Title: "Behavioral cardiovascular risk factors and prevalence of diabetes and hypertension in subjects with familial hypercholesterolemia"

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Abstract

Cardiovascular disease (CVD) is highly prevalent in familial hypercholesterolemia (FH), hence this condition usually implies family concern and requires early medical intervention including promotion of healthy lifestyle. Whether the lower prevalence of type 2 diabetes mellitus (T2DM) reported in FH is associated with a healthier lifestyle has not been explored.

Methods. This cross-sectional study was designed to determine the prevalence of lifestyle-related cardiovascular risk factors in patients with heterozygous FH (HeFH) drawn from the Dyslipidemia Registry of the Spanish Atherosclerosis Society and to compare this prevalence to that observed in the ENRICA study, a representative sample of the adult Spanish general population, weighted to match the age and sex distribution of the HeFH sample.

Results. A total of 2185 patients with HeFH and 11856 individuals from the ENRICA study were included. Patients with HeFH had lower body mass index (BMI) and fewer current smokers than the reference Spanish population. A model adjusted for age, sex, and BMI showed that compared with the Spanish population, HeFH patients more frequently had CVD (odds ratio (OR) 23.98; 95% confidence interval (CI) 18.40 to 31.23), hypertension (OR 1.20; 95% CI 1.07 to 1.35), took anti-hypertensive medication (OR 1.36; 95% CI 1.18 to 1.56) and anti-diabetic medication (OR 1.25; 95% CI 1.00 to 1.56), but less frequently were current smokers (OR 0.79; 95% CI 0.71 to 0.89). In a HeFH subsample (n=513) with complete blood glucose information, those patients without CVD showed lower prevalence of current smoking and T2DM, lower BMI and blood glucose, and higher diastolic blood pressure than the general Spanish population. The differences in T2DM disappeared after adjustment for age, sex and

BMI, however, this adjustment also showed higher prevalence of hypertension and use of anti-hypertensive drugs in HeFH.

Conclusions. HeFH patients had lower BMI, which may contribute to explain the lower prevalence of diabetes in this population, and lower current smoking but higher hypertension prevalence than the general population.

Introduction

Familial hypercholesterolemia (FH) is one of the most common genetic diseases worldwide (1). The estimated prevalence of heterozygous FH (HeFH) is one in every 200-250 persons (2,3) and it is even higher in areas with some genetic isolation (4). FH patients 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 mutations in the genes encoding for the LDL particle receptor (*LDLR*) (6), apolipoprotein B (*APOB*) (7), proprotein convertase subtilisin/kexin type 9 (*PCSK9*) (8) and apolipoprotein E (*APOE*) (9). Untreated individuals have a markedly elevated long-term CHD risk, with hazard ratios up to 5.0 with respect to the general population and 100-fold increase in early CHD mortality in young adults (10).

Several recent studies have found that patients with HeFH might be less prone to develop type 2 diabetes mellitus (T2DM) (11,12). This protection is puzzling because these patients are frequently treated with high doses of potent statins for long periods, and it is well established that hydroxy-methyl-glutaryl-CoA reductase (HMGCR) inhibition increases the risk of T2DM (13). Several observational studies (13,14) and a large meta-analysis of statin trials showed that statins increase the risk for new onset T2DM by 9-12% with a dose-dependent effect, supporting a causal relationship (15). The mechanism of this apparent protection of HeFH for the development of T2DM is unknown. It has been hypothesized that a reduction of cholesterol uptake by pancreatic β cells in HeFH may improve insulin secretion (11,16).

T2DM is the result of complex interactions between genetic and environmental factors. The genetic component is very polygenic in most cases, and attributable to modest deleterious effects of common regulatory gene variants present in the general

population (17). However, the genes causing FH have not been found associated with T2DM, neither in the genome-wide association studies (GWAS) (18) nor in exome sequencing of T2DM populations (19). These data suggest that T2DM protection is not related with the primary defect of FH (20).

On the contrary, some reports suggest that certain CVD risk factors could be diminished in the HeFH population, especially those related to healthy behaviors (11,12) such as diet and physical activity. Maintaining a healthy lifestyle throughout young adulthood is strongly associated with a low cardiometabolic risk later in life (21). It is plausible that the low prevalence of T2DM in patients with HeFH could be related to a healthy lifestyle early in life, which can modulate the risk of T2DM. Hence, the aim of this study is to assess the frequency of some behavioral CVD risk factors in patients with HeFH and compare it against the general population.

Methods

Study design and participants. This observational, multicenter, national study in Spain was designed to determine the prevalence of lifestyle-related cardiovascular risk factors in patients with HeFH and to compare it against that observed in the general population. Data on HeFH patients and general population information were obtained from the Dyslipidemia Registry of the Spanish Atherosclerosis Society (SEA) (22) and the ENRICA study (23), respectively. 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 (22). Anonymous clinical data collection in this registry was approved by a central ethics committee (Comité Ético de Investigación Clínica de Aragón, CEICA) and participants gave their informed written consent. The ENRICA study was conducted between June 2008 and October 2010 on a

representative sample of the Spanish population aged 18 years and older. The information was collected by trained personnel in participants' homes in three sequential stages: i) computer-assisted telephone interview, ii) first home visit to obtain biological samples (blood and urine), and iii) second home visit to measure anthropometric variables and blood pressure as well as to take a dietary history. The ENRICA protocol was approved by the Clinical Research Ethics Committees of the 'La Paz' University Hospital in Madrid and the Hospital 'Clinic' in Barcelona; all study participants gave informed written consent.

Subjects in both studies were eligible for inclusion in this analysis if they were 18 years of age or older and, in the case of the Dyslipidemia Registry cohort, 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 (DLCN): 6-8 points (probable), and >8 points (definitive) (1). Genetic diagnosis was based on tested carrier status of a known pathogenic mutation for FH. Homozygous FH were not included in this study.

Study variables. Arterial hypertension was defined as having 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 was defined as fasting plasma glucose ≥126 mg/dl, HbA1c > 6.5% or self-reported treatment with antidiabetic medication. Smoking was defined as smoking in the present or having ever smoked. Former smoker was defined as a subject having smoked at least one year in his lifetime and but being non-smoker in the last year.

Subgroup analysis. In the Dyslipidemia Registry of the SEA the information about current blood glucose, although is present in over 95% of cases, is not mandatory, in contrast to the diagnosis of T2DM and the anti-diabetic medication use. To avoid any

selection bias, we selected a subgroup of HeFH cases from those lipid clinics in which 100% of HeFH cases had full information of current blood glucose.

Statistical analysis

Summaries of risk factors, continuous and categorical variables, were calculated as mean (standard deviation) and percentages, respectively. Estimations with 95% confidence intervals (CI) were calculated using Student's t and binomial distributions, as appropriate. Sex and age strata were created in both studies and weights were created for the ENRICA study so that the sample would have the same age and sex composition of HeFH patients in the Dyslipidemia Registry, i.e., ENRICA estimations would provide summary values for the registry sample should they have been members of the general population instead of HeFH patients. This approach dealt with the sparse and irregular demographics distribution of the registry sample and avoided assumptions on linearity of the association of risk factors with age and absence of sex interactions implied by adjustment using linear models.

Differences between the registry and ENRICA samples were tested with weighted Student's t and Chi squared tests. Plots of sex and 5-years-of-age strata provided detailed information on which patients were most different. Linear models were used to analyze the differences adjusting for pre-hypothesized variables that could justify environmental influences on the differences observed, like BMI, and preventive drugs. Given that some of these variables are associated with demographics, conditioning on them reintroduces an artificial confounding association in spite of weighting, which was resolved including age and sex in the adjusted regression models.

Analyses were performed with the statistical software R version 3.4.4.

Results

A total of 2185 subjects with HeFH from the Dyslipidemia Registry and 11856 subjects as reference population from the ENRICA were selected according to inclusion criteria. In a first analysis where the ENRICA population was weighted to match age and sex distribution of FH sample, HeFH patients had lower levels of systolic blood pressure, lower BMI and fewer current smokers and ever smokers than the reference population (Table 1). The prevalence of CVD was much higher in HeFH than in the general population (15.1% vs. 0.9%). Antihypertensive medication was more frequently taken by HeFH subjects than the reference population (20.0% vs 17.7%), in spite of similar prevalence of hypertension in both cohorts. There were not any differences in the prevalence of T2DM or in the consumption of antidiabetic drugs (Table 1).

After adjusting for age, sex, and BMI, HeFH patients more frequently had CHD (odds ratio (OR) 23.98; 95% confidence interval (CI) 18.40 to 31.23), hypertension (OR 1.20; 95% CI 1.07 to 1.35), took anti-hypertensive medication (OR 1.36; 95% CI 1.18 to 1.56) and anti-diabetic medication (OR 1.25; 95% CI 1.00 to 1.56), but less frequently were current smokers (OR 0.79; 95% CI 0.71 to 0.89) (Table 2). There was also a trend to take more anti-diabetic medication in HeFH (OR 1.25; 95% CI 1.00 to 1.56).

The differences in CVD risk factors were compared by age and sex strata (Figure 1). For any stratum of age, both men and women, the HeFH subjects had lower BMI; higher CVD prevalence; and lower tobacco consumption (except in the case of women in the decade of 60 to 70 years) than the general population. Regarding anti-hypertensive medication, the differences in consumption increase as the age increases in men and women, just as it happens with anti-diabetic medication.

Subsequently, we performed a sub-analysis with identical methodology, selecting 513 HeFH subjects from lipid clinics where 100% of HeFH subjects had

information of blood glucose, as described in methods, and 11856 subjects of the reference population. The existing differences were the same as in the first analysis (Tables 3 and 4). Finally, in this subgroup, we analyzed whether the higher prevalence of CVD among HeFH could be responsible of the CVD risk factors differences found between cohorts. HeFH subjects without CVD showed lower BMI, blood glucose and prevalence of current smokers and T2DM, and higher mean diastolic blood pressure than the general population (Table 5). The differences in T2DM disappeared after adjustment for age, gender and BMI, however this adjustment showed higher prevalence of hypertension and anti-hypertensive drugs in HeFH (Table 5).

Comentado [FRA1]: OJO, en la tabla 4, hay un modelo 1 y un modelo 2 que no se describieron ni en métodos ni en os primeros análisis más arriba. Probablemente requiere más explicación.

Comentado [FRA2]: ¿Po qué no se hace en el total de la muestra en lugar de este subgrupo?)

Comentado [FRA3]: OJO en la tabla 5, hay un modelo 1 y un modelo 2 que no se comentan en los resultados. Probablemente requiere más explicación (¿por qué se hacen y que se encuentra en cada modelo?).

Discussion

In the present work, we have analyzed different CVD risk factors in subjects with HeFH and compared their prevalence with a representative population of the same country. The main results are that patients with HeFH have lower prevalence of certain CVD risk factors than the general population in spite of a much higher prevalence of CVD. Considering that these risk factors have a strong environmental influence (24), our results suggest, that a healthier lifestyle in HeFH may be a major contributor to these results. These differences are present early in life supporting the idea that the familial environment induces a higher consciousness about CVD. This seems to be obvious because the history of precocious CVD in most of these families (25). In addition, the medical intervention seems to play a major role. A relevant fact of our data is that the treatment of risk factors not directly dependent on cholesterol, that is, T2DM, hypertension, and smoking is higher in subjects with HeFH than in subjects of the general population, which probably translates into a greater contact with a highly specialized health system that favors the diagnosis and treatment of all risk factors (26).

It is well known that the prevalence of T2DM is lower with patients with HeFH (11,12). In our study it appears that a large part of this lower prevalence could be related to the fact that BMI is lower at any age than in the general population. Without being able to rule out that FH cause itself or plays a role in the control of the weight of these subjects, it does not seem that this is a relevant factor since the genes related to the FH (*LDLR, APOB, PCSK9,* and *APOE*) have not been related to the inter-individual variation of weight in the general population (27,28). Therefore, it seems reasonable that the HeFH subjects are thinner due to environmental factors probably due to a healthier lifestyle since childhood and the aforementioned medical intervention. This environmental action is in line with the observed data of smoking that shows that HeFH subjects are less smokers from early ages of life, as previously reported (29). However, previous studies use different populations as controls, including non-affected relatives, and the bias of a shared environment makes interpretation difficult (11,30).

The differences attributed to the prevalence of T2DM in subjects with FH have been attributed to a lower disposition of the pancreatic beta cell when the LDL cholesterol uptake is defective in the presence of mutations in the receptor (31). This defect in peripheral uptake would be compensated for by an increase in endogenous cholesterol synthesis. In accordance with this hypothesis, the genetic variation in *HMGCoA*, the gene responsible for intracellular cholesterol synthesis, is associated with the risk of T2DM and variants with gain of function are associated with T2DM (32). However, if this phenomenon is responsible for the lower risk in FH is unknown. Without being able to rule out that the genetic factors that cause FH play a causal role, our data suggest that environmental factors are important to explain a large part of the phenomenon. This would explain that the lower prevalence of T2DM occurs in subjects with FH with independence in the presence or absence of a mutation in the candidate

gene, which suggests that it is the FH itself and not the cause what is responsible for this minor prevalence of diabetes (12).

The use of statins is associated with the risk of T2DM (33). Our HeFH cohort, where most subjects take statins for many years, show that prevalence of T2DM is not increased, which emphasizes the value of a heathy lifestyle in the prevention of T2DM in subjects with high doses of statins.

It is noteworthy that subjects with HeFH have higher diastolic blood pressure, higher prevalence of hypertension and higher anti-hypertensive drug treatment than the general population in spite a lower BMI. The association of hypertension with FH has not been studied in deep previously. A recent report from our country in a different population has found almost double prevalence of hypertension among clinically defined HeFH (34). If this phenomenon is related to a closer medical control, a misdiagnosis of some clinically defined HeFH with familial combined hyperlipidemia, a phenotype highly related to hypertension (35), or, it is causally associated with FH cannot be concluded from our study.

Our study has some limitations. The diagnosis of tobacco consumption is referred by patients this may condition that may be underestimated in the medical environment of a lipid unit and there may be biases with respect to an interviewer in your own home. However, the differences are important enough to be hardly explained by this phenomenon. Similarly, the diagnosis of hypertension has been made in both studies according to the figures of blood pressure and medication and blood pressure at home are usually lower than the clinical blood pressure. However, our study has important strengths. The high number of subjects studied in both studies, the control population is representative of the same population from which the population of

subjects with FH is extracted, and the same criteria used for the diagnosis of the main risk factors.

In conclusion, subjects with HeFH have a different prevalence of CVD risk factors than the general population. This includes a lower mean BMI, which could explain much of the lower prevalence of diabetes in this population, lower frequency of current smoking but higher hypertension prevalence.

Declarations

Ethics approval and consent to participate: Anonymous clinical data collection was approved by a central ethical committees 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.

Competing interests: All authors declare that they have no conflict of interest.

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Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed all authors. The first draft of the manuscript was written by Sofía Pérez-Calahorra, Fernando Civeira and Martín Laclaustra, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figure legend

Plots of sex and 5-years-of-age strata of means and percentages of relevant variables. Two plots (one per sex) are created for each variable. Circles (solid for familial hypercholesterolemia, and hollow for the reference population) denote the mean or percentage for each 5-year interval. Vertical lines are the 95% CI of the estimation.

Comentado [4]: Cuidado, parece que los paneles están descolocados. Es mejor configurar las imagenes "como caracter, en linea" y colocarlas en una tabla, donde tú controlas su posición mejor.