ, Holst JJ, Rehfeld JF, Astrup A. Contribution of gastroenteropancreatic appetite hormones to protein-induced satiety. Am J Clin Nutr 2013;97:980–9.
[22] Giezenaar C, Luscombe-Marsh ND, Hutchison AT, Standfield S, Feinle-Bisset C, Horowitz M, et al. Dose-dependent effects of randomized intraduodenal whey-protein loads on glucose, gut hormone, and amino acid concentrations in healthy older and younger men. Nutrients 2018;10.
[23] Gannon MC, Nuttall FQ, Saeed A, Jordan K, Hoover H. An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. Am J Clin Nutr 2003;78:734–41.
[24] Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. Ann Intern Med 2005;142:403–11.
[25] Brinkworth GD, Noakes M, Parker B, Foster P, Clifton PM. Long-term effects of advice to consume a high-protein, low-fat diet, rather than a conventional weight-loss diet, in obese adults with type 2 diabetes: one-year follow-up of a randomised trial. Diabetologia 2004;47:1677–86.
[26] Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. Am J Clin Nutr 2003;78:31–9.
[27] de Luis DA, Izaola O, Aller R, de la Fuente B, Bachiller R, Romero E. Effects of a high-protein/low carbohydrate versus a standard hypocaloric diet on adipocytokine levels and insulin resistance in obese patients along 9 months. J Diabetes Complicat 2015;29:950–4.
[28] van der Klaauw AA, Keogh JM, Henning E, Trowse VM, Dhillo WS, Ghatei MA, et al. High protein intake stimulates postprandial GLP1 and PYY release. Obes Silver Spring 2013;21:1602–7.
[29] Fernández-Real JM, López-Bermejo A, Casamitjana R, Ricart W. Novel interactions of adiponectin with the endocrine system and inflammatory parameters. J Clin Endocrinol Metab 2003;88:2714–8.
[30] Balagopal PB, de Ferranti SD, Cook S, Daniels SR, Gidding SS, Hayman LL, et al. American Heart Association Committee on Atherosclerosis Hypertension and Obesity in Youth of the Council on Cardiovascular Disease in the Young, et al. Nontraditional risk factors and biomarkers for cardiovascular disease: mechanistic, research, and clinical considerations for youth: a scientific statement from the American Heart Association. Circulation 2011;123:2749–69.
[31] Westerterp-Plantenga MS, Nieuwenhuizen A, Tomé D, Soenen S, Westerterp KR. Dietary protein, weight loss, and weight maintenance. Annu Rev Nutr 2009;29:21–41.
[32] Ozawa M, Yoshida D, Hata J, Ohara T, Mukai N, Shibata M, et al. Dietary protein intake and stroke risk in a general Japanese population: the Hisayama study. Stroke 2017;48:1478–86.
[33] Chalvon-Demersay T, Azzout-Marniche D, Arfsten J, Egli L, Gaudichon C, Karagounis LG, et al. A systematic review of the effects of plant compared with animal protein sources on features of metabolic syndrome. J Nutr 2017;147:281–92.
[34] Tay J, Thompson CH, Luscombe-Marsh ND, Wycherley TP, Noakes M, Buckley JD, et al. Effects of an energy-restricted low-carbohydrate, high unsaturated fat/low saturated fat diet versus a high-carbohydrate, low-fat diet in type 2 diabetes: a 2-year randomized clinical trial. Diabetes Obes Metab 2018;20:858–71.
[35] van Zuuren EJ, Fedorowicz Z, Kuijpers T, Pijl H. Effects of low-carbohydrate-compared with low-fat-diet interventions on metabolic control in people with type 2 diabetes: a systematic review including GRADE assessments. Am J Clin Nutr 2018;108:300–31.

Atherosclerosis 284 (2019) 245–252

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia

Sofía Perez-Calahorra[a], Martín Laclaustra[a], Victoria Marco-Benedí[a], Itziar Lamiquiz-Moneo[a], Juan Pedro-Botet[b], Núria Plana[c], Rosa M. Sanchez-Hernandez[d], Antonio J. Amor[e], Fátima Almagro[f], Francisco Fuentes[g], Manuel Suarez-Tembra[h], Fernando Civeira[a,i,*], on behalf of the Dyslipemia Registry of Spanish Arteriosclerosis Society

[a] Lipid Unit. Hospital Universitario Miguel Servet, IIS Aragon, CIBERCV, Zaragoza, Spain
[b] Lipid and Vascular Risk Unit, Department of Endocrinology and Nutrition, Hospital del Mar, Universitat Autònoma de Barcelona, Barcelona, Spain
[c] Unitat de Medicina Vascular i Metabolism, Hospital Universitari Sant Joan, Institut d'Investigación Sanitaria Pere Virgili (IISPV), CIBERDEM, Reus, Tarragona, Spain
[d] Lipid Unit, Endocrinology Department, Hospital Universitario Insular de Gran Canaria, Instituto Universitario de Investigaciones Biomédicas y Sanitarias, Universidad de Las Palmas de Gran Canaria, Las Palmas de, Gran Canaria, Spain
[e] Lipid Unit, Endocrinology and Nutrition Service, Institut d'Investigacions Biomèdiques August Pi Sunyer, Hospital Clínic, CIBEROBN, Barcelona, Spain
[f] Lipid Unit, Hospital Universitario Donostia, San Sebastián, Spain
[g] Lipid Unit, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Hospital Universitario Reina Sofía, CIBEROBN, Universidad de Córdoba, Spain
[h] Lipid Unit, Hospital San Rafael, A Coruña, Spain
[i] Universidad de Zaragoza, Zaragoza, Spain

HIGHLIGHTS

- Statins have changed the natural history of cardiovascular disease (CVD) in heterozygous familial hypercholesterolemia (HeFH).
- We report the current CVD prevalence in HeFH after an average of 9.7 years of statins.
- 10% of HeFH suffered a CVD event after more than 12 years of statin treatment.
- HeFH at high risk with high-intensity statins are those with > 3 risk factors.
- This study identifies HeFH patients susceptible for more intensive treatment.

ARTICLE INFO

Keywords:
Familial hypercholesterolemia
Cardiovascular disease
Lipid-lowering
Statins

ABSTRACT

Background and aims: The impact on heterozygous familial hypercholesterolemia (HeFH) health led by high-intensity lipid-lowering therapy (HILLT) is unknown, and the question remains if there is still an unacceptably high residual risk to justify treatment with new lipid-lowering drugs.
Methods: This observational, retrospective, multicenter, national study in Spain, whose information was obtained from a national dyslipemia registry, was designed to establish the current prevalence of cardiovascular disease (CVD) in HeFH and to define the impact of HILLT on CVD in this population. Odds were estimated using several logistic regression models with progressive adjustment.
Results: 1958 HeFH, mean age 49.3 ± 14.3 years, were included in the analysis. At inclusion in the registry, 295 patients (15.1%) had suffered CVD and 164 (55.6%) had suffered the first event before the onset lipid-lowering treatment. Exposition to treatment associated more than ten times lower odds for CVD than in subjects naïve to treatment (OR 0.085, 95% CI 0.063–0.114, $p < 0.001$). A first CVD event after a mean treatment period of 9.1 ± 7.2 years occurred in 131 out of 1615 (8.1%) HeFH subjects, and 115 (87.8%) of them were on HILLT.
Conclusions: Current prevalence of CVD among HeFH is one third of that reported before the statins era. Early initiation and prolonged lipid-lowering treatment was associated with a reduction in CVD. New cases of CVD, in spite of HILLT, appeared mostly among patients accumulating risk factors and probably they may be considered for further lipid-lowering drugs.

* Corresponding author. Unidad de Lípidos, Hospital Universitario Miguel Servet, Paseo Isabel La Católica 1-3, 50009, Zaragoza, Spain.
E-mail address: civeira@unizar.es (F. Civeira).

https://doi.org/10.1016/j.atherosclerosis.2019.02.003
Received 13 October 2018; Received in revised form 9 January 2019; Accepted 1 February 2019
Available online 12 February 2019
0021-9150/

1. Introduction

Familial hypercholesterolemia (FH) is one of the most common genetic diseases in the world [1]. The estimated prevalence of heterozygotes FH (HeFH) is one in every 200–250 persons [2,3] and it is even higher in areas with some genetic isolation [4]. FH subjects are characterized by very high plasma concentration of low-density lipoprotein (LDL) cholesterol with autosomal co-dominant pattern of transmission, tendon xanthomas and high risk of premature coronary heart disease (CHD) [5]. Most cases of FH are caused by loss-of-function mutations in the genes encoding the LDL particle receptor (*LDLR*) [6], or apolipoprotein B (*APOB*) [7], but also by gain-of-function mutations in the genes encoding for proprotein convertase subtilisin/kexin type 9 (*PCSK9*) [8] or apolipoprotein E (*APOE*) [9].

Untreated affected subjects have a markedly elevated long-term CHD risk, with hazard ratios up to 5.0 with respect to the general population [10,11], and early mortality with up to 100-fold increase from CHD in young adults. This high CHD risk reduces life expectancy of 20 years for men and 12 years for women [10]. Consequently, international clinical guidelines classified HeFH as a high-risk condition, which deserves early diagnosis and treatment [1,5,12].

The advent of potent lipid-lowering drugs, especially 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors or statins, has been a landmark for people suffering from FH. Since the late 1980s, this pharmacological therapy, sometimes in association with ezetimibe, has substantially reduced or even normalized LDL cholesterol concentrations in HeFH, and the natural history of the disease has been importantly modified [5]. Different reports from United Kingdom [13], Norway [14], Denmark [15] and The Netherlands [16] indicate that, although cardiovascular disease (CVD) remains significantly higher in treated heterozygous FH than in subjects from the general population, CVD has substantially improved in HeFH in recent years. However, the impact on HeFH health led by high-intensity lipid lowering therapy is basically unknown, as well as the question whether this treatment is sufficient or still the residual risk is unacceptably high to justify treatment with new lipid-lowering drugs, such as PCSK9 inhibitors.

Most diagnosed cases of HeFH in Spain, especially those with genetic diagnosis, are controlled in specialized lipid units distributed throughout the country, organized in a network within the Spanish Atherosclerosis Society (SEA). SEA created a National Registry in 2013 that includes primary dyslipidemias using homogeneous clinical diagnostic criteria [17,18]. We hypothesized that lipid-lowering therapy has improved HeFH cardiovascular prognosis in recent years. Thus, the objective of this analysis was to establish the current prevalence of CVD in HeFH adults, and to assess the impact of high intensity lipid lowering treatment of CVD in this population.

2. Materials and methods

2.1. Study characteristics

This observational, retrospective, multicenter, national study in Spain was designed to determine current prevalence of CVD in patients with HeFH in the era of statin treatment. The impact of lipid lowering treatment was studied with a case-control approach. The information was obtained from the Dyslipidemia Registry of the SEA. This is an active online registry, where 50 certified lipid clinics distributed throughout all regions of Spain report cases of various types of primary hyperlipidemias [17]. The anonymous clinical data collection in this registry was approved by a central ethical committee (Comité Ético de Investigación Clínica de Aragón, CEICA). Inclusion criteria were standardized in 5 training sessions before case recruitment. For HeFH, the registry includes personal and family health history, anthropometry, physical examination, laboratory data, presence of CVD, age at which CVD events occurred, age at which statin treatment began, history of lipid-lowering treatment, and genetic data regarding mutations in *LDLR, APOB* or *PCSK9* (positive, negative or unknown). Patients were eligible for inclusion in this study if they were 18 years of age or older, with clinical or genetic diagnosis of HeFH. Clinical diagnosis was based on the diagnostic criteria proposed by the Dutch Lipid Clinics Network (DLCN): 6–8 points (probable), and > 8 points (definite) [1]. Genetic diagnosis was based on tested carrier status of a known pathogenic mutation for FH. Pathogenicity definition of mutations followed the American College of Medical Genetics ACMG recommendations [18]. Only pathogenic and likely pathogenic mutations were considered as causal in this analysis. Additional written informed consent was required for genetic analysis. Homozygous FH were not included in this study. CVD is defined as: coronary (myocardial infarction, coronary revascularization procedure, sudden death); cerebral (stroke with > 24 h neurological deficit without evidence of bleeding in brain imaging tests); peripheral vascular disease (intermittent claudication with ankle arm index < 0.9, or arterial revascularization of lower limbs) or symptomatic or asymptomatic abdominal aortic aneurysm. Arterial hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or self-reported use of antihypertensive medication. Diabetes was defined as fasting plasma glucose ≥126 mg/dl, HbA1c ≥ 6.5% or self-reported treatment with antidiabetic medications. Current smoking was defined as current smoking or having smoked in the last year. Former smoker was defined as a subject having smoked at least 50 cigarettes in his lifetime, but not having smoked in the last year. Severe high LDL cholesterol was considered when > 250 mg/dl in the absence on lipid-lowering drugs [12].

Lipid-lowering treatment was classified into three categories according to the type of drug and the daily dose: low intensity treatment (ezetimibe 5–10 mg, simvastatin 5–10 mg, lovastatin 20 mg, pravastatin 10–20 mg, fluvastatin 20–40 mg or pitavastatin 1 mg), moderate intensity treatment (atorvastatin 10–20 mg, rosuvastatin 5–10 mg, simvastatin 20–40 mg, fluvastatin 80 mg, lovastatin 40 mg, pravastatin 40 mg or pitavastatin 2–4 mg) and high intensity treatment (rosuvastatin 20–40 mg, atorvastatin 40–80 mg, or any daily statin doses plus ezetimibe) [19]. Only extended lipid-lowering (> 6 months) at entry was considered.

We conducted this study in accordance with the Declaration of Helsinki for the protection of the rights and welfare of people participating in biomedical research.

2.2. Statistical analysis

Prevalence and HeFH description used the information at the time of data collection. Cases were participants who had suffered a first CVD event and controls were the remainder participants. Lipid-lowering treatment use defined the exposure variable. A participant was deemed exposed to lipid-lowering treatment if the recorded treatment start age predated the first CVD event in cases, and in all cases of treatment in controls. Duration of exposure was used for a dose-effect analysis. Years of lipid-lowering therapy were calculated by subtracting age of treatment to age of event among cases, and age of treatment to age at the time of data collection among controls. This variable was categorized in non-exposed, and tertiles of duration among those exposed (resulting in cutoff values at 5 and 12 years of treatment). Case-control age, for adjustment, was that of the first CVD event for cases and that at the time of data collection for controls. Other clinical variables used as potential determinants of CVD were values present at the time of data collection for both cases and controls.

Odds ratios (OR) were estimated using several logistic regression models with progressive adjustment: Model 1 was unadjusted, model 2 was adjusted for gender and age, model 3 was further adjusted for hypertension, diabetes, tobacco, HDL cholesterol (mg/dl), LDL cholesterol (mg/dl), and body mass index (BMI) (kg/m^2), and model 4 additionally for lipoprotein (a) [Lp(a) mg/dl].

A second logistic regression analysis with the same progressive adjustments was performed restricted to those exposed to high intensity treatment (as defined above) to study factors that influence CVD among those under such treatment.

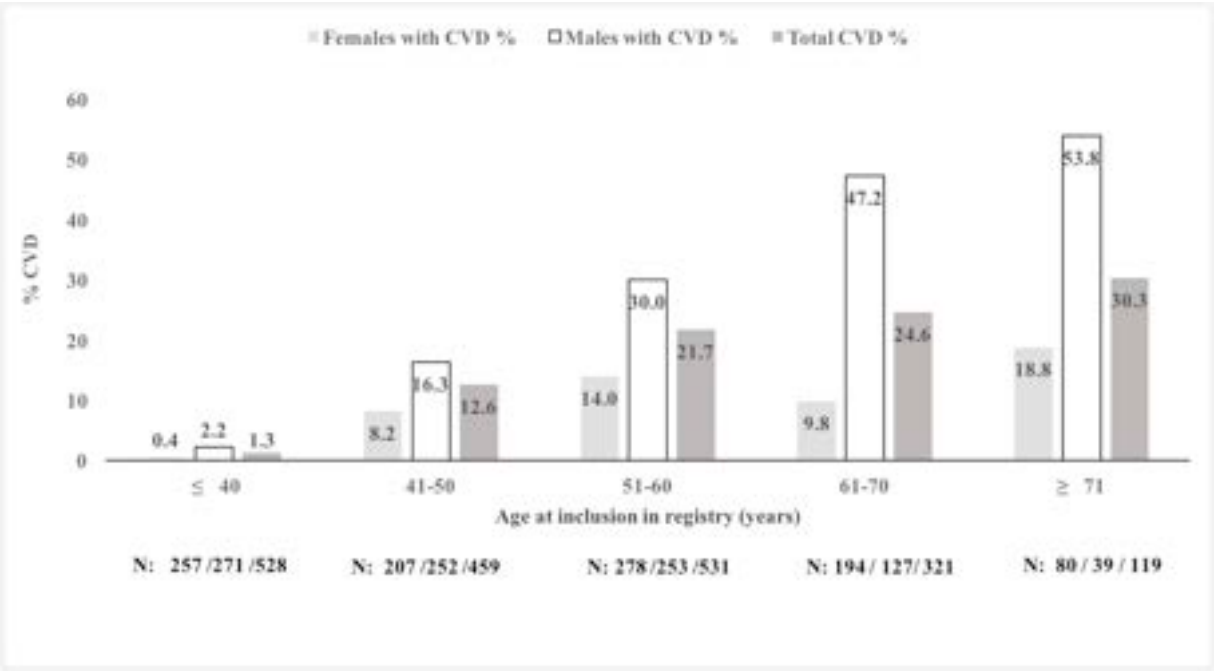


Fig. 1. Prevalence of cardiovascular disease according to age at the entry in the registry stratified by gender.
CVD, cardiovascular disease.

Table 1
Anthropometric, clinical and biochemical characteristic of HeFH subjects at registry inclusion.

Variables	Total (n = 1958)	Non-CVD (n = 1663)	CVD (n = 295)	p^a
Age at registry, years	49.3 ± (14.3)	47.8 ± (14.3)	58.0 ± (10.2)	< 0.001
Case-control age [b], years	47.7 ± (13.7)	47.8 ± (14.3)	47.6 ± (9.9)	0.267
Gender (male)	48.1 (942)	44.4 (738)	69.2 (204)	< 0.001
Body mass index, (Kg/m^2)	26.2 ± (4.4)	25.9 ± (4.4)	28.1 ± (4.3)	< 0.001
Xanthomas, % (n)	32.0 (626)	31.9 (530)	32.5 (96)	0.545
Tobacco consumption				0.001
Never smoke, % (n)	53.9 (1056)	56.0 (932)	42.0 (124)	
Ever smoke, % (n)	46.1 (902)	44.0 (731)	58.0 (171)	
Hypertension, % (n)	19.6 (383)	15.5 (258)	42.4 (125)	< 0.001
Diabetes, % (n)	6.5 (128)	4.4 (73)	18.6 (55)	< 0.001
Lipids without treatment				
Total cholesterol, mg/dl	348 ± (76.2)	345 ± (72.0)	362 ± (95.0)	< 0.001
HDL cholesterol, mg/dl	54.8 ± (15.7)	55.8 ± (15.5)	49.2 ± (15.8)	< 0.001
LDL cholesterol, mg/dl	269 ± (74.7)	267 ± (69.9)	285 ± (96.3)	< 0.001
Triglycerides, mg/dl	132 ± (118)	128 ± (120)	157 ± (98.2)	< 0.001
Lipoprotein (a), mg/dl (n = 1360)	49.4 ± (56.9)	47.8 ± (57.0)	59.0 ± (55.6)	0.004
Clinical HeFH diagnosis				0.101
Probable (6–8 DLCN points), % (n)	18.1 (354)	17.4 (290)	21.7 (64)	
Definite (> 8 DLCN points), % (n)	81.9 (1604)	82.6 (1373)	78.3 (231)	
Genetic test				< 0.001
Unknown, % (n)	24.4 (478)	22.9 (381)	32.9 (97)	
Negative, % (n)	10.6 (207)	11.3 (188)	6.4 (19)	
Positive, % (n)	65.0 (1273)	65.8 (1094)	60.7 (179)	

Values are numbers (%), mean ± (SD), as applicable.
CVD, cardiovascular disease; HDL, high-density lipoprotein; HeFH, heterozygous familial hypercholesterolemia; LDL, low-density lipoprotein; DLCN, Dutch Lipid Clinic Network.
[a] *p* values refer to differences calculated after adjusting gender and case-control age, as appropriate.
[b] Case-control age refers to the age of controls at their inclusion in the registry, and the age of the first CVD event in the group of cases.

3. Results

3.1. Study population

A total of 1958 HeFH patients, 1016 women and 942 men, with a mean age of 49.3 years fulfilled the inclusion criteria. Probable (6–8 DLCN points) and definite HeFH (> 8 DLCN points) was diagnosed in 354 (18.12%) and 1604 (81.98%) subjects, respectively. A positive genetic diagnosis was present in 1273 (65.0%) subjects. At inclusion in the registry, 295 patients (15.1%) had suffered CVD. Prevalence of CVD among HeFH increased with age and male gender (Fig. 1). Subjects with CVD were older, more frequently men, had higher BMI, tobacco consumption, and had more frequently hypertension and diabetes than those HeFH without CVD. In addition, HeFH patients with CVD had lower HDL cholesterol and higher LDL cholesterol, triglycerides and Lp (a) concentrations without lipid-lowering treatment (Table 1).

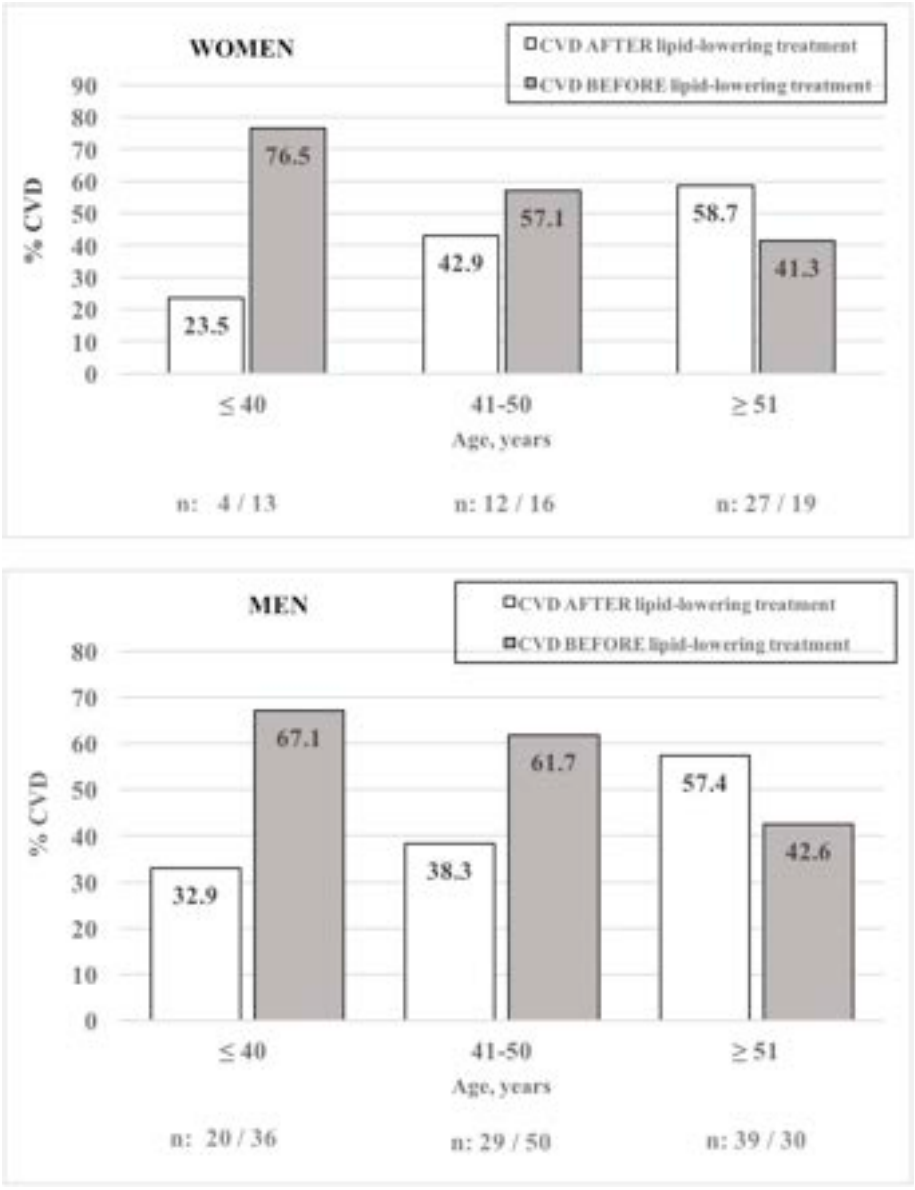


Fig. 2. Distribution of a first cardiovascular event in relation to the onset of lipid-lowering treatment stratified by age and gender. CVD, cardiovascular disease.

3.2. Effect of exposure to lipid-lowering treatment on CVD

Among HeFH patients with CVD, the first event occurred before the onset lipid-lowering treatment in 164 (55.6%) of the subjects. This percentage was slightly higher for men (56.9%) than for women (52.7%) and had a tendency to decrease with age in both genders (Fig. 2).

The mean exposure time to lipid-lowering treatment was 9.7 years, being lower in subjects with CVD, although without reaching statistical significance (Table 2).

To better establish the impact of lipid-lowering treatment on CVD, we calculated the risk of presenting a first CVD event according to previous exposure to lipid-lowering treatment and its duration. Treatment exposure was associated with more than ten times, gender and age adjusted, lower odds of CVD than in treatment naïve patients (OR 0.085, 95% CI 0.063 to 0.114, $p < 0.001$) (Supplemental Fig.1). This CVD protection was also analyzed as lipid-lowering treatment exposure increased. According to tertiles of years of exposure to statins, with respect to those not exposed to statins, the OR for those subjects with exposure < 5 years, between 5 and 12 years and > 12 years was 0.095 (95% CI 0.065 to 0.139), 0.086 (95% CI 0.058 to 0.128) and 0.071 (95% CI 0.047 to 0.108), respectively, with significant differences *versus* non-exposed ($p < 0.001$ in all cases) (Table 3). There was a trend for a greater protection among longer exposures but, due to the small number of events among those exposed, the differences did not reach statistical significance. These ORs remained similar after further adjustment for sex, age, BMI, hypertension, diabetes, tobacco, HDL cholesterol and LDL cholesterol levels without treatment. Adjustment for Lp(a) concentration did not modify ORs in the regression (Table 3).

3.3. Risk factors for CVD in subjects with lipid-lowering treatment

A first CVD event occurred in 131 out of 1615 (8.1%) HeFH subjects already in extended lipid-lowering (> 6 months) treatment. Among them, 115 (84.6%) were on high intensity therapy, including 75 (65.2%) combining ezetimibe, and 93 (71.0%) had a positive genetic diagnosis. Clinical characteristics of these groups are shown in Table 2. There were more men and they were older, with higher BMI, more

Table 2
Anthropometric, clinical and biochemical characteristics of HeFH subjects with and without CVD after lipid-lowering treatment.

Variables	No CVD n = 1484	CVD n = 131	*p*
Control-cases age, years	48.3 ± (14.1)	50.0 ± (10.8)	0.022
Gender (male), % (n)	45.3% (672)	67.2% (88)	< 0.001
Body Mass Index, Kg/m^2	25.9 ± (4.3)	28.3 ± (4.5)	< 0.001
Xanthomas, % (n)	33.7% (484)	37.3% (47)	0.660
Tobacco consumption			0.848
Never smoker, % (n)	55.3% (821)	51.1% (67)	
Ever smoker, % (n)	44.7% (663)	48.9% (64)	
Hypertension, % (n)	15.5% (230)	38.9% (51)	< 0.001
Diabetes, % (n)	4.2% (63)	20.6% (27)	< 0.001
Pre-treatment LDL-cholesterol, mg/dl	268 ± (69.0)	295 ± (102)	< 0.001
Post-treatment LDL-cholesterol, mg/dL	140.1 ± (49.9)	126.7 ± (49.1)	0.016
Lp(a), mg/dl (n = 1169)	47.7 ± (57.4)	55.8 ± (50.8)	0.152
Treatment intensity			0.014
Unknown, % (n)	8.5 (126)	7.6 (10)	
Low intensity, % (n)	1.9% (28)	0.8% (1)	
Moderate intensity, % (n)	20.8% (309)	3.8% (5)	
High intensity, % (n)	68.8% (1021)	87.8 (115)	
Time with lipid-lowering treatment, years	9.8 ± (7.4)	9.1 ± (7.2)	0.085
Probable FH, % (n)	17.0% (253)	16.0% (21)	0.540
Definite FH, % (n)	83.0% (1231)	84.0% (110)	
Genetic test			0.324
Positive test genetic, % (n)	67.0% (994)	71.0% (93)	
Negative test genetic, % (n)	11.8% (175)	7.6% (10)	
Unknown, % (n)	21.2% (315)	8.2% (28)	

Values are numbers (%), mean ± (SD), as applicable. *p* values refer to differences calculated after adjusting by gender and age, as appropriate.
CVD denotes cardiovascular disease; HeFH, heterozygous familial hypercholesterolemia; LDL, low-density lipoprotein; Lp(a), lipoprotein (a).

prevalence of hypertension and diabetes, more often confirmed genetically, and with higher LDL cholesterol levels without lipid-lowering treatment among HeFH with CVD event than among HeFH without it. There were nodifferences in the presence of xanthomas, tobacco consumption, Lp(a) concentration and years of exposure to lipid-lowering treatment.

Two regression analyses calculating OR for CVD after lipid-lowering treatment are shown (Supplemental Table 1). The first analysis included all HeFH subjects exposed to treatment while the second, only those subjects treated with high intensity therapy. Independent risk factors were male gender, BMI, LDL cholesterol without treatment, history of hypertension or diabetes, less than 5 years of lipid-lowering treatment, onset of lipid-lowering at age > 30 years, and a positive genetic test for FH. In subjects with high intensity treatment, the intensity of association of these factors with CVD was similar to that in the whole group.

In subjects treated with high intensity therapy, the mean dose of atorvastatin or rosuvastatin was 41.8 ± 0.61 or 24.8 ± 0.44 mg/day, respectively, and 738 (64.9%) subjects were also taking ezetimibe. We calculated the proportion of subjects who developed CVD according to the count of independent risk factors found in the previous logistic regression analysis. CVD prevalence increases as the number of risk factors increases (Fig. 3). Among our HeFH population with statin treatment previous to CVD, and treated with high intensity therapy, 56.2% showed ≤3 risk factors, but still 34 (5.3%) had developed CHD in spite of having undergone an average of 9.7 years of previous lipid lowering treatment.

4. Discussion

In the present study, we describe the prevalence of CVD in a registry of HeFH patients treated in specialized lipid units and the effect on CVD of prolonged treatment with lipid-lowering drugs. This is the first work where we can describe the characteristics of HeFH that suffered CVD in spite of lipid-lowering treatment, even with some of them on high intensity treatment, and provide information to assess the potential role

Table 3
Odds ratio (OR) for cardiovascular disease according to lipid-lowering treatment in subjects with heterozygous familial hypercholesterolemia.

	OR (CI 95%)	*p*	Lipid-lowering treatment				*p*
	Exposure Yes *vs.* No		Non-exposure	< 5 years	5.12 years	> 12 years	
n/N % subjects with CHD	131/1615 vs 164/343		164/343	48/575	42/499	41/541	
	8.1% vs 47.8%	< 0.001	47.8%	8.3%	8.4%	7.6%	< 0.001
Model 1 N = 1958	0.096 (0.073–0.121)	< 0.001	REF	0.099 (0.069–0.143)	0.100 (0.069–0.147)	0.090 (0.061–0.131)	< 0.001
Model 2 N = 1958	0.085 (0.063–0.114)	< 0.001	REF	0.095 (0.065–0.139)	0.086 (0.058–0.128)	0.071 (0.047–0.108)	< 0.001
Model 3 N = 1958	0.092 (0.067–0.126)	< 0.001	REF	0.115 (0.077–0.171)	0.089 (0.058–0.136)	0.070 (0.045–0.110)	< 0.001
Model 4 n = 1360	0.082 (0.054–0.123)	< 0.001	REF	0.096 (0.058–0.160)	0.076 (0.045–0.130)	0.070 (0.040–0.121)	< 0.001

n/N, number with CVD/number in the exposure group.
Model 1: Univariate analysis.
Model 2: After adjustment for gender and age.
Model 3: After adjustment for gender, age, hypertension, diabetes, tobacco consumption, HDL cholesterol (mg/dl), LDL cholesterol (mg/dl), and body mass index (Kg/m^2).
Model 4: After adjustment for gender, age, hypertension, diabetes, tobacco consumption, HDL cholesterol (mg/dl), LDL cholesterol (mg/dl), body mass index (Kg/m^2), and lipoprotein (a) (mg/dl).
F-test *p* are those for the lipid-lowering treatment variables.

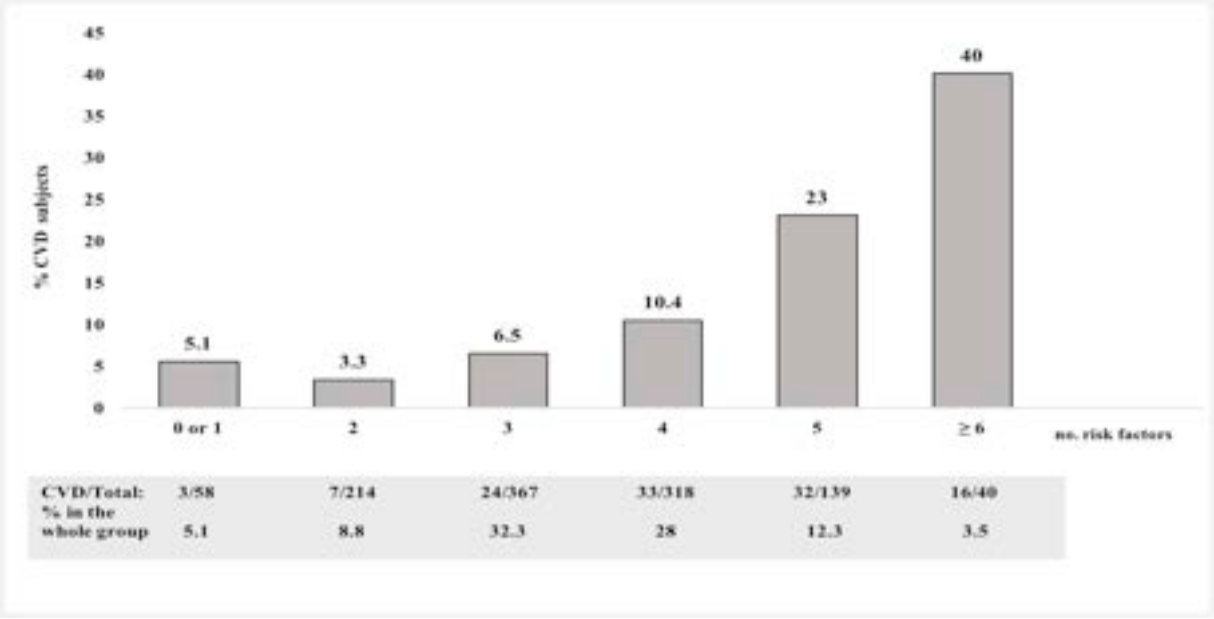


Fig. 3. Prevalence of cardiovascular disease among HeFH in high-intensity lipid-lowering treatment stratified by the count of cardiovascular risk factors. CVD, cardiovascular disease.
The risk factors considered were those that were statistically significant in regression 1 described in Supplemental Table 1.

of new drugs in the treatment of this disease.

In our opinion, three important conclusions can be drawn from our work. First, current CVD prevalence in HeFH is much lower in the statin era than reported several decades ago; second, CVD in HeFH patients is highly dependent on the moment when lipid-lowering is started; and third, new cases of CVD under prolonged statin treatment are uncommon and concentrated in subjects with certain risk factors. Altogether, our results would indicate that when lipid-lowering treatment is started early in life, HeFH is no longer a high-risk CVD condition.

CVD prevalence estimated from this study is similar to that reported in other current registries from specialized lipid centers and, as expected, is highly dependent on the mean age of the cohort. The HeFH cohort from The Netherlands with a mean age of 38.3 years showed a CVD prevalence of 9.2% among 14,283 HeFH [20]; in a cohort from Canada with a mean age of 43.9 years, it was 12.1% [21]; in our cohort with a mean age of 49.3 years, it was 15.1%; and in Norway, with a mean age of 58 years, it increased to 24% [22]. These prevalences are clearly much lower than those reported years ago [23], and could probably be related to multiple factors, including reduction of smoking habit that has been reduced near 50% in males in Spain in the last 20 years [24,25]; better medical cardiovascular risk factor control, especially hypertension [26]; and changes in the diagnosis of HeFH from clinical diagnosis, where the weight of the family and personal history of coronary disease is very strong and favors the selection of more serious cases, *versus* diagnosis based on the genetic diagnosis that eliminates these potential biases. In addition, early initiation of statin therapy seems to play a major role [16]. In fact, in our sample, in over 50% of HeFH subjects CVD developed before initiating lipid-lowering treatment, while among all the patients that initiated treatment while free from CVD, only 8% had a subsequent CVD event.

We quantified the impact of lipid-lowering drugs, mainly statins, on CVD prevention in HeFH. In the absence of randomized clinical trials with clinical events as main end-point, observational studies contribute to analyze this effect. Considering that the mean reduction of LDL cholesterol in our cohort was approximately 50%, which corresponds approximately to 135 mg/dl (3.5 mmol/L), the Cholesterol Treatment Trialists' (CTT) Collaboration [27] and epidemiological prospective studies [28] would predict a reduction of approximately 59% in CVD incidence in 5 years, because in mathematical terms, the decrease in CVD risk should be 0.78 to the power of the LDL cholesterol reduction in mmol/L [29]. Applying the formula *relative risk* ≈ *OR/[1 − absolute risk + (absolute risk · OR)]*, an OR = 0.10, that we find in our study, projecting from, as an example, and an untreated absolute risk of 50% in HeFH, our OR would correspond to a relative risk of 19%, which corresponds to an 81% reduction. The impressing magnitude of the protection found in our work may be explained because the mean treatment duration in our study is almost 10 years. The estimation obtained in the present study overcomes that figure probably because the mean treatment duration in our study was almost 10 years, LDL cholesterol is the major, if not the only, risk factor in many HeFH patients, and the treatment was started in most cases in primary prevention to avoid the development of atherosclerosis, which is probably more effective than in subjects with advanced disease [29]. This result is in agreement with the large CVD benefit observed with LDL cholesterol lowering effect of a certain genetic variation that reduced LDL cholesterol early in life [28,30]. Our results emphasize the importance of an early-in-life diagnosis and intense treatment of HeFH [1,31].

Although CVD is drastically reduced with high intensity lipid-lowering treatment in our study, approximately 10% of HeFH patients that started treatment free of CVD events still had one event in spite of treatment, some of them even after more than 12 years of treatment. Probably, this group of patients are good candidates for more potent lipid-lowering treatments such as inhibition of PCSK9 with monoclonal antibodies. The analysis of our cohort would indicate that HeFH subjects with 4 or more risk factors including male gender, statin treatment duration less than 5 years, obesity, diabetes, hypertension, LDL cholesterol > 250 mg/dl without treatment, the presence of a causative mutation in candidate genes, or late-in-life initiation of statin treatment would be probably the best candidates for such approach. These conditions are well-recognized risk factors in the general population and HeFH [32]. In contrast, the CVD risk for HeFH subjects, with early-in-life initiation, more than 5 years of treatment, and free from other risk factor, is reasonably good.

In conclusion, current prevalence of CVD among treated HeFH in specialized lipid clinics for long periods of time is one third of that reported before statins were available. Early initiation and prolonged lipid-lowering treatment are associated with most of this benefit.

However, new cases of CVD appear in spite of high-intensity statin but these episodes occur among high risk patients that should be considered for further lipid-lowering drugs such as PCSK9 inhibitors.

4.1. Limitations

The design of our registry does not reliably allow calculating the cumulative LDL cholesterol of the subjects. This calculation has been related to the risk of cardiovascular disease [1]. However, the fact that CVD decreases so significantly with treatment suggests that cumulative LDL cholesterol above a certain threshold that many HeFH get with high-intensity lipid-lowering treatment is even more important than the total cumulative LDL cholesterol. During the period of registered treatment (approximately 10 years in average), it may not remain constant. We have information about the time of treatment onset but covariates are collected at the time of inclusion in the registry. However, these patients are usually treated with potent therapies from the very beginning. Although all lipids clinics in the network follow homogeneous recommendations for the treatment of HeFH, some differences may be present. In addition, HeFH patients in our registry are followed at specialized lipid clinics, and perhaps, their phenotype or their management does not fully represent the whole spectrum of HeFH in the population. Finally, the retrospective study design implies that only HeFH who lived enough time to be registered in our sites are included, and thus most severe phenotypes leading to premature death, as well as mortal CVD episodes, have not been considered, although cardiovascular death has been reported very low in HeFH under high intensity treatment [33].

Conflicts of interest

J. Pedro-Botet received advisory and/or lecture fees from AstraZeneca, Esteve, Ferrer, Mylan, MSD and Sanofi. N. Plana received honoraria from Amgen, Merck, Ferrer, Alexion and Sanofi. A. J. Amor received lecture fees from Mylan and consulting fees from Sanofi-Aventis and Astra-Zeneca. F. Civeira receives grants, consulting fees, and/or honoraria from Amgen, Merck, Pfizer, and Sanofi-Aventis. All identified disclosures are modest. The other researcher did not receive any specific grant from agencies in the public, commercial, or not-for-profit sectors.

Author contributions

SP-C contributed to the data acquisition, analyzed the data and wrote the manuscript; ML analyzed the data and wrote the manuscript; VM-B, IL-M, JP-B, NP, RMS-H, AJA, FA, FF and MS-T contributed to the data acquisition and critically reviewed the manuscript; and FC designed the study, supervised the data analysis and drafted the manuscript.

Financial support

This study was supported by grants from the Spanish Ministry of Economy and Competitiveness PI15/01983 and CIBERCV. These projects are co-financed by Instituto de Salud Carlos III and the European Regional Development Fund (ERDF) of the European Union "A way to make Europe". Dr. Laclaustra's research activity is funded by Agencia Aragonesa para la Investigación y el Desarrollo (ARAID).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.02.003.

References

[1] B.G. Nordestgaard, M.J. Chapman, S.E. Humphries, H.N. Ginsberg, L. Masana, et al., European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society, Eur. Heart J. 34 (2013) 3478–3490.
[2] S.D. de Ferranti, A.M. Rodday, M.M. Mendelson, J.B. Wong, L.K. Leslie, et al., Prevalence of familial hypercholesterolemia in the 1999 to 2012 United States national health and nutrition examination surveys (NHANES), Circulation 133 (2016) 1067–1072.
[3] M. Benn, G.F. Watts, A. Tybjaerg-Hansen, B.G. Nordestgaard, Mutations causative of familial hypercholesterolaemia: screening of 98 098 individuals from the Copenhagen General Population Study estimated a prevalence of 1 in 217, Eur. Heart J. 37 (2016) 1384–1394.
[4] G.K. Hovingh, M.H. Davidson, J.J. Kastelein, A.M. O'Connor, Diagnosis and treatment of familial hypercholesterolaemia, Eur. Heart J. 34 (2013) 962–971.
[5] F. Civeira, International Panel on Management of Familial Hypercholesterolemia. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia, Atherosclerosis 173 (2004) 55–68.
[6] M.S. Brown, J.L. Goldstein, Familial hypercholesterolemia: a genetic defect in the low-density lipoprotein receptor, N. Engl. J. Med. 294 (1976) 1386–1390.
[7] T.L. Innerarity, K.H. Weisgraber, K.S. Arnold, R.W. Mahley, R.M. Krauss, et al., Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding, Proc. Natl. Acad. Sci. U.S.A. 84 (1987) 6919–6923.
[8] M. Abifadel, J.P. Rabès, M. Devillers, A. Munnich, D. Erlich, et al., Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (PCSK9) gene in cholesterol metabolism and disease, Hum. Mutat. 30 (2009) 520–529.
[9] A. Cenarro, A. Etxebarria, I. de Castro-Orós, M. Stef, A.M. Bea, et al., The p.Leu167del Mutation in APOE Gene Causes Autosomal Dominant Hypercholesterolemia by Down-regulation of LDL Receptor Expression in Hepatocytes, J. Clin. Endocrinol. Metab. 101 (2016) 2113–2121.
[10] A.M. Perak, H. Ning, S.D. de Ferranti, H.C. Gooding, J.T. Wilkins, et al., Long-term risk of atherosclerotic cardiovascular disease in US adults with the familial hypercholesterolemia phenotype, Circulation 134 (2016) 9–19.
[11] Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group, BMJ 303 (1991) 893–896.
[12] A.C. Goldberg, P.N. Hopkins, P.P. Toth, C.M. Ballantyne, D.J. Rader, et al., Familial hypercholesterolemia: screening, diagnosis and management of pediatric and adult patients: clinical guidance from the National Lipid Association Expert Panel on Familial Hypercholesterolemia, J. Clin. Lipidol. 5 (2011) 133–140.
[13] Mortality in treated heterozygous familial hypercholesterolaemia: implications for clinical management. Scientific Steering Committee on behalf of the Simon Broome Register Group, Atherosclerosis 142 (1999) 105–112.
[14] L. Mundal, J. Igland, L. Ose, K.B. Holven, M.B. Veierød, et al., Cardiovascular disease mortality in patients with genetically verified familial hypercholesterolemia in Norway during 1992-2013, Eur. J. Prev. Cardiol. 24 (2017) 137–144.
[15] K.A. Kjærgaard, M.K. Christiansen, M. Schmidt, M.S. Olsen, H.K. Jensen, Long-term cardiovascular risk in heterozygous familial hypercholesterolemia relatives identified by cascade screening, J. Am. Heart Assoc. 6 (6) (2017) e005435, https://doi.org/10.1161/JAHA.116.005435 2017;6(6).
[16] J. Besseling, G.K. Hovingh, R. Huijgen, J.J.P. Kastelein, B.A. Hutten, Statins in familial hypercholesterolemia: consequences for coronary artery disease and all-cause mortality, J. Am. Coll. Cardiol. 68 (2016) 252–260.
[17] S. Pérez-Calahorra, R.M. Sánchez-Hernández, N. Plana, P. Valdivielso, F. Civeira, National dyslipidemia registry of the Spanish arteriosclerosis society: current status, Clín. Invest. Arterioscler. 29 (2017) 248–253.
[18] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, et al., ACMG Laboratory Quality Assurance Committee, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology, Genet. Med. 17 (2015) 405–424.
[19] L. Masana, N. Plana, S. Pérez-Calahorra, D. Ibarretxe, I. Lamiquiz-Moneo, et al., Dyslipidemia Registry of the Spanish Arteriosclerosis Society. How many familial hypercholesterolemia patients are eligible for PCSK9 inhibition? Atherosclerosis 262 (2017) 107–112.
[20] J. Besseling, I. Kindt, M. Hof, J.J. Kastelein, B.A. Hutten, et al., Severe heterozygous familial hypercholesterolemia and risk for cardiovascular disease: a study of a cohort of 14,000 mutation carriers, Atherosclerosis 233 (2014) 219–223.
[21] L.R. Brunham, L. Cermakova, T. Lee, I. Priecelova, K. Alloul, et al., Contemporary trends in the management and outcomes of patients with familial hypercholesterolemia in Canada: a prospective observational study, Can. J. Cardiol. 33 (2017) 385–392.
[22] L. Mundal, M.B. Veierød, T. Halvorsen, K.B. Holven, L. Ose, et al., Cardiovascular disease in patients with genotyped familial hypercholesterolemia in Norway during 1994-2009, a registry study, Eur. J. Prev. Cardiol. 23 (2016) 1962–1969.
[23] J.L. Goldstein, H.H. Hobbs, M.S. Brown, Familial hypercholesterolemia, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic Basis of Inherited Disease, vol. 120, McGraw-Hill, New York, 2001, pp. 2863–2913.
[24] E. Regidor, J.L. Gutierrez-Fisac, M.E. Calle, P. Navarro, V. Domínguez, Trends in cigarette smoking in Spain by social class, Prev. Med. 33 (2001) 241–248.
[25] B. Pérez-Hernández, E. García-Esquinas, A. Graciani, P. Guallar-Castillón, E. López-García, et al., Social inequalities in cardiovascular risk factors among older adults in Spain: the seniors-ENRICA study, Rev. Esp. Cardiol. 70 (2017) 145–154.

[26] J.R. Banegas, E. Lopez-Garcia, J. Dallongeville, E. Guallar, J.P. Halcox, et al., Achievement of treatment goals for primary prevention of cardiovascular disease in clinical practice across Europe: the EURIKA study, Eur. Heart J. 32 (2011) 2143–2152.

[27] Cholesterol Treatment Trialists' (CTT) Collaboration, C. Baigent, L. Blackwell, J. Emberson, L.E. Holland, C. Reith, et al., Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170 000 participants in 26 randomised trials, Lancet 376 (2010) 1670–1681.

[28] B.A. Ference, H.N. Ginsberg, I. Graham, K.K. Ray, C.J. Packard, et al., Low density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel, Eur. Heart J. 38 (2017) 2459–2472.

[29] H. Soran, J.D. Schofield, P.N. Durrington, Cholesterol, not just cardiovascular risk, is important in deciding who should receive statin treatment, Eur. Heart J. 36 (2015) 2975–2983.

[30] B.A. Ference, W. Yoo, I. Alesh, N. Mahajan, K.K. Mirowska, et al., Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis, J. Am. Coll. Cardiol. 60 (2012) 2631–2639.

[31] G.K. Hovingh, J.J. Kastelein, Diagnosis and management of individuals with heterozygous familial hypercholesterolemia: too late and too little, Circulation 134 (2016) 710–712.

[32] J. Wang, J.S. Dron, M.R. Ban, J.F. Robinson, A.D. McIntyre, et al., Polygenic versus monogenic causes of hypercholesterolemia ascertained clinically, Arterioscler. Thromb. Vasc. Biol. 36 (2016) 2439–2445.

[33] A.M. Bea, F. Civeira, E. Jarauta, I. Lamiquiz-Moneo, S. Pérez-Calahorra, et al., Association between the presence of carotid artery plaque and cardiovascular events in patients with genetic hypercholesterolemia, Rev. Esp. Cardiol. 70 (2017) 551–558.

Lipids in Health and Disease

SHORT REPORT Open Access

Comparative efficacy between atorvastatin and rosuvastatin in the prevention of cardiovascular disease recurrence

Sofía Perez-Calahorra[1], Martin Laclaustra[1], Victoria Marco-Benedi[1], Xavier Pinto[2], Rosa M. Sanchez-Hernandez[3], Núria Plana[4], Emilio Ortega[5], Francisco Fuentes[6] and Fernando Civeira[1,7*]

Abstract

Background: There is no randomized clinical trials with recurrence of atherosclerotic cardiovascular disease (ASCVD) as a major outcome with rosuvastatin. In order to analyze potential differences in the clinical response to atorvastatin and rosuvastatin in secondary ASCVD prevention, we have analyzed the clinical evolution of those subjects of the Dyslipemia Registry of the Spanish Society of Arteriosclerosis (SEA) who at the time of inclusion in the Registry had already suffered an ASCVD.

Methods: This observational, retrospective, multicenter, national study was designed to determine potential differences between the use of atorvastatin and rosuvastatin in the ASCVD recurrence. Three different follow-up start-times were performed: time of inclusion in the registry; time of first event if this occurred after 2005, and time of first event without date restriction.

Results: Baseline characteristics were similar between treatment groups. Among atorvastatin or rosuvastatin users, 89 recurrences of ASCVD were recorded (21.9%), of which 85.4% were coronary. At the inclusion of the subject in the registry, 345 participants had not suffered a recurrence yet. These 345 subjects accumulated 1050 person-years in a mean follow-up of 3 years. Event rates were 2.73 (95% CI: 1.63, 4.25) cases/100 person-years and 2.34 (95% CI: 1.17, 4.10) cases/100 person-years in the atorvastatin and rosuvastatin groups, respectively. There were no statistically significant differences between the two groups independently of the follow-up start-time.

Conclusions: This study does not find differences between high doses of rosuvastatin and atorvastatin in the recurrence of ASCVD, and supports their use as clinically equivalent in secondary prevention of ASCVD.

Keywords: Rosuvastatin, Atorvastatin, Secondary prevention, High-potent statin

Background

Reduction of cholesterol transported in low-density lipoproteins (LDLc) is one of the mainstays of atherosclerotic cardiovascular disease (ASCVD) prevention, since multiple studies have demonstrated the causal role of LDLc in the pathogenesis of atherosclerosis, and the benefit of LDLc reduction in blood [1].

One central idea in ASCVD prevention is that the type and intensity of any preventive measure should be conditioned by the risk of developing ASCVD over time, especially in the short and medium term [2]. For LDLc reduction, the main international scientific societies recommend undertaking hygienic-dietary measures as the first step of lipid-lowering treatment in all patients, but also concomitantly initiating hypolipidemic treatment with potent statins in high-risk groups: subjects with very high concentrations of LDLc, subjects affected by severe genetic form of hypercholesterolemia, and patients who have already suffered an ASCVD event [3, 4]. In all of these cases, these guidelines recommend aiming to LDLc reduction > 50% with the use of high potency statins at high doses. The American College of Cardiology/American Heart Association guideline on the

* Correspondence: civeira@unizar.es
[1]Lipid Unit, Hospital Universitario Miguel Servet, IIS Aragon, CIBERCV, Zaragoza, Spain
[7]Universidad de Zaragoza, Zaragoza, Spain
Full list of author information is available at the end of the article

treatment of blood cholesterol to reduce ASCVD risk in adults, after analyzing the hypolipidemic efficacy of different statins in multiple clinical trials and performing head-to-head comparison among statins, classify them according to their hypolipidemic effect in statins of low, medium, and high potency. The latter group encompasses rosuvastatin at doses of 20 mg/day and 40 mg/day, and atorvastatin at doses of 40 mg/day and 80 mg/day. High potency statins allow LDLc reduction > 50% and for that intensity, a similar clinical benefit is assumed [3].

However, there are few observational reports and no randomized clinical trials in secondary prevention with recurrence of ASCVD as a major outcome with rosuvastatin, in contrast to atorvastatin [5–8]. So, the assumption of equivalent clinical benefit is based on their lipid-lowering capacity and the clinical benefit of rosuvastatin demonstrated in subjects in primary prevention. Given that subjects in secondary prevention have different clinical characteristics, such as the currently high prevalences of diabetes [9] and vascular revascularization [10] among them, and different concomitant medications, from subjects in primary prevention, it would be good to know whether the benefit of both statins is similar in secondary prevention in real life.

In order to analyze potential differences in the clinical response to atorvastatin and rosuvastatin in subjects in secondary ASCVD prevention, we have analyzed the clinical evolution of those subjects of the Dyslipemia Registry of the Spanish Society of Arteriosclerosis (SEA) who at the time of inclusion in the Registry had already suffered an ASCVD.

Material and methods

This observational, retrospective, multicenter, national study in Spain was designed to determine potential differences between the use of atorvastatin and rosuvastatin in the ASCVD recurrence. The information was obtained from the Dyslipidemia Registry of the SEA [11]. This is an active online registry, where 50 certified lipid clinics distributed throughout all regions of Spain report cases of various types of primary hyperlipidemias. Anonymous clinical data collection in this registry was approved by a central ethical committee (Comité Ético de Investigación Clínica de Aragón, CEICA) and participants gave their written informed consent. Inclusion criteria were standardized in 5 training sessions before case recruitment. For patients in secondary prevention, the registry collects personal and family health history, anthropometry, physical examination, laboratory data, type of ASCVD, age at which the ASCVD event occurred, age at which statin treatment began, and history of lipid-lowering treatment [12]. Patients were eligible for inclusion in this study if they were 18 years of age or older with previous ASCVD at inclusion in the registry. ASCVD was defined as: coronary (myocardial infarction, coronary revascularization procedure, sudden death); cerebral (ischemic stroke with > 24-h neurological deficit without evidence of bleeding in brain imaging tests); peripheral vascular disease (PAD) (intermittent claudication with ankle arm index< 0.9, or arterial revascularization of lower limbs) or symptomatic or asymptomatic abdominal aortic aneurysm. Arterial hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or self-reported use of antihypertensive medication. Diabetes was defined as fasting plasma glucose ≥126 mg/dl, HbA1c ≥6.5%, or self-reported treatment with antidiabetic medications. Current smoking was defined as current smoking or having smoked in the last year. Former smoker was defined as a subject having smoked at least 50 cigarettes in his lifetime, but not having smoked in the last year.

Follow up

The registry is designed so that at least once a year the data on the clinical evolution of the included patients are updated, with new anthropometric data, changes in risk factors or medication, and the appearance of new ASCVD events.

The main endpoint was defined as the occurrence of a new major ASCVD event composed of coronary heart disease (coronary death, acute coronary syndrome requiring hospitalization, or coronary revascularization due to angina), cerebrovascular (fatal and non-fatal stroke, or carotid revascularization), and peripheral arterial disease (arterial revascularization of the lower extremities).

Participants were divided according to the type of statins recorded at the time of inclusion in the registry. The statin documented in the registry represented the treatment for the follow-up years prior to the recurrence or censoring. Recurrent ASCVD event dates were collected and, in their absence, participants were censored at the date the follow-up data was obtained from the registry. Three different follow-up start-times were performed: starting from the time of inclusion in the registry (all participants had a previous event), starting from the time of first event if this occurred after 2005, and starting from the time of first event without date restriction.

Statistical analysis

Data are expressed as mean and standard deviation for continuous variables with normal distribution and they were analyzed with the Student's t test. Categorical variables are expressed as a percentage and analyzed by the × 2 test. The rates of adverse events up to the end of the follow-up were calculated by considering observed person-time and survival curves were created by Kaplan-Meier estimation, and the groups were compared by log rank test. The association between type of statin and

ASCVD events was calculated using Poisson regression. Multivariable Poisson regression models were fitted including the covariates: age and sex (model 1), diabetes, hypertension, smoking status, body mass index (BMI), non-high-density lipoprotein (non-HDL) cholesterol, HDL cholesterol and ezetimibe use.

We conducted this study in accordance with the Declaration of Helsinki for the protection of the rights and welfare of people participating in biomedical research.

Results

Patient characteristics

In the registry, 985 subjects had had an ASCVD event at the time of their inclusion. On March 31st, 2019, follow-up data were evaluated and 475 subjects were excluded due to incomplete data, changes in the lipid-lowering drugs, follow-up less than 1 year, or loss to follow-up. There were no relevant clinical differences at registry between those included and excluded for the analysis (Additional file 3: Table S1). Only those subjects under continuous treatment with atorvastatin (n = 243) or rosuvastatin (n = 164) were included in this analysis (Fig. 1). Clinical characteristics at the moment of inclusion in the registry only differed in the gender proportion between both treatment groups (Table 1). In the atorvastatin group men were more frequent. At inclusion, the mean age in both groups was 61 years, there were no differences in body mass index, the prevalence of hypertension, diabetes, or smoking history between those patients on atorvastatin and rosuvastatin. The age of the first ASCVD and the type of ASCVD were also similar between the groups (Table 1).

Total cholesterol and non-HDLc were higher before treatment in those subjects to whom rosuvastatin was prescribed. After treatment, HDLc has higher in the rosuvastatin group, and the differences in total cholesterol and non-HDLc were reduced to a level at which they did not reach statistical significance any more (Table 1). The mean dose of atorvastatin and rosuvastatin were 50.8 (24.7) mg/day and 21.4 (9.6) mg/day, respectively, corresponding to a medium dose of a high potency statin and they were equivalent with respect to their lipid-lowering efficacy. At the highest doses marketed in Spain (rosuvastatin 20 mg and atorvastatin 80 mg), there were no significant differences in the reduction of LDLc. The concomitant use of ezetimibe was very high among patients on atorvastatin, but higher in those patients on rosuvastatin, 57.9 and 69.5%, respectively (p = 0.023) (Table 1).

Recurrences

In the registry, among atorvastatin and rosuvastatin users, 89 recurrences of ASCVD after a first event were recorded (21.9%), of which 85.4% were coronary, 11.2% ischemic stroke, and 3.4% PAD; there were no hemorrhagic strokes or abdominal aortic aneurism surgery during evolution. At the inclusion of the subject in the registry, 345 participants had not suffered a recurrence yet. Thus 62 recurrences

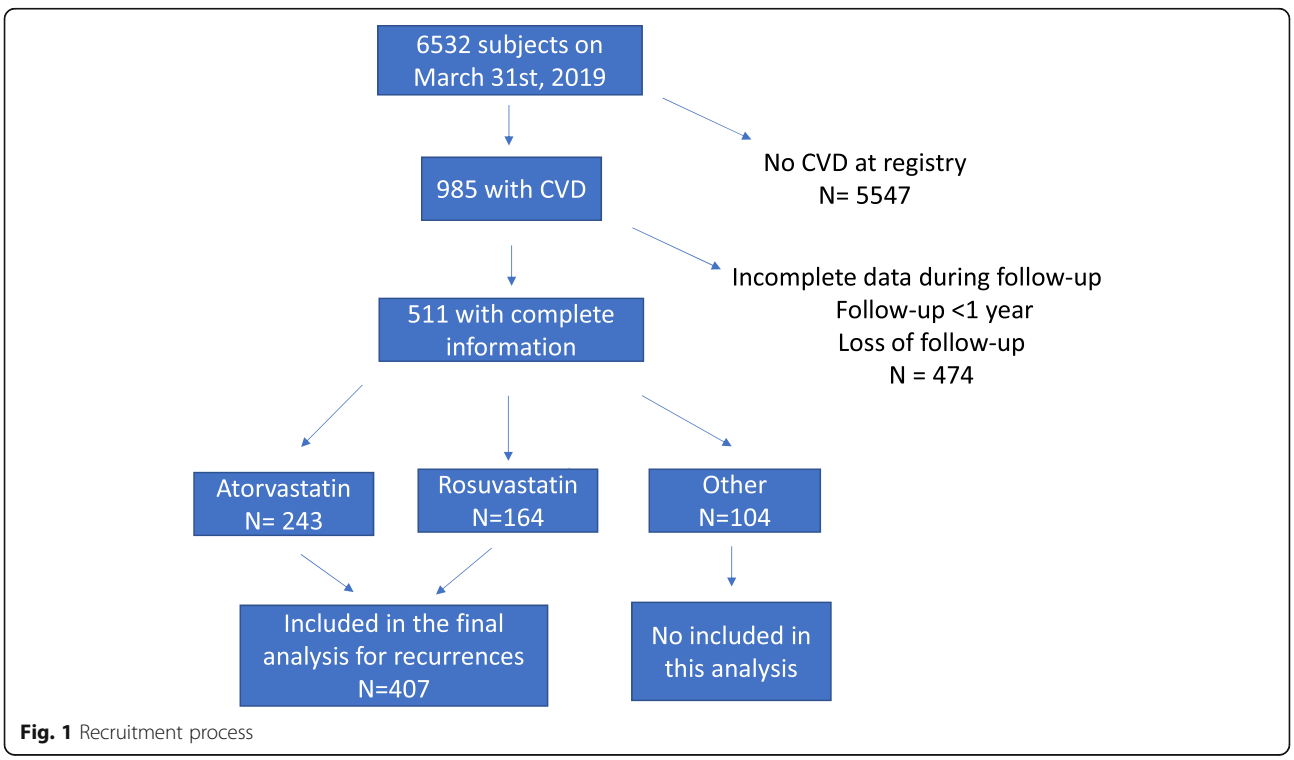


Fig. 1 Recruitment process

Table 1 Clinical characteristic of subjects with CVD at inclusion in the Registry according to statin prescribed, and lipid values at diagnosis of dyslipidemia in the Lipid Clinic without lipid-lowering treatment and after lipid-lowering treatment recorded at inclusion in the Registry

Variables	Atorvastatin ($n = 243$)	Rosuvastatin ($n = 164$)	*P*
Gender (Male), % (n)	78.2 [190]	68.9 [113]	0.046
Age at inclusion, years	60.9 (11.1)	60.6 (9.9)	0.743
Body mass index, (Kg/m^2)	28.9 (4.1)	28.6 (4.3)	0.595
ASCVD type (CHD/Stroke/PAD), %	77.3/14.5/7.0	79.3/12.2/5.5	0.460
Age first ASCVD event	51.7 (11.4)	51.1 (10.5)	0.561
Tobacco consumption, % (n)	18.9 [45]	15.5 [25]	0.461
Hypertension, % (n)	48.6 [118]	56.1 [92]	0.164
Diabetes, % (n)	30.9 [75]	31.7 [52]	0.943
Glucose, mg/dL	113.6 (33.5)	108.4 (32.4)	0.130
Age statin onset	48.4 (12.3)	49.4 (11.2)	0.477
Total cholesterol, mg/dl			
Pre-treatment	296.8 (102.6)	322.1 (111.1)	0.020
Post-treatment	172.9 (55.6)	182.6 (49.8)	0.065
HDL cholesterol, mg/dl			
Pre-treatment	45.2 (14.3)	46.6 (12.8)	0.285
Post-treatment	47.1 (13.8)	49.6 (11.6)	0.048
Non-HDL cholesterol, mg/dl			
Pre-treatment	251.6 (100.8)	275.5 (109.9)	0.027
Post-treatment	110.6 (39.9)	119.0 (49.5)	0.072
Triglycerides, mg/dl			
Pre-treatment	241.5 (291.3)	209.2 (229.4)	0.213
Post-treatment	161.5 (175.2)	160.2 (153.8)	0.938
Statin daily dose, mg/day	50.8 (24.7)	21.4 (9.6)	–
Ezetimibe use, % (n)	57.9 [140]	69.5 [114]	0.023

Values are percentage [count], mean (SD), as applicable. *ASCVD* Denotes arteriosclerotic cardiovascular disease, *CHD* Coronary heart disease, *PAD* Peripheral artery disease, *HDL* High-density lipoprotein

occurred before and 27 after inclusion on the registry. These 345 subjects accumulated 1050 person-years in a mean follow-up of 3 years. Event rates were 2.73 (95% CI: 1.63, 4.25) cases/100 person-years and 2.34 (95% CI: 1.17, 4.10) cases/100 person-years in the atorvastatin and rosuvastatin groups respectively (Fig. 2). There were no statistically significant differences between the two groups (crude, adjusted for age and sex, and for major cardiovascular risk factors Poisson models as described in methods). Subjects with recurrent ASCVD presented higher pre-treatment concentration of non-HDLc than those subjects without recurrences during the follow-up. All other clinical and biochemical variables did not differ between those who suffered recurrence and those who did not (Additional file 4: Table S2).

Among the patients in the registry with ASCVD (n = 407), 287 had their first episode in the last 15 years (year 2004 or later). Among them, 176 took atorvastatin at the time of inclusion in the registry and 111 subjects rosuvastatin. The Kaplan–Meier survival estimates for the end-point from the moment of the first event are shown in Additional file 1: Figure S1. Within an average follow-up of 7.5 years, 47 (16.4%) patients (28 in the atorvastatin group and 19 in the rosuvastatin group) suffered a second episode of ASCVD. Crude rates for this follow-up of 2154 person-years were 2.2 (95% CI 1.5, 3.1) and 2.2 (95% CI 1.3, 3.3) episodes per 100 person-years for the atorvastatin and rosuvastatin groups respectively, without finding statistically significant differences between the two groups (crude models, adjusted for age and sex, and for major cardiovascular risk factors as described in methods). Results did not differ when subjects with first events prior to 2004 (10.8 years mean follow-up) were included in the model (Additional file 2: Figure S2).

Discussion

The present work shows that the recurrence of ASCVD events in the Registry of Dyslipemias of the SEA does not reveal relevant differences between those subjects in

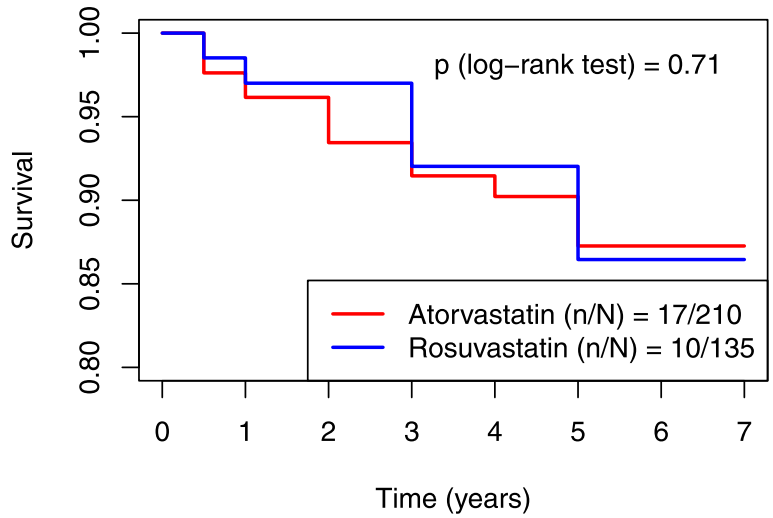


Fig. 2 Cumulative incidence of the composite primary end point after inclusion in the registry

treatment with rosuvastatin or atorvastatin at the beginning of the follow-up. These results support the recommendation to use them as clinically equivalent in the secondary prevention of ASCVD when used at appropriate dose.

There are very limited studies that have analyzed the differences in clinical ASCVD events between statins. The Pravastatin or Atorvastatin Evaluation and Infection Trial (PROVE IT) analyzed the efficacy of atorvastatin 80 mg/day and pravastatin 40 mg/day in the prevention of cardiovascular recurrence after acute coronary syndrome. Atorvastatin provided greater protection against death or major cardiovascular events than pravastatin did. However, they used different doses, not equivalent with respect to their lipid-lowering potency, so their results support the use of powerful statins at high doses compared to statins of intermediate potency [12]. The Treating to New Targets (TNT) demonstrated that intensive lipid-lowering therapy with 80 mg of atorvastatin per day in patients with stable coronary disease provides benefit when compared with 10 mg of atorvastatin per day. Again, they used different doses, although with identical conclusions [5]. The IDEAL study, enrolled patients with a history of acute MI and were randomly assigned to receive a high dose of atorvastatin (80 mg/day) or simvastatin (20 mg/day). The intensive lowering of LDLc did not result in a significant reduction in the primary outcome of major coronary events, but did reduce the risk of other composite secondary end points and nonfatal acute MI [6]. Hence, there is high quality evidence that intensive lipid-lowering treatment with further reductions in LDLc produce further reductions in ASCVD [13], but there is no evidence of clinically meaningful differences between statins with the same lipid-lowering potency. In this study, we show that when using similarly powered statins in a high-risk population, rates of second events are similar, no matter the statin used.

An added value to our data is the high use of combined treatment in our registry. It must be kept in mind that these are specialized units and that many patients in the registry have severe primary dyslipidemias, many of them familial hypercholesterolemia. The fact that the results are similar in those subjects after adjusting for ezetimibe in the treatment gives more information about the clinical equivalence of both statins at equipotent doses.

Our study has several limitations. The main one is that it is an observational study and therefore subject to biases in the use of one or another statin. However, the data have been adjusted with the different potentially confounding variables without modifying the results. The follow-up of the subjects is also variable and it is not possible to analyze the therapeutic compliance during the follow-up. However, no differences in compliance between drugs have been described, so it does not seem to be a major problem. Finally, changes in treatment have not been covered during the period of follow-up previous to inclusion in the registry and some subjects have been able to change from atorvastatin to rosuvastatin and vice versa. This extreme is exceptional in the registry since the usual is the addition of ezetimibe in case of not achieving therapeutic goals, and the use of ezetimibe in both groups is well balanced [14].

Conclusion

This observational and retrospective analysis of ASCVD recurrences does not find appreciable clinical differences between high doses of rosuvastatin and atorvastatin, and supports their use as clinically equivalent in secondary prevention of ASCVD.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12944-019-1153-x.

Additional file 1: Figure S1 Cumulative incidence of the composite primary end point in those 287 subjects with a first episode in the last 15 years (year 2004 or later).

Additional file 2: Figure S2 Cumulative incidence of the composite primary end point in all subjects with a first ASCVD included in the Registry

Additional file 3: Table S1 Clinical and biochemical differences at Registry between subjects with and without ASCVD recurrence after inclusion in the Registry

Additional file 4: Table S2 Characteristics of patients with ASCVD at inclusion in the Registry according to inclusion or exclusion in the follow-up.

Acknowledgements
Not applicable.

Authors' contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by FC and ML, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding
This study was funded by CIBERCV (grant number CB16/11/00451), and Sociedad Española de Arteriosclerosis (SEA 2019).

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Anonymous clinical data collection in this registry was approved by a central ethical committee (Comité Ético de Investigación Clínica de Aragón, CEICA) and participants gave their written informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication
Not applicable.

Competing interests
Fernando Civeira has received a speaker honorarium from Ferrer. The other authors declare that they have no conflict of interest.

Author details
[1]Lipid Unit, Hospital Universitario Miguel Servet, IIS Aragon, CIBERCV, Zaragoza, Spain. [2]Lipid and Vascular Risk Unit. Internal Medicine Service, CIBEROBN, Hospital de Bellvitge, Hospitalet de Llobregat, Spain. [3]Lipid Unit, Endocrinology Department, Hospital Universitario Insular de Gran Canaria, Instituto Universitario de Investigaciones Biomédicas y Sanitarias, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain. [4]Unitat de Medicina Vascular i Metabolism, Hospital Universitari Sant Joan, Institut d'Investigació Sanitària Pere Virgili (IISPV), CIBERDEM, Reus, Tarragona, Spain. [5]Lipid Unit, Endocrinology and Nutrition Service, Institut d'Investigacions Biomèdiques August Pi Sunyer, CIBEROBN, Hospital Clínic, Barcelona, Spain. [6]Lipid Unit, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), CIBEROBN, Universidad de Córdoba, Hospital Universitario Reina Sofía, Córdoba, Spain. [7]Universidad de Zaragoza, Zaragoza, Spain.

Received: 27 July 2019 Accepted: 26 November 2019
Published online: 11 December 2019

References

1. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38:2459–72.
2. Domanski M, Lloyd-Jones D, Fuster V, Grundy S. Can we dramatically reduce the incidence of coronary heart disease? Nat Rev Cardiol. 2011;8:721–5.
3. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults. J Am Coll Cardiol. 2014;63(25 pt. B):2889–934.
4. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, ESC Scientific Document Group, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J. 2016;37(29):2315–21.
5. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med. 2005;352:1425–35.
6. Pedersen TR, Faergeman O, Kastelein JJ, Olsson AG, Tikkanen MJ, Holme I, et al. High-dose atorvastatin vs usual dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. JAMA. 2005;294:2437–45.
7. Schwartz GG, Olsson AG, Ezekowitz MD, Ganz P, Oliver MF, Waters D, et al. Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) Study Investigators. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the MIRACL study: a randomized controlled trial. JAMA. 2001;285:1711–8.
8. Amarenco P, Bogousslavsky J, Callahan A 3rd, Goldstein LB, Hennerici M, Rudolph AE, et al. Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) Investigators. High-dose atorvastatin after stroke or transient ischemic attack. N Engl J Med. 2006;355:549–59.
9. Kim SH, Chunawala L, Linde R, Reaven GM. Comparison of the 1997 and 2003 American Diabetes Association classification of impaired fasting glucose: impact on prevalence of impaired fasting glucose, coronary heart disease risk factors, and coronary heart disease in a community-based medical practice. J Am Coll Cardiol. 2006;48:293–7.
10. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, ODYSSEY OUTCOMES Committees and Investigators, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. N Engl J Med. 2018;379:2097–107.
11. Perez-Calahorra S, Laclaustra M, Marco-Benedí V, Lamiquiz-Moneo I, Pedro-Botet J, Plana N, et al. Dyslipemia Registry of Spanish Arteriosclerosis Society. Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia. Atherosclerosis. 2019;284:245–52.
12. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, et al. Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 Investigators. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med. 2004;350:1495–504.
13. Cholesterol Treatment Trialists' (CTT) Collaboration, Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376:1670–81.
14. Pérez-Calahorra S, Sánchez-Hernández RM, Plana N, Valdivielso P, Civeira F. National Dyslipidemia Registry of the Spanish Arteriosclerosis Society: Current status. Clin Investig Arterioscler. 2017;29:248–53.

Publisher's Note

Clin Investig Arterioscler. 2019;31(2):89–92

Sociedad Española de Arteriosclerosis

CLÍNICA E INVESTIGACIÓN EN ARTERIOSCLEROSIS

www.elsevier.es/arterio

CLINICAL REPORT

Treatment of a high cardiovascular risk patient with McArdle's disease with PCSK9 inhibitors☆

Victoria Marco-Benedí[a,*], Estíbaliz Jarauta[a,b], Sofía Pérez-Calahorra[a], Ana M. Bea[a], Fernando Civeira[a,b]

[a] *Clinical Lipid and Arteriosclerosis Research Unit, Hospital Universitario Miguel Servet, IIS (Instituto de Investigación Sanitaria [Health Research Institute]) Aragón, CIBERCV (Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares [Biomedical Research Centre for Cardiovascular Diseases]), Zaragoza, Spain*
[b] *Universidad de Zaragoza, Zaragoza, Spain*

Received 25 July 2018; accepted 18 November 2018
Available online 9 March 2019

KEYWORDS
McArdle's disease;
Rhabdomyolysis;
Statin intolerance;
PCSK9 inhibitors

Abstract A 60-year-old male with familial combined hyperlipidemia, ischaemic heart disease and type 2 diabetes. Since childhood, intolerance to intense exercise. The patient was diagnosed of McArdle's disease after an episode of rhabdomyolysis associated with statins as treatment after a myocardial infarction. Since then, he had been treated with diet, fibrates and ezetimibe with good tolerance, despite this, LDL cholesterol (cLDL) remained >180 mg/dl. He started to be treated with alirocumab 150 mg/sc every 14 days, with excellent clinical response and a decrease in cLDL to 15 mg/dl. Our case shows that PCSK9 inhibitors are effective and safe in patients with muscle diseases who have statin contraindication, and they are a good therapeutic tool for these patients.

PALABRAS CLAVE
Enfermedad de McArdle;
Rabdomiólisis;
Intolerancia a estatinas;
Inhibidores de PCSK9

Tratamiento de un varón con enfermedad de McArdle y muy alto riesgo cardiovascular con inhibidores de PCSK9

Resumen Varón de 60 años con hiperlipidemia familiar combinada, cardiopatía isquémica y diabetes tipo 2. Desde la infancia, intolerancia al esfuerzo intenso. Se le diagnosticó enfermedad de McArdle a raíz de rabdomiólisis asociada a estatinas tras un infarto de miocardio. Desde entonces había seguido tratamiento con dieta, fibratos y ezetimiba con buena tolerancia,

DOI of original article: https://doi.org/10.1016/j.arteri.2018.11.005
☆ Please cite this article as: Marco-Benedí V, Jarauta E, Pérez-Calahorra S, Bea AM, Civeira F. Tratamiento de un varón con enfermedad de McArdle y muy alto riesgo cardiovascular con inhibidores de PCSK9. Clín Investig Arterioscler. 2019;31:89–92.
* Corresponding author.
E-mail address: vmarcobenedi@gmail.com (V. Marco-Benedí).

pero a pesar de ello las concentraciones de colesterol LDL (cLDL) eran >180 mg/dl. Se asoció al tratamiento alirocumab 150 mg subcutáneos cada 14 días, con excelente respuesta clínica y descenso de cLDL a 15 mg/dl, manteniéndose estable desde entonces. Nuestro caso demuestra que los inhibidores de PCSK9 son eficaces y seguros en pacientes con enfermedades musculares que contraindican las estatinas y que son una alternativa terapéutica ideal para este tipo de pacientes.

Introduction

McArdle's disease, also known as glycogenosis type V, glycogen storage disease type V or myophosphorylase deficiency, is a rare disease that causes muscle pain on minimal exertion.[1,2] McArdle's disease is an autosomal recessive disease and affected patients present mutations in both alleles of the PYGM gene, which encodes myophosphorylase. To date, over 65 mutations of the PYGM gene have been identified to cause McArdle's disease.[2,3] Myophosphorylase initiates the breakdown of glycogen in the muscles, as a result of a deficiency in this enzyme's activity. Those who suffer from the condition find it difficult to obtain energy from their glycogen stores.[1,2] Consuming complex carbohydrates (vegetables, fruit, grains, bread, pasta and rice) before exercise and a total daily calorie intake of fat of 20% seems to improve tolerance.[2] At present, there is no known definitive cure, and for patients diagnosed with dyslipidaemia who also have coronary artery disease, new treatments are proposed which may achieve therapeutic objectives in these high-risk patients. Consequently, anti-PCSK9 monoclonal antibodies may be used as a therapeutic alternative in these patients.

Material and methods

We present the case of a 60-year-old male with a very high cardiovascular risk and a history of rhabdomyolysis from statins, who first attended our clinic at the Lipid Unit of the Hospital Universitario Miguel Servet in Zaragoza approximately 16 years ago. He was referred by his primary care physician for the investigation of his dyslipidaemia, with fasting total cholesterol levels of around 300 mg/dl and triglycerides of 534 mg/dl. The patient had been diagnosed with type 2 diabetes around 6 years prior.

Since childhood, he has reported myalgia and exercise intolerance. On several occasions, he presented elevated resting levels of creatine kinase (CK) and episodes of myoglobinuria. At 52 years of age he suffered a myocardial infarction. A few weeks later, he had symptoms of rhabdomyolysis with haematuria, intense myalgia and CK levels of >10,000 IU/l, which appeared two weeks after he began treatment with atorvastatin 20 mg/day.

When the patient first attended our clinic, he presented with severe asthenia which had been ongoing for several months. His physical examination was normal and he weighed 81.7 kg, with a body mass index (BMI) of 27.2 kg/m^2. He had no corneal arcus or xanthomas. He did, however, exhibit telangiectases on the cheeks. He had been following a strict diet low in saturated fat and high in complex carbohydrates, and undergoing pharmacological treatment with colestipol 5 g per day for four years. Nevertheless, his lipid profile revealed the following: total cholesterol 267 mg/dl, triglycerides 189 mg/dl, HDL cholesterol (HDL-C) 36 mg/dl, LDL cholesterol (LDL-C) 193.2 mg/dl, apolipoprotein B (apoB) 186 mg/dl. His liver enzymes were also raised: GGT 82 U/l, GPT 82 U/l, GOT 53 U/l, and he also presented elevated CK levels of 4505 U/l and lactate dehydrogenase (LDH) of 523 U/l. His other biochemical parameters, including thyroid hormones, glucose and creatinine, were within the normal ranges. In light of suspected primary muscle disease, a muscle biopsy confirmed the diagnosis of McArdle's disease or glycogen storage disease type V.

It was recommended that he begin treatment with fenofibrate 145 mg per day and his colestipol dose was doubled to 10 g per day. He was also told to limit his physical activity. The nutritionist reinforced the importance of following a diet high in complex carbohydrates, limiting the intake of saturated fats, simple sugars and alcohol, and consuming oily fish at least once a week as well as nuts (at least three units) every day. At the next visit a year later, the patient had lost 5 kg, but still felt tired and suffered from muscle pain. His CK levels were still raised, at 3000 U/l, while his LDL-C was measured at 212 mg/dl and glycosylated haemoglobin (HbA1c) 6.3%. At this stage, treatment with ezetimibe 10 mg was also introduced. The patient was periodically reviewed by our unit, with no substantial changes in the physical examination or lipid profile.

Results

Since the patient was far off his LDL-C targets, which are below 70 mg/dl due to him being very high risk, and given the possibility of prescribing proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, in early 2017 the patient began treatment with alirocumab 150 mg administered subcutaneously every 14 days, while continuing on ezetimibe 10 mg per day and fenofibrate 145 mg per day. Colestipol was voluntarily withdrawn due to digestive discomfort. He presented no clinical changes and remained angina-free,

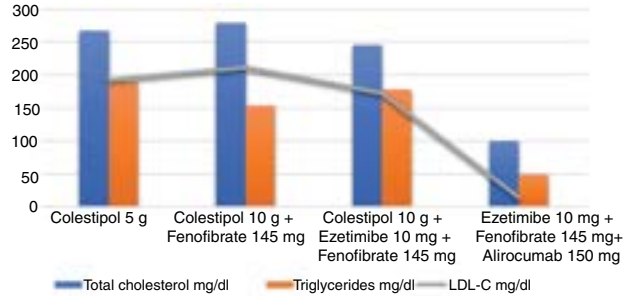


Figure 1 Evolution of lipid profile with the different treatments.
LDL-C: low-density lipoprotein-bound cholesterol.

leading a normal life and tolerating light exercise on a daily basis. His CK levels have stayed between 1212 and 6000 U/l, his liver enzymes have remained normal and there has been a marked decrease in his LDL-C, with levels of 15 mg/dl. His latest blood work revealed the following: total cholesterol 100 mg/dl, triglycerides 175 mg/dl, HDL-C 50 mg/dl, LDL-C 15 mg/dl, apoB 41.4 mg/dl, HbA1c 6.6%. Enzymes: GGT 28 U/l, GPT 51 U/l, GOT 47 U/l, CK 1212 U/l, LDH 222 U/l. He is currently on the same treatment as stated above (Fig. 1).

Discussion

This case report depicts a patient with McArdle's disease whose LDL-C levels improved on PCSK9 inhibitors, with excellent clinical tolerance.

McArdle's disease is a hereditary autosomal recessive disease caused by a deficiency in myophosphorylase, the enzyme in charge of skeletal muscle glycogen breakdown.[4] It is characterised by asthenia, muscle weakness, cramping, myalgia and exercise intolerance, as well as high resting CK levels and episodes of myoglobinuria, particularly after exertion.[1,3]

This is the first case described in the literature with a favourable evolution of dyslipidaemia in the context of this disease. It also shows that PCSK9 inhibitors may be used as a treatment in these patients. Statins tend to aggravate muscular symptoms in patients with myopathies. It is therefore recommended that they be avoided or, where applicable, that low doses be used and strict follow-up performed.[1,5] Our patient had episodes of myalgia and malaise when he performed exercise, which worsened while receiving statin therapy. He even developed an episode of severe rhabdomyolysis. However, on treatment with PCSK9 inhibitors, his lipid and enzyme profiles improved favourably, with no effects on his muscles. In spite of the very low LDL-C levels achieved in the patient, his doses of lipid-lowering agents were maintained in light of the excellent tolerance results and the clinical benefit of low LDL-C levels with PCSK9 inhibitors.[6]

The LDL receptor concentration on the surface of the liver is controlled by the PCSK9 protein.[7] This protein reduces the uptake of LDL particles, which leads to an increase in plasma LDL-C levels. Monoclonal antibodies act by inhibiting PCSK9 binding to the LDL receptor. They have shown dose-dependent reductions of LDL-C (44–65%), apoB (48–59%) and lipoprotein(a) (27–50%), with no significant adverse effects, including in patients who are intolerant to statins.[8] In the Odyssey Alternative study, 314 statin-intolerant patients were randomised to receive alirocumab 75 mg every two weeks, ezetimibe 10 mg/day or atorvastatin 20 mg/day. If the LDL-C targets were not achieved, the alirocumab dose was increased to 150 mg every two weeks. Muscular side effects were less prevalent with alirocumab than with atorvastatin.[9]

These findings suggest that PCSK9 inhibitors could be a promising alternative to reduce LDL-C in patients with contraindications for statins.[10]

Conclusions

McArdle's disease is a myopathy caused by myophosphorylase deficiency. Affected patients experience acute muscle crises after intense exercise. There is currently no treatment, but a diet high in complex carbohydrates and limiting intense exercise are recommended. Statin therapy is contraindicated, so in the presence of high vascular risk and raised LDL-C levels, PCSK9 inhibitors represent a therapeutic alternative, as shown in our case.

Conflicts of interest

Fernando Civeira received financial contributions from Amgen and Sanofi for conferences and advisory committees. Victoria Marco-Benedí and Estíbaliz Jarauta received travel bursaries from Sanofi to attend congresses.

Acknowledgements

This case was granted a prize by the Spanish Society of Atherosclerosis (*Sociedad Española de Arteriosclerosis, SEA*) at the SEA National Congress held in Girona in June 2018.

References

1. Quinlivan R, Buckley J, James M, Twist A, Ball S, Duno M, et al. McArdle disease: a clinical review. J Neurol Neurosurg Psychiatry. 2010;81:1182–8, http://dx.doi.org/10.1136/jnnp.2009.195040.
2. Leite A, Oliveira N, Rocha M. McArdle disease: a case report and review. Int Med Case Rep J. 2012;5:1–4, http://dx.doi.org/10.2147/IMCRJ.S28664.
3. Andreu A, Nogales-Gadea G, Cassandrini D, Arenas J, Bruno C. McArdle disease: molecular genetic update. Acta Myol. 2007;26:53–7. PMCID: PMC2949323.
4. Özen H. Glycogen storage diseases: new perspectives. World J Gastroenterol. 2007;13:2541–53, http://dx.doi.org/10.3748/wjg.v13.i18.2541.
5. Stroes ES, Thompson PD, Corsini A, Vladutiu GD, Raal FJ, Ray KK, et al. Statin-associated muscle symptoms: impact on statin therapy. Eur Heart J. 2015;36:1012–22, http://dx.doi.org/10.1093/eurheartj/ehv043.
6. Giugliano RP, Pedersen TR, Park JG, de Ferrari GM, Gaciong ZA, Ceska R, et al. Clinical efficacy and safety of achieving very low LDL-cholesterol concentrations with the PCSK9 inhibitor evolocumab: a prespecified secondary analysis of the FOURIER trial. Lancet. 2017;390(10106):1962–71, http://dx.doi.org/10.1016/S0140-6736(17)32290-0.
7. Schulz R, Schlüter KD, Laufs U. Molecular and cellular function of the proprotein convertase subtilisin/kexin type

9 (PCSK9). Basic Res Cardiol. 2015;110:4, http://dx.doi.org/10.1007/s00395-015-0463-z.
8. Bergeron N, Phan BA, Ding Y, Fong A, Krauss RM. Proprotein convertase subtilisin/kexin type 9 inhibition: a new therapeutic mechanism for reducing cardiovascular disease risk. Circulation. 2015;132:1648–66, http://dx.doi.org/10.1161/circulationaha.115.016080.
9. Moriarty PM, Jacobson TA, Bruckert E, Thompson PD, Guyton JR, Baccara-Dinet MT, et al. Efficacy and safety of alirocumab, a monoclonal antibody to PCSK9, in statin-intolerant patients: design and rationale of Odyssey Alternative, a randomized phase 3 trial. J Clin Lipidol. 2014;8:554–61, http://dx.doi.org/10.1016/j.jacl.2014.09.007.
10. Stroes E, Colquhoun D, Sullivan D, Civeira F, Rosenson RS, Watts GF, et al. Anti-PCSK9 antibody effectively lowers cholesterol in patients with statin intolerance: the Gauss-2 randomized, placebo-controlled phase 3 clinical trial of evolocumab. J Am Coll Cardiol. 2014;63:2541–8, http://dx.doi.org/10.1016/j.jacc.2014.03.019.

Accepted Manuscript

Disappearance of recurrent pancreatitis after splenectomy in Familial Chylomicronemia Syndrome

Victoria Marco-Benedí, Itziar Lamiquiz-Moneo, Luis A. Álvarez-Sala, Fernando Civeira

PII: S0021-9150(18)31182-1

DOI: 10.1016/j.atherosclerosis.2018.06.870

Reference: ATH 15593

To appear in: *Atherosclerosis*

Received Date: 4 February 2018

Revised Date: 6 June 2018

Accepted Date: 19 June 2018

Please cite this article as: Marco-Benedí V, Lamiquiz-Moneo I, Álvarez-Sala LA, Civeira F, Disappearance of recurrent pancreatitis after splenectomy in Familial Chylomicronemia Syndrome, *Atherosclerosis* (2018), doi: 10.1016/j.atherosclerosis.2018.06.870.

Title

Disappearance of recurrent pancreatitis after splenectomy in Familial Chylomicronemia Syndrome

Authors

Victoria Marco-Benedí[1], Itziar Lamiquiz-Moneo[1], Luis A. Álvarez-Sala[2], Fernando Civeira[1,3]

Centers

[1] Lipid Clinic, Hospital Universitario Miguel Servet, IIS Aragón, CIBERCV, Zaragoza, Spain

2 Lipid Clinic, Hospital Universitario Gregorio Marañón, IIS Gregorio Marañón (IiSGM), Departmento de Medicina. School of Medicine. Universidad Complutense de Madrid, Madrid, Spain

[3] Universidad de Zaragoza, Zaragoza, Spain

Corresponding author. Prof. Fernando Civeira, Unidad de Lípidos, Servicio de Medicina Interna, Hospital Universitario Miguel Servet, Avda Isabel La Católica 1-3, 50009, Zaragoza, Spain

E-mail address: civeira@unizar.es

Abstract:

Background and aims: Recurrent pancreatitis is a severe complication of Familial Chylomicronemia Syndrome (FCS) mainly secondary to lipoprotein lipase deficiency. The mechanism and interindividual variability of pancreatitis in FCS is not fully understood, but abnormalities in the drainage system of pancreatic veins could be involved.

Methods and results: Two cases of typical FCS are described with a past history of recurrent pancreatitis that dramatically improved after splenectomy performed in both cases for reasons non-related to FCS.

Conclusion: These are the first reports of the disappearance of pancreatitis after splenectomy in FCS and they should be considered of anecdotal nature at this time. The disappearance of pancreatitis following splenectomy could be in part due to subsequent improvements in pancreatic drainage. Extrahepatic portal hypertension induced by hypertriglyceridemic splenomegaly leading to pancreatic congestion could also be a contributing factor.

Keywords:

Familial Chylomicronemia; Lipoprotein lipase deficiency; Pancreatitis; Splenectomy

Introduction

Familial Chylomicronemia Syndrome (FCS) deficiency is a rare autosomal recessive disorder characterized by the absence or the severe reduction of lipoprotein lipase (LPL) activity and a massive accumulation of chylomicrons in plasma leading to a large increase of plasma triglyceride concentration, usually greater than 22.5 mmol/L (2000 mg/dl) in the fasting state (1). FCS is clinically characterized by repeated abdominal pain episodes, recurrent acute pancreatitis, eruptive cutaneous xanthomatosis, *lipemia retinalis* and hepatosplenomegaly. FCS is mostly identified in childhood due to recurrent episodes of pancreatitis and high fasting triglycerides (2). Pancreatitis is the most serious complication of LPL deficiency. The underlying pathophysiological mechanisms are not fully understood (3). FCS is a very rare disease with a frequency at about one per million in the general population, although it may be higher in some populations due to a founder effect (4).

The degree of hyperchylomicronemia in FCS depends, at least in part, on dietary fat intake, but genetic heterogeneity can play a role (5). It has been observed that a severe restriction of dietary fat to less than 20 g/day is enough to control the symptoms in some cases (6). In contrast, FCS is usually not responsive to conventional lipid-lowering therapy. Acute pancreatitis characterizes early stages of the disease while some patients may develop recurrent abdominal pain and chronic pancreatitis as the disease progresses. Hepatomegaly and splenomegaly could appear over time when the chylomicronemia becomes chronic. Splenomegaly is less frequently observed than hepatomegaly and can be notably hard. The organomegaly occurs as result of triglyceride uptake by macrophages. These individuals might show anemia and/or thrombocytopenia due to secondary hypersplenism (1,7).

In this study we describe two unrelated cases of typical FCS with a past history of recurrent pancreatitis controlled in two different lipid clinics in Spain. Both cases have shown a dramatic improvement in pancreatitis after a splenectomy was performed due to reasons non-related to FCS.

Material and methods

Lipoprotein Activity. The LPL activity assay was done on post-heparin samples on an Intralipid 10% emulsion as previously described (8). Each sample for LPL activity was assayed in triplicate and two standard samples were analyzed in each assay.

Genetic analysis. DNA was extracted by standard methods. Promoters, coding regions and intron-exon boundaries of *LPL*, *APOA5*, *APOC2*, and *GPIHBP1* were amplified by PCR and purified by ExoSap-IT (USB) using primers previously described (9). Amplified fragments were sequenced by Sanger method using the BigDye 3.1 sequencing kit (Applied Biosystems) in an automated ABI 3500xL sequencer (Applied Biosystems).

Results

The first case is a 68-year-old man who first visited our Lipid Unit at the Hospital Universitario Miguel Servet, Zaragoza, Spain, approximately 20 years ago. He was referred by his physician for the study of severe hypertriglyceridemia (HTG) with fasting plasma concentrations between 22.5-45 mmol/l (2000-4000 mg/dl from, at least, the age of 30 years old. At his first visit the patient was asymptomatic and the physical exam was normal except for low weight and body mass index (61.4 Kg and 18 kg/m^2, respectively). He was following a low-fat diet and his fasting lipid profile showed triglycerides 39.2 mmol/l (3473 mg/dl); total cholesterol 7.8 mmol/l (303 mg/dl); HDL

cholesterol 0.62 mmol/l (24 mg/dl), apolipoprotein B (apoB) 90 mg/dl, and plasma Lp(a) 3.56 mg/dl. Other biochemical parameters including thyroid hormones, glucose, liver enzymes, and creatinine were in the normal range. His post-heparin lipoprotein lipase activity was undetectable in two occasions (<10 µU/ml, normal values 22-47.6 µU/ml). Years later, the sequencing analysis of *LPL*, *APOC2*, *APOA5*, *LMF1* and *GPIHBP1* showed that the patient was double heterozygous with a pathogenic mutation in *LPL* gene (p.Pro234Leu) and two different pathogenic mutations in *LMF1* (p.Arg354Trp) and (p.Arg364Gln) (9). He was diagnosed of FCS secondary to LPL deficiency. He has been reviewed periodically in our unit, remaining asymptomatic and without substantial changes in his physical exam or lipid phenotype.

He began at the age of 22 with recurrent episodes of abdominal pain. These episodes repeated once or twice a year, lasting 4-12 hours, ceasing after prolonged fasting. At the age of 28, the patient was admitted to our hospital because of another episode of diffuse abdominal pain, more intense and prolonged than previous, with maximal pain in the upper left quadrant of the abdomen. His physical exam was normal except for mild splenomegaly. He was treated with fasting, analgesia and intravenous fluids and the pain resolved within a few hours. Triglycerides and pancreatic enzymes were not analyzed during his hospital stay. However, mild leukocytosis and lipemic plasma was observed at admission. He was discharged with the diagnosis of abdominal pain secondary to splenomegaly.

At the age of 31 years and following a parachuting accident, he began to experience severe abdominal pain and underwent emergency splenectomy in another center under the suspicion of a spleen rupture. The pathological study of the spleen was not performed. After the splenectomy, the pain disappeared and he has remained

asymptomatic ever since. His current lipid profile shows: triglycerides 31.0 mmol/l (2748 mg/dl); total cholesterol 10.9 mmol/l (423 mg/dl); and apo B 82.6 mg/dl.

Second case presentation

A 43 year old woman was reviewed at the Lipid Unit, Hospital Universitario Gregorio Marañon in Madrid, Spain, where she was diagnosed with FCS due to severe HTG from birth, recurrent episodes of acute pancreatitis and very low post-heparin LPL activity: 5.2 µU/ml. Sequencing analysis of the *LPL* gene showed that the patient was homozygous for a functional *LPL* mutation (G188E/G188E). Her parents had a pattern of mild combined hyperlipidemia and were heterozygous to G188E mutation in *LPL*.

The patient started suffering from abdominal pain at 2 months of age, with triglycerides above 22.5 mmol/l (2000 mg/dl) in several controls. Breast-feeding was stopped and she started receiving an artificial milk formula.

Along her infancy she experienced several episodes of abdominal pain. No registries of clear pancreatitis were reported up to the age of 13 years. In analytical controls she always had chylomicronemia with values of triglycerides higher than 22.5 mmol/l (2000 mg/dl). In two reports at the age of 12 and 13 she presented eruptive xanthomas in the shoulders, neck and thorax. At least six cases of acute pancreatitis were documented between the ages of 13-26. Additionally, she reported frequent episodes of mild to moderate abdominal pain.

She became pregnant at 26 years old and suffered a mild pancreatitis after 4 months of pregnancy. During her 5^{th} month of pregnancy she reported severe pancreatitis and developed a pseudocyst and later a pancreatic abscess. An elective cesarean was performed during her 25^{th} week of pregnancy. The child who is nowadays alive and healthy (no lipids data are available). Image studies following the caesarean

showed multiple gallstones. Part of the body and tail of the pancreas had disappeared due to autodigestion following the last severe pancreatic episode. Ten months after the caesarean, an elective surgery was done due to mechanical small bowel obstruction secondary to abdominal adhesions. Y Roux gastro-jejunostomy, cholecystectomy and splenectomy were performed. Splenectomy was due to suspected spleen vein thrombosis in presence of splenomegaly and spleen congestion. The spleen was well encapsulated, 15 cm long and 500 g weight. Histopathologically, the capsule was thickened; red pulp was predominant, there was endothelial hyperplasia, enlargement of the splenic sinuses, and fibrosis of intersinusal spaces. Penicilliary arterioles showed marked sclerosis. Lipid-laded macrophages were not observed. The pathological diagnosis was chronic congestive splenomegaly (Figure). Few months later she was admitted in the hospital due to mild episodes of pancreatitis twice (the last one not clearly confirmed). Since then, 11 years later, the patient has had no further pancreatitis episodes in spite of chylomicronemia with high triglycerides values 29.6 mmol/l (2629 mg/dl) found on Oct 29th, 2015 and 37.4 mmol/l (3316 mg/dl) on Dec 18th, 2015).

Discussion

The two cases presented in this report illustrates the disappearance of abdominal pain after performing a splenectomy in patients with FCS. It is the first description in the literature of this favorable evolution in this disease and thus generates hypotheses that can help to identify some of the mechanisms associated with the onset of pancreatitis and chronic pain in these patients.

Abdominal pain is very common in FCS and is due in most cases to episodes of recurrent acute pancreatitis or subsequent complications (10). Approximately 50% of subjects with FCS have at least one episode of severe pancreatitis (11). Alcohol

consumption, pregnancy and poor compliance with a low-fat diet are risk factors for the development of pancreatitis in FCS; however, even in absence of these factors, pancreatitis may develop with large differences among subjects, even those sharing the same pathogenic mutation (11). Recently, two loci: the chymotrypsinogen C (CTRC) and serine protease inhibitor Kazal-type1 (SPINK1) have been associated with a risk of recurrent hospitalization for acute pancreatitis in severe HTG due to FCS (17). However, these genes are poorly studied and their plausible association with pancreatitis is not related to pancreatic secretion or drainage, or to spleen function. Furthermore, the improvement of pancreatic episodes after splenectomy in our cases does not support a genetic predisposition to pancreatitis in our patients (12).

In the two cases reported here, episodes of recurrent pancreatitis seem evident given the characteristics of the episodes of pain and the confirmation of LPL deficiency. However, because the first case happened 40 years ago, it is difficult to verify at present time regardless of the episodes very suggestive nature.

The mechanism by which very severe HTG leads to pancreatitis has not been fully elucidated, but it is likely related to the liberation by pancreatic lipase of free fatty acids (FFA) from triglycerides and lysophosphatidylcholine from phosphatidycholine, when the pancreas is exposed to severe hyperchylomicronemia in the pancreatic capillaries (7). High local concentrations of FFA overwhelm the binding capacity of albumin with resultant aggregation into micellar structures with detergent properties (13).

The association of chronic pancreatitis with splenomegaly has been well-documented for some time (14). On the one hand, splenomegaly and pancreatitis are more frequent in the presence of very high triglyceride levels, so they could be concomitant manifestations in nature without having a causal association between them.

Splenomegaly can also be the result of complications from pancreatitis such as pseudocysts, abscesses, infarcts, hemorrhages or vascular lesions causing splenic portal hypertension (15). However, the association between splenomegaly and pancreatitis appears even in the absence of HTG and sometimes precedes the development of pancreatitis in the presence of splenic venous thrombosis, and so other mechanisms may be involved. As pointed out by Francesco and cols (16), it is difficult to establish which came first, the pancreatitis or the splenomegaly, what they call the chicken or the egg causality dilemma.

The most plausible explanation for the disappearance of episodes of pancreatitis in the exposed cases would be a hemodynamic mechanism. The pancreatic veins drain into the splenic vein and the latter into the vena cava. Splenomegaly frequently associates extrahepatic portal hypertension that hinders pancreatic drainage (17). In a retrospective analysis, Ramesh et al reported the effect of different surgical techniques to get pain relief in patients with chronic pancreatitis and signs of portal hypertension. In their work, 15 out of 57 patients had chronic pancreatitis with extrahepatic portal hypertension secondary to thrombosis of the splenic vein, and in these cases, splenectomy was performed along with pancreatic drainage. The authors observed that the surgery significantly improved the symptoms of these patients (18). It would be possible that in the presence of defective pancreatic venous drainage increased FFA accumulation and pancreatic aggression may occur. Splenectomy improves pancreatic venous drainage and could favor the decrease of pancreatitis in certain cases of hypertriglyceridemic pancreatitis associated with extra hepatic portal hypertension. It would be interesting to know if there are more cases of hypertriglyceridemic pancreatitis and splenectomy worldwide, in order to further confirm our observation.

These two observations cannot induce one to perform splenectomy in FCS patients with recurrent pancreatitis. They should be considered of anecdotal nature and without cause-and-effect relationship between splenectomy and recurrent pancreatitis. This association would require a definite demonstration of the deleterious effect of elevated extrahepatic portal pressure in FCS subjects. Furthermore, the effect of splenectomy in FCS animal models (19,20) should be profoundly studied before any human intervention study should commence.

Conflicts of interest

The authors declared that they do not have any conflict of interest to disclose with respect to this research.

Funding

This study was supported in part by grants from the Carlos III Research Institute: CIBERCV (co-supported by the European Regional Development Fund (ERDF) which is allocated by the European Union; IIS16/0114, PI15/01983 and PIE16/00022.

Contribution Statement.

All authors have made substantial contributions to all of the following: the conception and design of the study (FC); acquisition of data and analysis and interpretation of data (VM-B, LA A-S, I L-M, FC), drafting the article (V M-B, FC), and revising it critically for important intellectual content (LA A-S, I L-M). All authors have approved the final the version of the manuscript. Authors report no conflicts of interest.

Acknowledgement

Authors thank Chanel A. Ramirez for her English language editing.

Bibliography

[1] Brunzell D, Deeb SS. Familial lipase deficiency, ApoC-II deficiency and hepatic lipase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, ed. The metabolic basis of inherited disease. 8th ed. New York, NY: McGraw-Hill; 2001;2789-816.

[2] Stroes E, Moulin P, Parhofer KG, Rebours V, Löhr JM, Averna M. Diagnostic algorithm for familial chylomicronemia syndrome. Atheroscler. Suppl. 23 (2017) 1-7.

[3] Scherer J, Singh VP, Pitchumoni CS, Yadav D. Issues in hypertriglyceridemic pancreatitis: an update. J. Clin. Gastroenterol. 48 (2014) 195-203.

[4] Santamarina-Fojo S. The familial chylomicronemia syndrome. Endocrinol. Metab. Clin. North. Am. 27 (1998) 551-67.

[5] Chokshi N, Blumenschein SD, Ahmad Z, Garg A. Genotype-phenotype relationships in patients with type I hyperlipoproteinemia. J. Clin. Lipidol. 8 (2014) 287-95.

[6] Brahm AJ, Hegele R. Chylomicronaemia current diagnosis and future therapies. Nat. Rev. Endocrinol. 11 (2015) 352-62.

[7] Chait A, Subramanian S, Brunzell JD. Genetic Disorders of Triglyceride Metabolism. In: De Groot LJ, Chrousos G, Dungan K, et al, editors. Endotext [Internet]. South Dartmouth, MA: MDText.com, Inc.; 2015: Available from http://www.ncbi.nlm.nih.gov/books/NBK326743.

[8] Coca-Prieto I, Kroupa O, Gonzalez-Santos P, Magne J, Olivecrona G, Ehrenborg E, Valdivielso P. Childhood-onset chylomicronaemia with reduced plasma lipoprotein lipase activity and mass: identification of a novel GPIHBP1 mutation.

J. Intern. Med. 270 (2011) 224-8.

[9] Lamiquiz-Moneo I, Blanco-Torrecilla C, Bea AM, et al. Frequency of rare mutations and common genetic variations in severe hypertriglyceridemia in the general population of Spain. Lipids Health Dis.15 (2016) 82.

[10] Davidson M, Stevenson M, Hsieh A, Ahmad Z, Crowson C, Witztum JL. The burden of familial chylomicronemia syndrome: interim results from the IN-FOCUS study. Expert Rev. Cardiovasc. Ther. 15 (2017) 415-23.

[11] Rabacchi C, Pisciotta L, Cefalù AB, et al. Spectrum of mutations of the LPL gene identified in Italy in patients with severe hypertriglyceridemia. Atherosclerosis 241 (2015) 79-86.

[12] Tremblay K, Dubois-Bouchard C, Brisson D, Gaudet D. Association of CTRC and SPINK1 gene variants with recurrent hospitalizations for pancreatitis or acute abdominal pain in lipoprotein lipase deficiency. Front. Genet. 5 (2014) 90.

[13] Valdivielso P, Ramírez-Bueno A, Ewald N. Current knowledge of hypertriglyceridemic pancreatitis. Eur. J. Intern. Med. 25 (2014) 689-94.

[14] Izbicki JR, Yekebas EF, Strate T, et al. Extrahepatic portal hypertension in chronic pancreatitis: an old problem revisited. Ann. Surg. 236 (2002) 82-9.

[15] Fishman EK, Soyer P, Bliss DF, Bluemke DA, Devine N. Splenic involvement in pancreatitis: spectrum of CT findings. AJR Am. J. Roentgenol. 164 (1995) 631-5.

[16] di Francesco F, Del Prete L, Grimaldi C, Monti L, de Ville de Goyet J. Pancreatitis and portal vein thrombosis in children: the chicken or the egg causality dilemma. J. Pediatr. Surg. 50 (2015) 565-9.

[17] Singh IK, Bhatnagar V, Gupta AK, Seith A. Correlation of splenic volume with hematological parameters, splenic vein diameter, portal pressure and grade of varices in extrahepatic portal vein obstruction in children. Pediatr. Surg. Int. 27 (2011) 467-71.

[18] Ramesh H, Jacob G, Venugopal A, Lekha V, Jacob M. Surgical management of chronic pancreatitis with portal hypertension--a 19-year experience. Surgery. 143 (2008) 252-8.

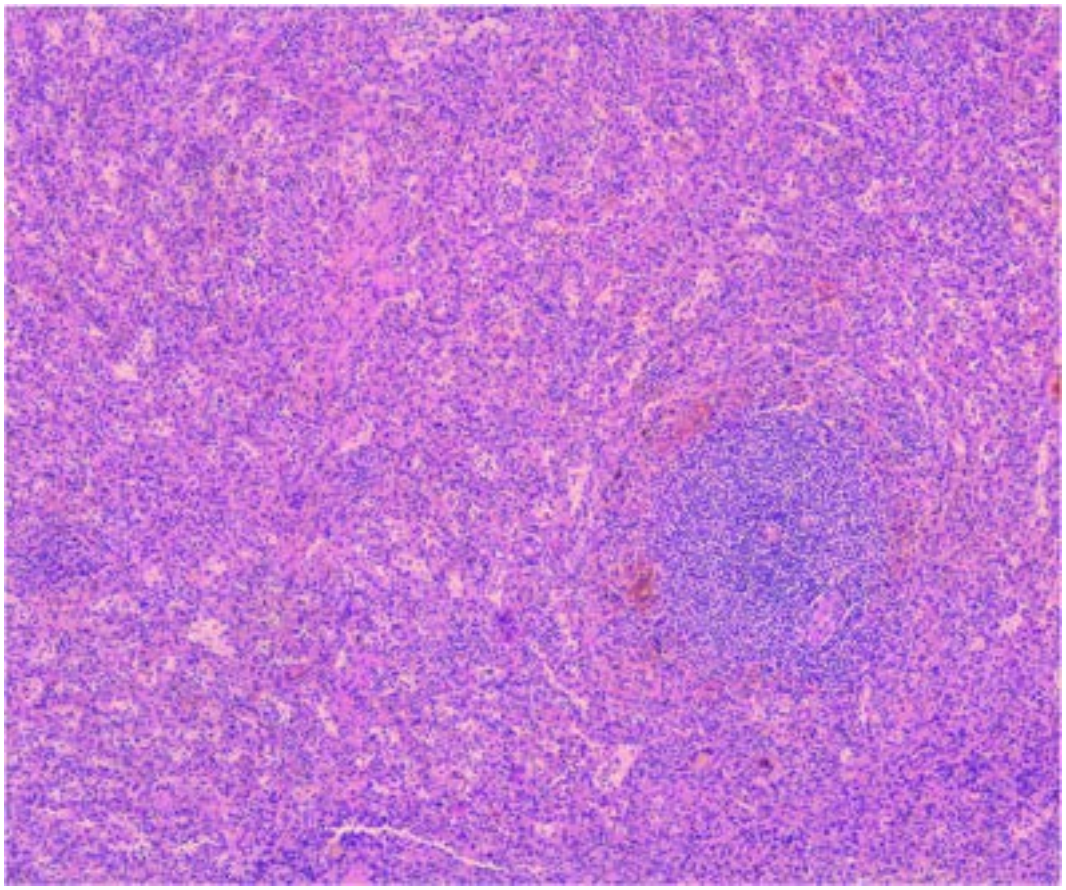
[19] Liu C, Gates KP, Fang L, et al. Apoc2 loss-of-function zebrafish mutant as a genetic model of hyperlipidemia. Dis. Model. Mech. 8 (2015) 989-98.

[20] Nordstoga K, Christophersen B, Ytrehus B, Espenes A, Osmundsen H, Landsverk T, Olivecrona T, Olivecrona G. Pancreatitis associated with hyperlipoproteinaemia type I in mink (Mustela vison): earliest detectable changes occur in mitochondria of exocrine cells. J. Comp. Pathol. 134 (2006) 320-8.

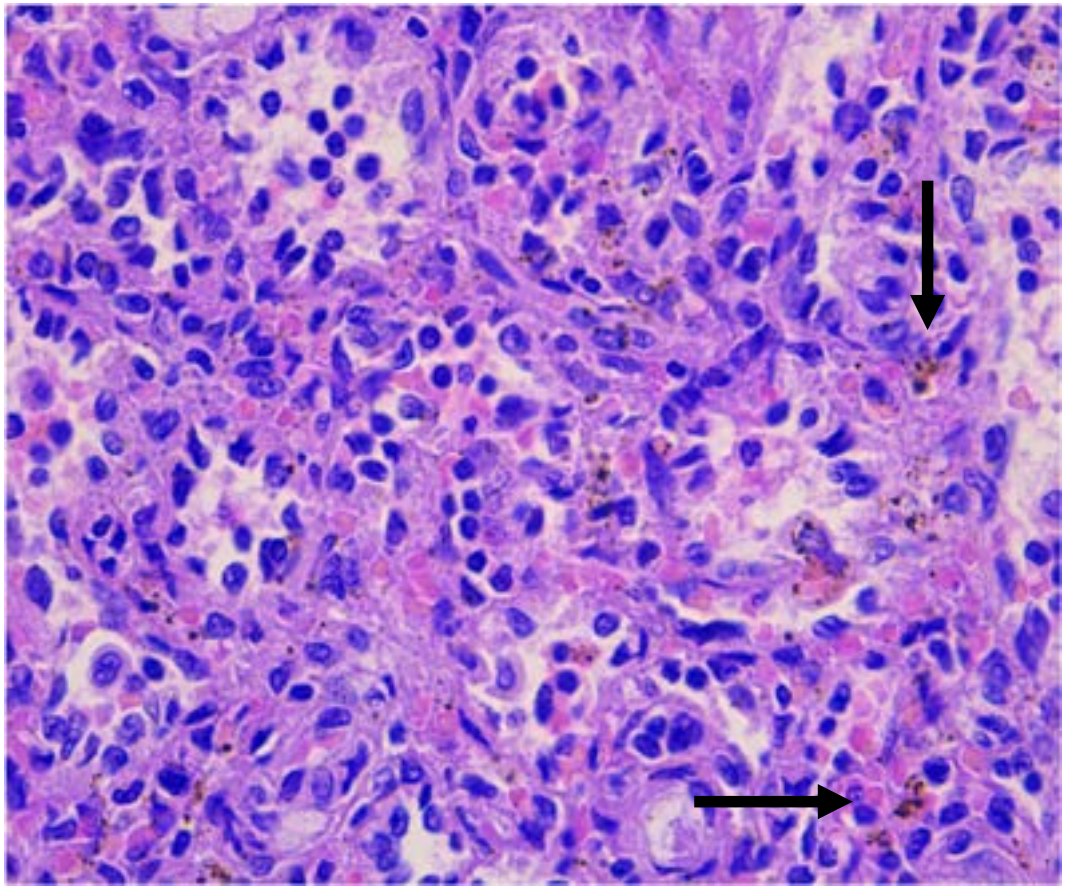
Figure legend

Histopathological findings of the spleen of the case 2 stained with hematoxylin and eosin. A (x 100) and B (x 400). Red pulp is predominant. Endothelial hyperplasia, enlargement of the splenic sinuses, and brown areas indicating hemosiderin deposits (arrows) are present. The pathological diagnosis was chronic congestive splenomegaly.

A



B



- Pancreatitis is a frequent and serious complication in familial chylomicronemia
- We present two cases in which recurrent pancreatitis disappeared after splenectomy
- Extrahepatic portal hypertension favors the development of pancreatitis
- Improvement of portal drainage could explain this favorable evolution