Association of Polymorphisms at the SR-BI Gene Locus With Plasma Lipid Levels and Body Mass Index in a White Population

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Abstract

The scavenger receptor class B type I (SR-BI) is a lipoprotein receptor that has been shown to be important in high density lipoprotein cholesterol (HDL-C) metabolism in mice. To determine its role in humans, we have characterized the human SR-BI gene and investigated its genetic variation in 489 white men and women. Five variants were demonstrated: 2 in introns (3 and 5) and 3 in exons (1, 8, and 11). Three variants at exons 1 and 8 and intron 5 with allele frequencies >0.1 were used to examine associations with lipid or anthropometric variables. The exon 1 variant was significantly ($P<0.05$) associated with increased HDL-C and lower low density lipoprotein cholesterol (LDL-C) values in men, but no associations were observed in women. The exon 8 variant was associated in women with lower LDL-C concentrations (3.05±0.98 mmol/L and 3.00±0.93 mmol/L for heterozygotes and homozygotes, respectively) compared with women homozygous for the common allele (3.39±1.09 mmol/L, $P=0.043$). No associations for this variant were observed in men. Women carriers of the intron 5 variant showed a higher body mass index (23.8±3.8 kg/m2, $P=0.031$) than those women homozygous for the common allele (22.4±3.4 kg/m2). Similar results were observed after haplotype analysis. Multiple regression analysis using HDL-C, LDL-C, and body mass index as dependent variables and age, sex, and each of the genetic variants as predictors also provided similar results. The associations found with both LDL-C and HDL-C suggest that SR-BI may play a role in the metabolism of both lipoprotein classes in humans.

Key Words: scavenger receptor class B type I, single-nucleotide polymorphism, body mass index, LDL cholesterol, HDL cholesterol

The scavenger receptor class B type I (SR-BI) is a multilipoprotein receptor found in the liver and steroidogenic glands of both mice[1] and humans[2,3] (for a review, see Reference 4). The cDNA for human SR-BI (also known as CLA-1) was originally cloned by homology to human CD36 and rat LIMPII, which are members of a family of transmembrane proteins.[5] An independent expression cloning study identified the hamster homologue by its ability to mediate the binding of modified LDL, and it was also shown to bind native LDL.[6] Subsequently, murine SR-BI was shown to mediate the uptake of lipid, but not apoprotein, from HDL into cells,[1] a process described as selective uptake.[7–9] This finding established SR-BI as the first HDL transmembrane receptor to be identified and cloned. Further studies of the human homologue demonstrated that it also is a multilipoprotein receptor that binds HDL, LDL, and VLDL.[2,10]

Further analysis in vivo in mice and rats has supported a role for SR-BI in cholesterol metabolism. Targeted disruption of apoAI, the major protein component of HDL, leads to an increase in SR-BI expression in the adrenal glands of mice,[11] where HDL-C is used for steroid hormone synthesis. In addition, SR-BI expression levels in the adrenal glands are increased in response to adrenocorticotropic hormone and decreased in response to dexamethasone.[12] Estrogen treatment at high doses in rats greatly reduces SR-BI expression in the liver while it increases SR-BI expression in the adrenal gland and ovarian corpus luteal cells.[13] Transient overexpression of SR-BI in the livers of mice by adenoviral infection leads to a marked reduction in plasma HDL levels and a concomitant increase in plasma LDL/IDL cholesterol levels.[14] Finally, targeted disruption of the SR-BI gene in mice leads to a significant increase in plasma HDL[15,16] and reduced selective uptake of cholesterol from HDL into the liver.[16] Thus, SR-BI has clearly been shown to be a very important player in HDL metabolism in mice. However, although mice have HDL as the major cholesterol-carrying lipoprotein in plasma, adult humans carry the bulk of plasma cholesterol in LDL and VLDL particles.

As a first step to determine the physiological role of SR-BI in humans, we have isolated the gene and determined the intron-exon boundaries. This information was used to search for genetic variability at this gene locus in a random population of unrelated white individuals. Moreover, in this well-characterized population, we investigated associations between common polymorphisms at this gene locus and plasma lipid levels as well as anthropometric characteristics.

Methods

Subjects

Blood samples for DNA isolation and biochemical measurements were collected from 489 unrelated subjects (201 men and 288 women) living in Zaragoza, Spain. All subjects were apparently healthy factory and hospital workers and students at the local university. They were recruited during their regular physical examinations, and they gave informed consent to their participation in the study. Height and weight for each participant were measured with the individual dressed in an examining gown and wearing no shoes. Body mass index (BMI) was expressed as weight in kilograms divided by the square of height in meters. Cigarette use was based on smoking status in the year before the study and included assessment of the number of cigarettes smoked per day. Alcohol intake was based on average weekly intake during the same period. All subjects were of white descent. The study was approved by the institutional review committee at the University of Zaragoza, Zaragoza, Spain.

Plasma Lipid Measurements

Plasma lipids were measured after a 12- to 14-hour overnight fast by using blood collected in tubes containing 0.1% EDTA. Plasma HDL-C was determined after precipitation of plasma apoB-containing lipoproteins with dextran sulfate–Mg^{2+}. Plasma total cholesterol, HDL-C, and triglyceride levels were measured using standard enzymatic procedures. LDL-C was calculated by the Friedewald equation when triglyceride levels were <400 mg/dL (4.52 mmol/L). CVs between runs for all lipid assays were <5%.

Isolation of the Human SR-BI Gene

A probe consisting of a 474-bp fragment of the human SR-BI cDNA was given to Research Genetics (Huntsville, Ala) to isolate bacterial artificial chromosomes (BACs) containing genomic DNA encoding the human SR-BI protein from a human BAC library (catalog No. 96041). Two BACs were isolated by hybridizing the probe to this library. These BACs were then sized by pulse field electrophoresis, and the inserts were found to be =80 and 70 kb for BAC 179 m10 and BAC 256i19, respectively. All further work was done using BAC 179 m10. BAC 179 m10 was digested with restriction enzymes, analyzed by Southern blot hybridization with portions of human SR-BI cDNA, and shown to contain a large portion of the SR-BI sequence. This BAC was then sheared by nebulizing the DNA into fragments that were inserted into a vector for sequencing (pminisk), and the resulting insert sizes were shown to range from 1 to 3 kb. Initially, clones that hybridized to the coding sequence of the full-length human SR-BI cDNA were sequenced, leading to the identification of most of the exons of the gene. Further random sequencing of the BAC sheared library led to the identification of the remaining coding exons and the adjacent intron flanking sequences. Sequences were not polished unless they were considered important for selection of polymerase chain reaction (PCR) primers.

Amplification of Genomic DNA Fragments

Multiple pairs of primers were synthesized to amplify each of the exonic regions with intronic borders. Genomic DNA from a human subject was subjected to PCR in 25-μL reactions (1X PCR Amplitaq polymerase buffer, 0.1 mmol/L dNTPs, 0.8 mmol/L 5' primer, 0.8 mmol/L 3' primer, 0.75 U of Amplitaq polymerase, and 50 ng genomic DNA) by using each of the described pairs of primers under the following cycle conditions: 94°C for 2 minutes, 35 times (94°C for 40 seconds, annealing temperature for 30 seconds, and 72°C for 1 minute), 72°C for 5 minutes, and a 4°C hold. The resulting PCR products were analyzed on a 2% agarose gel. The identity of the PCR product was confirmed by digestion with a restriction enzyme and subsequent agarose electrophoresis. Thirteen pairs of oligomers were chosen to serve as PCR primers to amplify regions containing each of the 12 coding exons of the human SR-BI gene. The nucleotide sequence of these primers is indicated in Table 2. The optimum PCR annealing temperatures for each primer pair, the expected sizes of the PCR products, as well as diagnostic restriction sites, are indicated in Table 2.

Single-Strand Conformation Polymorphism (SSCP) Analysis

The amplified genomic DNA fragments were analyzed by SSCP[17–19] from 96 subjects. From each 25 μL reaction, 3 μL was taken and added to 7 μL of loading buffer. The mixture was heated to 94°C for 5 minutes and then immediately cooled in a slurry of ice water. Three to 4 μL was then loaded onto a 10% polyacrylamide gel containing 10% glycerol and subjected to electrophoresis, typically overnight at 4 W at room temperature or for 6 hours at 20 W at 4°C. The secondary structure of single-stranded nucleic acids varies according to sequence, thus allowing the detection of small differences in nucleic acid sequence between similar nucleic acids. At the end of the electrophoretic period, the DNA was detected by gently overlaying a mixture of dyes onto the gel (1X the manufacturers' recommended concentration of SYBR green I and SYBR green II in 0.5X Tris-borate-EDTA buffer; Molecular Probes) onto the gel for 5 minutes, followed by rinsing in distilled water and detection in a Fluorimager 575 (Molecular Dynamics).

Identification of Mutations by Direct Sequencing of PCR Products

On detection of a polymorphism in an amplified SR-BI genomic region by SSCP, this region was reamplified using the aforementioned primers that were modified to contain additional sequences that could be used to directly sequence the PCR product (M13 forward sequence [5'-TGT AAA ACG ACG GCC AGT-3'] on the 5' end of the 5' primer and M13 reverse sequence [5'-CAG GAA ACA GCT ATG ACC-3'] on the 5' end of the 3' primer). The genomic DNA from 3 to 6 subjects per polymorphism was subjected to PCR in 50μL reactions (1X PCR Amplitaq polymerase buffer, 0.1 mmol/L dNTPs, 0.8 mmol/L 5' primer, 0.8 mmol/L 3' primer, 0.75 U of Amplitaq polymerase, and 50 ng genomic DNA) by using each of the above-described pairs of primers under the following cycle conditions: 94°C for 2 minutes, 35 times (94°C for 40 seconds, annealing temperature for 30 seconds, 72°C for 1 minute), 72°C for 5 minutes, and a 4°C hold. The optimum PCR annealing temperatures are given in Table 2. The newly amplified products were then purified by agarose gel electrophoresis and subjected to sequencing by using M13 forward and reverse primers.

Genotyping of Single-Nucleotide Polymorphisms (SNPs) in the Population

After characterization of the SNPs by direct sequencing, subjects were typed by digestion of PCR products for exon 1, intron 5, and exon 8 by using the primers and enzymes listed in Table 5. Introns 3 and 11 were typed by SSCP.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS), version 8.0 for Windows, was used for the statistical analysis. Power analyses for the different patterns of association by sex were carried out using PC-Size Consultant, version 1.01, from Statools. Sample size was calculated a priori considering an α error <5% and a β error <0.2. Owing to missing genetic information for some of the subjects, the statistical power was recalculated, a posteriori, for each tested hypothesis. The statistical power for the different patterns of association by sex ranged from 0.745 to 0.847, and the average was 0.8024, suggesting that our sample size was large enough to minimize type I errors and to assume standard type II errors.

A normal distribution for all continuous variables was checked by graphical methods and by hypotheses tests. Only triglyceride concentrations were markedly skewed, and this variable was logarithmically transformed to improve normality for statistical testing. To assess mean differences of lipid and anthropometric variables be-tween sexes and between genotypes, Student's *t* test for independent samples was used after determining the homogeneity of variances by the Levene statistic. All probability values were calculated using the assumption of potentially 2-sided differences. For multiple comparisons of means, 1-way ANOVAs were performed. Once established that differences existed among means, to determine which mean differed with correction for multiple comparisons, Tukey's test was applied. The correction is needed because when several *t* tests are made, each at the α=0.05 level, then the probability of incorrectly rejecting at least 1 null hypothesis will be much larger than α and will increase with the number of tests made. Unadjusted probability values (obtained by Student's *t* test) and adjusted probability values (obtained by Tukey's test) are reported in the tables to show the extent of the correction. To test the null hypotheses of no association between genotypes and lipid or anthropometric variables, controlling for 1 or more potential confounders (such as age, sex, BMI, alcohol intake, and tobacco use), multiple linear regression models with dummy variables were fitted. To improve statistical power, data from men and women were analyzed together, including a dummy variable for sex (1=male, 0=female) into the model. Several regression models were fitted for each outcome variable: BMI, total cholesterol, LDL-C, HDL-C, and triglycerides (after logarithmic transformation). Genotypes or haplotypes were included as *k*-1 dummy variables (*k* is the number of categories) to avoid collinearity, because the regression model contains an intercept and the 1/1 genotype was considered for each intron or exon as the reference category. Age was considered a control variable, and alcohol intake, tobacco use, or BMI was retained in the model when the increase in R^2 (F of change) was statistically significant (P<0.05). Taking into account the differences observed in mean values of lipids for men and women, additional interactions terms (with dummies) between sex and each genotype were included into the models. Because the power to detect associations is generally lower when testing inter-action terms, a probability value <0.08 was used as the criterion to retain these terms. Regression coefficients, SEs, 95% CIs, as well as probability values were estimated for each independent variable. When the interaction terms between sex and genotype were statistically significant (indicative that the relationship of interest was different at different categories of the variable sex), separate linear regression models, by applying the criterion mentioned above, were computed for men and women. Regression diagnostics were employed to check the assumptions and to assess the accuracy of computations. Finally, to calculate adjusted means for each outcome variable, controlling for the effects of the variables retained in the corresponding fitted linear regression models, covariance analyses with the general linear model procedure were carried out. To test the statistical significance and to estimate the 95% CIs for multiple comparisons of adjusted means, Tukey's method was applied. The allele and haplotype frequencies were estimated using the EH linkage utility program.[20] Significance of linkage disequilibrium between SNPs within SR-BI was assessed by x^2.

Results

Gene Structure and SNP Characterization

To begin to understand the physiological role of SR-BI in humans, we have defined the structure of the human gene by complete sequencing of the coding region as well as the majority of the noncoding region of the gene (see Methods and Reference 3). Thirteen exons were identified, ranging in size from 52 to 204 bp (Table 1). The first exon contained all 5' untranslated sequences found in SR-BI cDNAs as well as a portion of the coding region. The last exon comprised the entire 3' untranslated sequence. The exonic structure was remarkably similar to that of human CD36 (data not shown and References 3 and 21), another member of the CD36 family of proteins. To amplify the exonic regions as well as the splice junction sites, PCR primers were chosen in the intronic regions = 25 to 60 bp from the intron-exon boundaries (Table 2). The resulting PCR products were then analyzed by SSCP or restriction endonuclease analysis on a sample of 489 unrelated white men and women who were born and living in Spain (Table 3). The age range was between 18 and 75 years with a mean value of 37 years. These subjects were not selected on the basis of any preestablished disease. Polymorphisms were found in regions in or near exons 1, 3, 5, 8, and 11 (Table 4) and further identified by direct sequencing (see Methods). All polymorphisms were SNPs. SNPs near exons 5 and 11 were found in adjacent introns but were not in canonical splice-site sequences. The SNP in the region of exon 8 found by SSCP was determined to constitute a change in base position 1050 (cDNA position 1 is the first base of the initiator methionine) encoding amino acid 350, but there was no resultant change in the amino acid. The SNP in exon 3 at bp position 403 encoded a change from valine to isoleucine at amino acid 135. The SNP in exon 1 at bp 4 encoded a change from glycine to serine at the second amino acid position.

TABLE 1. Exonic Structure of the Human SR-BI Gene

Exon No.	cDNA Nucleotide Position*	Amino Acid Position
Exon 1	1–126	1–42
Exon 2	127–284	43–95†
Exon 3	285–426	95†–142
Exon 4	427–630	143–210
Exon 5	631–726	211–242
Exon 6	727–842	243–281†
Exon 7	843–1009	281†–337†
Exon 8	1010–1128	337†–376
Exon 9	1129–1202	377–401†
Exon 10	1203–1254	401†–418
Exon 11	1255–1401	419–467
Exon 12	1402–1530	468–509
Exon 13	1531–2512	z z

*Nucleotide position 1 is the first base in the initiator methionine.
†Indicates that the exon contains a portion of the codon for the amino acid.

TABLE 2. PCR Primers, PCR and SSCP Conditions, and Restriction Enzyme Analysis of Human SR-BI Gene Exons

Exon	Primers	Sequence (5'33')	T, °C	Product Length, bp	Enzyme Check	SSCP Conditions
1a	5p13srb1	TCCTGGGTGGGCTGGCGAAGTC	63	247	*Bst* XI (200, 47)	4°C, O/N
	3p13srb1	GTTTTGGGGCGGGAGCTGATGAAG				
1b	5e1.6srb1	CCCCTGCCGCCGGAATCCTGAAG	65	162	*Bam* HI (144, 118)	RT
	3e1.6srb1	CGCTTTGGCGGAGCAGCCCATGTC	(DMSO)			
2	5e22srb1	TGGGGCCCTCATCACTCTCCTCAC	64	294	*Apa* I (189, 98, 7)	RT
	3e22srb1	GCAGCCTCCCCATCCCGTCCACT				
3	5e30srb1	ATTGCAGGCGAGTAGAAG	57	281	*Xho* I (153, 128)	4°C, 20 W, 5 h
	3e30srb1	CAGGCGGGAGGAGAGACA				
4	5e41srb1	TGGGCTCTTTGCTGTGAGGC	59	360	*Spe* I (292, 68)	4°C, 20 W, 5 h
	3e41srb1	CCAGGCTGTGTGAGGGGAAG				
5	5e50srb1	GCCCAGAATGTTCAGACCAG	57	291	*Bam* HI (157, 134)	RT
	3e50srb1	GCACCCTCTTCACGACAAAG				
6	5e60srb1	CACCTGAGAGGGCTTATTA	52	273	*Dra* II (179, 72, 22)	RT
	3e60srb1	CAAAATGCTTTCCAAGTGC				
7	5e71srb1	GCCGCCGGGTCTGGGTGTCC	59	290	*Eco* RI (184, 106)	RT
	3e71srb1	ACAGAGGCCAGAGATTAAGCAGAC				
8	5e81srb1	TTGTATGATGTCCCCTCCCT	59	261	*Hae* III (158, 103)	RT
	3e81srb1	TTCCCACCACCCCAGCCCAC				
9	5e91srb1	GGTTGACTGTGTCCCTGGAG	57	206	*Pst* I (107, 99)	RT
	3e91srb1	GGGAACACTGGAGCACTGAGC				
10	5e104srb1	GGTGGTGAGGGTTTAGTGTG	56	253	*Ava* II (148, 105)	RT
	3e104srb1	CTCCCCCCGCCTCCTGCCTC				
11	5e112srb1	AAGGTGTTGGGTGGCATCTG	60	327	*Nco* I (242, 85)	RT
	3e112srb1	GGCTCCAGGCTGCGGTTGGC				
12	5e100srb1	TTGAAGAACCGTGTAAAAC	51	303	*Pst* I (184, 119)	RT
	3e100srb1	TTGAGGCTGAAGGAATGA				

O/N indicates overnight; RT, room temperature.

Association of Common SNPs at the SR-BI Gene With Plasma Lipids and Anthropometric Parameters

The frequencies of the less-common allele (allele 2) for each of the SNPs described at the SR-BI gene locus were as follows: exon 1, 0.117; exon 3, 0.018; intron 5, 0.105; exon 8, 0.438; and intron 11, 0.043. Because the frequencies of the SNPs in exon 3 and intron 11 were low (<0.1), no further analyses were done on these. The associations between the common SNPs (exon 1, intron 5, and exon 8) and plasma lipid concentrations and BMI are presented in Table 6 for men and Table 7 for women (1/1 represents homozygosity for the most common allele, 1/2 represents heterozygosity, and

2/2 represents homozygosity for the less-common allele). Linkage disequilibrium analysis suggested that there was significant linkage disequilibrium between the intron 5 and exon 8 SNPs (x^2 P=0.003). The intron 5 allele 2 was found more often with the exon 8 allele 1, suggesting that they forma haplotype. No evidence of linkage disequilibrium was found between exon 1 and either intron 5 or exon 8.

For men, the allele 2 defined by the exon 1 SNP was associated with significantly lower mean LDL-C concentrations (125±38 mg/dL [3.23±0.98 mmol/L] and 82 mg/dL [2.12 mmol/L] for heterozygotes and the homozygote, respectively) than those observed in subjects homozygous for allele 1 (145±44 mg/dL [3.75±1.14 mmol/L]; P=0.029). Conversely, the presence of allele 2 at this SNP was associated with increased HDL-C concentrations (48±10 mg/dL [1.24±0.26 mmol/L] and 56 mg/dL [1.45 mmol/L] for heterozygotes and the homozygote, respectively) relative to the homozygotes for allele 1 (42±13 mg/dL [1.19±0.44 mmol/L]; P=0.035). Moreover, allele 2 was also associated with lower triglyceride concentrations (P=0.038). Allele 2 at the intron 5 SNP was associated in men only with reduced triglyceride concentrations (102±60 mg/dL [1.15±0.68mmol/L] versus 79±30 mg/dL [0.89±0.34 mmol/L]; P=0.017). No significant associations were observed between any of the variables examined and the exon 8 SNP in men. In women, no significant associations were noted for the exon 1 SNP; however, allele 2 at the exon 8 SNP was associated with significantly lower mean plasma LDL-C concentrations (118±38 mg/dL [3.05±0.98 mmol/L] and 116±36 mg/dL [3.00±0.93 mmol/L] for heterozygotes and homozygotes, respectively) than those observed in subjects homozygous for allele 1 (131±42 mg/dL [3.39±1.09 mmol/L]; P=0.043). No other significant associations were observed between these SNPs and other lipid variables.

TABLE 3. Anthropometric Characteristics and Plasma Lipid Concentrations of the Population Studied (Mean±SD)

	Men (n=201)	Women (n=288)	P *
Age, y	39±15	36±12	0.008
BMI, kg/m^2	25.3±2.9	22.8±3.6	<0.001
TC, mg/dL	208±49	198±45	0.026
TC, mmol/L	5.38±1.27	5.12±1.16	
LDL-C, mg/dL	142±47	122±39	<0.001
LDL-C, mmol/L	3.67±1.22	3.15±1.01	
HDL-C, mg/dL	46±17	63±17	<0.001
HDL-C, mmol/L	1.19±0.44	1.63±0.44	
TG, mg/dL	98±57	68±34	<0.001
TG, mmol/L	1.11±0.64	0.77±0.38	

*P values were obtained in the comparison of means between men and women (Student's t test for independent samples).

A significant association was observed between the intron 5 SNP and BMI. Women carriers of allele 2 showed a mean BMI value (23.8±3.8 kg/m^2) that was significantly greater (P=0.031) than that in those women homozygous for the most common allele (22.4±3.4 kg/m^2).

The association between these SNPs and plasma lipid levels and BMI was further explored using multiple regression analysis (Table 8). Regression models were examined for BMI, LDL-C, and HDL-C as dependent variables and sex, age, and specific SNPs (exon 1, intron 5, or exon 8) as independent variables. For BMI, the model including both men and women showed that sex and age were the most significant determinants of BMI (P<0.001), with the intron 5 SNP contributing significantly to this model (P=0.011). Age, sex, and the intron 5 SNP accounted for =28% of the BMI variance. Cigarettes and alcohol were consumed by most subjects, and for this reason, they were not significant determinants of the variability in this specific population and thus were excluded from the model (see Statistical Methods). When men and women were analyzed separately, the model predicted that the presence of 1 allele of the intron 5 SNP contributed positively to BMI in women (+1.09 kg/m^2) and in men (+0.45 kg/m^2), but this effect was statistically significant only in women (P=0.036 versus P=0.336 in men). For LDL-C levels, exon 8 was a significant determinant for women (-0.426 mmol/L, P=0.006 for 1/2 versus 1/1 subjects and -0.422 mmol/L, P=0.045 for 2/2 versus 1/1 subjects). The exon 1 SNP (P=0.053) was close to reaching the predetermined significance level in men. Regarding HDL-C levels, exon 1 was a significant predictor for men (+0.144 mmol/L, P=0.025) but not for women (-0.025 mmol/L, P=0.633). A significant genotypeXsex interaction was noted for this SNP (P=0.044).

TABLE 4. Locations of Polymorphisms in the Human SR-BI Gene

Polymorphism	cDNA Position	Amino Acid Position	Change	Location		
				1	2	3
Exon 1	4	2	Gly3Ser	ATG	(G/A)GC	TGC
				135	136	137

Exon 3	403	135	Val3Ile	(G/A)TC ATG CCC	
				240 241 242	
Intron 5	NA	NA	NA	CTGAGC AAGgtgagggcgagaggcgagggcccctgtcgccagggagagggagggtgggcc(c/t)g	
				350 351 352	
Exon 8	1050	350	None	GC(C/T) GAC CCG	
					419 420 421
Intron 10	NA	NA	NA	c(c/g)tgcggccccagctcatgtgtttgtcattctgtctcctcag	AGC GGG GCC

The polymorphisms are in parentheses. Introns are numbered after their corresponding exon number (eg, intron 1 is 3' of exon 1). cDNA position 1 is the first base of the initiator methionine; the numbers above the sequences refer to the amino acid number. NA indicates not applicable. Lower-case letters indicate intronic sequence.

We performed haplotype analyses using the 3 most com-mon SNPs identified at this locus (exon 1, intron 5, and exon 8). Six of the 8 possible haplotypes were identified according to the absence (1) or presence (2) of the variant allele at each of the 3 polymorphic sites. Four of the haplotypes were common: 111 (wild type), 112 (exon 8 variant), 121 (intron 5 variant), and 211 (exon 1 variant), whereas 2 of the estimated haplotypes were rare: 221 (variants at exon 1 and intron 5) and 212 (variants at exons 1 and 8). Each subject was assigned to the most plausible haplotype; however, because of the uncertainty associated with genotype assignments in double heterozygotes when studying unrelated subjects for whom the phase of the polymorphisms cannot be directly ascertained, in further analysis we used only those subjects with unequivocal haplotypes. For simplicity, 112/112 and 112/111 subjects were pooled together in these analyses (represented as 112/11X). Panel A of the Figure displays mean HDL-C differences for each genotype versus genotype 111/111 by sex. No significant genotype-related differences were noted for women; however, men with the 112/11X genotypes had lower HDL-C levels than those with the 211/111 (P=0.002) genotype, whereas those with the 211/ 111 genotype had significantly elevated HDL-C concentrations compared with 111/111 (P=0.009) and 112/11X (P=0.002) subjects. Panel B of the Figure presents mean LDL-C differences for each genotype versus genotype 111/ 111 by sex. Women with the 112/11X genotypes had significantly lower LDL-C concentrations compared with 111/111 women (P=0.008). No other differences were found to be statistically significant. For BMI, no differences were observed for men, but in women, the 121/111 genotype was associated with higher BMIs (25.0±3.3 kg/m^2) compared with 111/111 (22.8±3.2 kg/m^2, P=0.010), 112/11X (22.8±3.9 kg/m^2, P=0.004), and 121/111 (21.5±2.1 kg/m^2, P<0.01) subjects (panel C of the Figure).

TABLE 5. PCR Primers and Conditions and Restriction Enzyme Digestion to Detect Human SR-BI Gene Polymorphisms

Polymorphisms	Primers	T, °C	Digest	Product Sizes, bp
Exon 1 (G/A)	CCGGCGATGGGGCATAAAACCACT	68–62	*Alu* I	GG: 263
	CGCCCAGCACAGCGCACAGTAGC			GA: 263, 192, 71
				AA: 192, 71
Intron 5 (C/T)	GCCCAGAATGTTCAGACCAG	57	*Apa* I	CC: 194, 67, 30
	GCACCCTCTTCACGACAAAG			CT: 194, 97, 67, 30
				TT: 194, 97
Exon 8 (C/T)	CCTTGTTTCTCTCCCATCCTCACTTCCTCAAGGC	66–61	*Hae* III	CC: 154, 33, 31
	CACCACCCCAGCCCACAGCAGC			CT: 154, 64, 33, 31
				TT: 154, 64

TABLE 6. Anthropometric Characteristics and Plasma Lipid Concentrations of the Population Studied According to SR-BI Genotypes in Men (Mean±SD)

	Exon 1*				Intron 5			Exon 8			
	1/1 (n=150)	1/2 (n=30)	2/2 (n=1)	*P*†	1/1 (n=151)	1/2 (n=45)	*P*†	1/1 (n=54)	1/2 (n=104)	2/2 (n=34)	*P*†
Age, y	39±15	34±14	22	0.175	39±16	37±14	0.286	42±15	38±15	38±15	0.212
BMI, kg/m^2	25.4±3.3	25.3±2.9	23.3	0.777	25.2±3.1	25.1±3.5	0.857	25.5±3.4	25.2±3.0	24.7±3.5	0.565
TC, mg/dL	212±45‡	188±37‡	148	0.014	209±51	208±43	0.937	212±44	204±47	209±44	0.556
TC, mmol/L	5.48±1.16	4.86±0.96	3.83		5.40±1.32	5.38±1.11		5.48±1.14	5.28±1.21	5.40±1.14	
LDL-C, mg/dL	145±44§	125±38§	82	0.029	143±47	145±44	0.818	144±44	139±47	142±42	0.547
LDL-C, mmol/L	3.75±1.14	3.23±0.98	2.12		3.70±1.21	0.375±1.14		3.72±1.14	3.59±1.21	3.67±1.09	
HDL-C, mg/dL	42±13¶	48±10¶	56	0.035	46±19	48±15	0.508	45±14	46±19	48±15	0.646
HDL-C, mmol/L	1.19±0.44	1.24±0.26	1.45		1.19±0.49	1.24±0.39		1.16±0.36	1.19±0.49	1.24±0.39	
TG, mg/dL	102±57¡	75±37¡	51	0.038	102±60	79±30	0.017	98±58	96±53	94±59	0.945
TG, mmol/L	1.15±0.64	0.85±0.42	0.58		1.15±0.68	0.89±0.34		1.11±0.66	1.08±0.60	1.06±0.67	

*Tukey's tests were not performed for exon 1 because 1 group (2/2) has fewer than 2 cases.

†*P* values were obtained in the ANOVA test.

P values obtained in the Student's *t* test for 2 groups are as follows: ‡*P*=0.005; §*P*=0.013; ¶*P*=0.028; ¡*P*=0.014.

Discussion

The goals of this study were to identify genetic variants in the human SR-BI gene locus and to determine possible roles for SR-BI in lipid metabolism in humans by evaluating whether associations exist between these variants and lipid parameters in a healthy, white population. Although this receptor has been shown to play a significant role in the metabolism of HDL in mice,[15,16] lipoprotein metabolism in mice is significantly different from that in humans. Most notably, mice have HDL as their principal circulating lipoprotein, whereas humans carry a more significant proportion of their lipids within LDL and VLDL. It is precisely those high levels of circulating LDL that are thought to contribute to the higher atherosclerosis susceptibility observed in humans than in other animal species. Moreover, mice lack cholesterol ester transfer protein, a key element of reverse cholesterol transport in humans. Because SR-BI has been shown to bind LDL and VLDL in vitro, it is plausible that it may also recognize apoB-containing lipoproteins in vivo and may be a significant contributor to LDL and VLDL catabolism in humans.

Our analysis of the SR-BI gene, located in 12q24,[3] reveals that this locus is polymorphic in whites. We found evidence for significant associations between several SNPs and plasma lipids and anthropometric measures, and these associations were sex-specific. In men, we found significant associations between the exon 1 SNP and LDL-C and HDL-C levels, with allele 2 being associated with a less atherogenic lipid profile.

TABLE 7. Anthropometric Characteristics and Plasma Lipid Concentrations of the Population Studied According to SR-BI Genotypes in Women (Mean±SD)

	Exon 1*				Intron 5			Exon 8†			
	1/1 (n=181)	1/2 (n=66)	2/2 (n=1)	*P*¶	1/1 (n=216)	1/2 (n=53)	*P*¶	1/1 (n=73)	1/2 (n=148)	2/2 (n=37)	*P*¶
Age, y	35±12	38±12	40	0.218	36±12	38±12	0.333	37±12	36±12	34±12	0.458
BMI, kg/m^2	22.9±3.9	22.5±3.0	19.7	0.552	22.4±3.4	23.8±3.8	0.031	22.8±3.0	23.0±3.9	21.9±3.4	0.252
TC, mg/dL	197±45	198±42	211	0.932	198±46	204±44	0.446	206±49	196±42	192±42	0.215
TC, mmol/L	5.09±1.16	5.12±1.09	5.46		5.12±1.19	5.28±1.14		5.33±1.27	5.07±1.09	4.97±1.09	
LDL-C, mg/dL	121±39	120±38	134	0.928	122±39	125±42	0.711	131±42‡§	118±38‡§	116±36‡§	0.043
LDL-C, mmol/L	3.13±1.01	3.10±0.98	3.47		3.15±1.01	3.23±1.09		3.39±1.09	3.05±0.98	3.00±0.93	
HDL-C, mg/dL	63±17	64±19	63	0.930	62±19	64±21	0.145	61±16	64±17	64±22	0.500
HDL-C, mmol/L	1.63±0.44	1.66±0.49	1.63		1.60±0.49	1.66±0.54		1.58±0.41	1.66±0.44	1.66±0.57	
TG, mg/dL	64±28	71±37	72	0.317	68±35	64±30	0.423	67±35	70±38	57±22	0.146
TG, mmol/L	0.72±0.32	0.80±0.42	0.81		0.77±0.40	0.72±0.34		0.76±0.40	0.79±0.43	0.65±0.25	

*Tukey's tests were not performed for exon 1 because 1 group (2/2) has fewer than 2 cases.

†Tukey's tests were performed for exon 8, and *P* values were compared with *P* values obtained from Student's *t* test: ‡*P* values from Tukey's test (adjusted values): *P*=0.046 for 1/1 vs 1/2; *P*=0.141 for 1/1 vs 2/2; *P*=0.907 for 1/2 versus 2/2. §*P* values from Student's test (unadjusted values): *P*=0.021 for 1/1 vs 1/2; *P*=0.049 for 1/1 vs 2/2; *P*=0.815 for 1/2 versus 2/2.

¶*P* values were obtained in the ANOVA test.

TABLE 8. Association of Common Polymorphisms at the SR-BI Gene With BMI and Plasma Lipids: Multiple Linear Regression Analysis

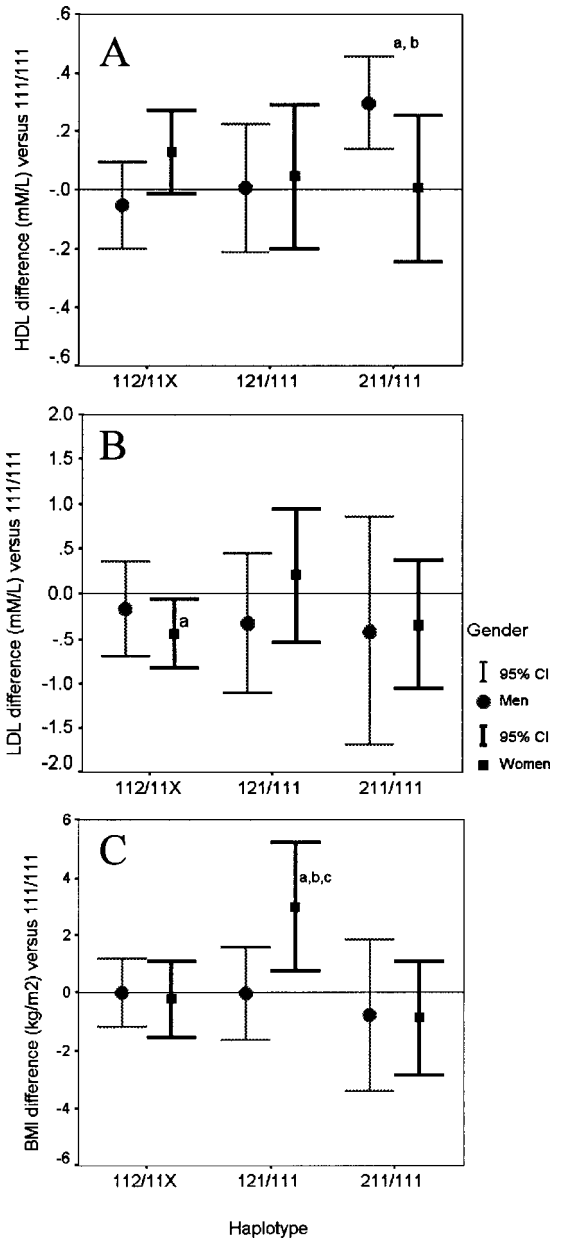
	Exon 1			Intron 5			Exon 8		
Models and Variables	B (SE)	*P*	R^2	B (SE)	*P*	R^2	B (SE)	*P*	R^2
Outcome variable: BMI									
Models for men and women									
Without interaction			0.30*			0.28*			0.29*
Sex (men vs women)	2.44 (0.29)	<0.001		2.26 (0.28)	<0.001		2.43 (0.28)	<0.001	
Age (years)	0.09 (0.12)	<0.001		0.07 (0.01)	<0.001		0.07 (0.01)	<0.001	
Genotype								0.134	
Dummy 1/2 vs 1/1	−0.12 (0.33)	0.725		0.89 (0.35)	0.011		0.22 (0.32)	0.505	
Dummy 2/2 vs 1/1							−0.56 (0.43)	0.190	
Interaction genotypeXsex		0.899	0.30*		0.220	0.28*		0.731	0.29*
Model for men†			0.29*			0.29*			0.29*
Genotype								0.328	
Dummy 1/2 vs 1/1	0.22 (0.51)	0.661		0.45 (0.46)	0.336		0.36 (0.46)	0.433	
Dummy 2/2 vs 1/1							−0.43 (0.59)	0.471	
Model for women†			0.20*			0.18*			0.19*
Genotype								0.440	
Dummy 1/2 vs 1/1	−0.52 (0.44)	0.239		1.09 (0.52)	0.036		0.02 (0.44)	0.961	
Dummy 2/2 vs 1/1							−0.65 (0.60)	0.277	
Outcome variable: LDL-C									
Models for men and women									
Without interaction			0.25*			0.24*			0.25*
Sex (men vs women)	0.409 (0.111)	<0.001		0.413 (0.127)	<0.001		0.416 (0.111)	<0.001	
Age (years)	0.028 (0.004)	<0.001		0.028 (0.004)	<0.001		0.028 (0.005)	<0.001	
BMI (kg/m^2)	0.056 (0.019)	0.003		0.058 (0.018)	<0.001		0.067 (0.002)	0.010	
Genotype								0.283	
Dummy 1/2 vs 1/1	−0.134 (0.116)	0.251		−0.016 (0.126)	0.899		−0.230 (0.142)	0.106	
Dummy 2/2 vs 1/1							−0.171 (0.130)	0.359	
Interaction genotypeXsex		0.078	0.26*		0.524	0.24*		0.360	0.26*
Model for men‡			0.17*			0.12*			0.06§
Genotype								0.872	
Dummy 1/2 vs 1/1	−0.433 (0.220)	0.053		0.054 (0.211)	0.800		−0.041 (0.243)	0.867	
Dummy 2/2 vs 1/1							0.062 (0.308)	0.841	
Model for women‡			0.19*			0.23*			0.34*
Genotype								0.034	
Dummy 1/2 vs 1/1	0.002 (0.129)	0.989		−0.085 (0.149)	0.571		−0.426 (0.153)	0.006	
Dummy 2/2 vs 1/1							−0.422 (0.208)	0.045	
Outcome variable: HDL-C									
Models for men and women									
Without interaction			0.32*			0.32*			0.31*
Sex (men vs women)	−0.309 (0.038)	<0.001		−0.338 (0.036)	<0.001		−0.344 (0.138)	<0.001	
Age (years)	0.001 (0.002)	0.839		−0.001 (0.001)	0.536		0.001 (0.002)	0.435	
BMI (kg/m^2)	−0.032 (0.006)	<0.001		−0.027 (0.006)	<0.001		−0.023 (0.006)	<0.001	
Genotype								0.495	
Dummy 1/2 vs 1/1	0.033 (0.040)	0.406		0.018 (0.006)	0.680		0.017 (0.039)	0.670	
Dummy 2/2 vs 1/1							0.062 (0.052)	0.242	
Interaction genotypeXsex		0.044	0.33*		0.904	0.32*		0.049	0.33*
Model for men‡			0.33*			0.06§			0.08§
Genotype								0.146	
Dummy 1/2 vs 1/1	0.144 (0.062)	0.025		0.023 (0.059)	0.692		−0.089 (0.057)	0.124	
Dummy 2/2 vs 1/1							0.022 (0.074)	0.762	
Model for women‡			0.08§			0.06§			0.06§
Genotype								0.180	
Dummy 1/2 vs 1/1	−0.025 (0.051)	0.633		0.012 (0.061)	0.848		0.098 (0.054)	0.070	
Dummy 2/2 vs 1/1							0.092 (0.072)	0.211	

Regression coefficients are expressed in kg/m^2 for BMI and in mmol/L for LDL-C and HDL-C.

†Models were additionally adjusted for age.

‡Models were additionally adjusted for age and BMI.

*$P<0.001$, §$P<0.05$.



A, Mean HDL-C differences (±95% CIs) between SR-BI genotypes carrying variant alleles and the wild-type genotype (111/111) by sex. a, Significantly different (P=0.009) from 111/111; b, signifi-cantly different (P=0.002) from 112/11X. For men, n=27 111/111; n=78 112/11X; n=16 121/111; and n=3 211/111. For women, n=29 111/111; n=100 112/11X; n=11 121/111; and n=12 211/111. B, Mean LDL-C differences (±95% CIs) between SR-BI genotypes carrying variant alleles and the wild-type genotype (111/111) by sex. a, Significantly different (P=0.008) from 111/111. For men, n=27 111/111; n=78 112/11X; n=16 121/111; and n=3 211/111. For women, n=29 111/111; n=100 112/11X; n=11 121/111; and n=12 211/111. C, Mean BMI differences (±95% CIs) between SR-BI genotypes carrying variant alleles and the wild-type geno-type (111/111) by sex. a, Significantly different (P=0.010) from 111/ 111; b, significantly different (P=0.004) from 112/11X; c, signifi-cantly different (P=0.003) from 211/111. For men, n=27 111/111; n=78 112/11X; n=16 121/111; and n=3211/111. For women, n=30 111/111; n=100 112/11X; n=11 121/111; and n=12 211/111.

This SNP did change the amino acid sequence, and therefore further work is warranted to examine the functionality of this mutation. Allele 2 at the intron 5 SNP was found to be associated with lower triglyceride levels, whereas no significant associations were noted for the exon 8 SNP. In women, the associations were different than those observed in men. Allele 2 at the exon 8 SNP was associated with lower LDL-C levels. This SNP, despite being within an exon, did not change the amino acid sequence and therefore, does not appear to be a functional mutation. These observations suggest that the SR-BI or a linked gene may play a role in LDL-C metabolism. Though less efficient than the LDL

receptor, SR-BI is able to mediate the degradation of LDL in vitro.[22] SR-BI may, however, play an indirect role in LDL-C metabolism in vivo by altering cholesterol homeostasis through its interaction with HDL and VLDL. Moreover, it has been shown that liver overexpression of SR-BI results in stimulation of excretion of cholesterol into the bile and suppresses the percentage of dietary cholesterol absorption.[23] All of these data suggest that the SR-BI is an important candidate gene in terms of cholesterol metabolism. However, we did not find any linked functional mutation in the SR-BI gene in carriers of the exon 8 SNP. This SNP could be in linkage disequilibrium with a functional mutation at a neigh-boring relevant locus. Several other candidate genes involved in lipid metabolism are localized in the 12q24 chromosomal region (ie, ACACB, PLA2, CLTA, MVK, ACADS, and TCF1). Moreover, the SR-BI gene has been assigned to mouse chromosome 5, in a region homologous with human chromosome 12 harboring the SR-BI locus. Several of the previously indicated candidate genes related to lipids have been found in the mouse homologous region (MVK, ACADS, and TCF1),[24] and more careful analysis of these loci appears to be warranted.

Additional analysis with the common SNPs revealed an association between the intron 5 polymorphism and BMI in women only. The finding was most significant in premenopausal women (not shown). None of the subjects had morbid obesity (BMI >40), and thus, this effect was observed in individuals within the common weight range. Some estimates suggest that 40% to 70% of the variation in obesity-related phenotypes in humans is heritable and probably the result of the interaction of multiple genes. Consequently, the effect of each single gene will be rather limited, making the search for obesity genes in humans especially challenging. Several common polymorphisms have been associated with BMI values in humans.[25–27] Moreover, evidence for linkage has been demonstrated for the following chromosomal regions: 1p, 6p (TNF-a), 7q (OB), 11q, and 20q (ADA and MC3R). An increased BMI has been associated with higher mortality from all causes and from cardiovascular disease in particular. For mortal-ity from cardiovascular disease, the relative risk associated with an increment of 1 in BMI in women in the age range of 30 to 44 years has been reported to be 1.08 (95% CI, 1.05 to 1.11).[28,29] Our data show that for an average woman, the presence of the 121 haplotype raises the BMI by =2.2 kg/m^2, which corresponds to =6 kg in body weight. This increase in BMI could result in an increase in coronary heart disease mortality of =17.6%, primarily due to a greater risk of developing non–insulin-dependent diabetes mellitus,[28,29] a major risk factor for coronary artery atherosclerosis.

It is not clear whether the SR-BI gene itself plays a role in weight control or whether a neighboring gene could be the cause of these observations. Sequencing of the entire coding portion in 3 individuals with the intron 5 polymorphism and 3 control subjects did not reveal a possible functional mutation in linkage with the SNP (not shown). Other genes in the chromosome 12q24 region are good candidates to be involved in the regulation of BMI and lipids, some of them acting through diabetic phenotypes. These are MODY3, NIDDM2, and ACACB. The lattermost is especially interest-ing, given the fact that ACACB may be involved in the regulation of fatty acid oxidation, thus affecting both lipid and energy metabolism.[30] It should be noted that the BMI, HDL-C, and LDL-C associations were found within different haplotypes. This result would suggest that there may be functional mutations linked separately to the SNPs in exon 1, intron 5, and exon 8. Linkage disequilibrium analysis would support this concept. The exon 1 SNP is in linkage equilib-rium with the other 2 SNPs; therefore, one would not expect to find similar associations for this SNP and those in intron 5 and exon 8. Linkage disequilibrium was detected between allele 2 of intron 5 and allele 1 of exon 8. Consequently, we would not expect to find similar patterns of associations for these SNPs.

SR-BI mRNA is expressed in adipocytes and adipose tissue, where SR-BI might mediate the uptake of lipids into those cells; however, steady-state levels of SR-BI protein are not high in adipose tissue.[1,6] In addition, SR-BI expression in adrenal cells is thought to be important for the uptake of cholesterol for glucocorticoid synthesis,[11–13] and excess production of glucocorticoids can lead to glucose intolerance, lipid alterations, and increased BMI.[31] Because the associa-ion with BMI was most evident in premenopausal women, there may be hormonal regulation of the SR-BI gene in humans. Indeed, estrogen (albeit at nonphysiological doses) causes SR-BI expression to be significantly reduced in the liver and dramatically increased in the adrenal glands of male rats.[13]

This study is the first to demonstrate genetic variation at this gene locus and a possible role for SR-BI in humans. Our data suggest that future studies should focus on LDL metabolism and weight regulation, in addition to HDL lipid metabolism. Moreover, these associations are sex-specific, suggesting that the role of SR-BI in steroidogenesis and its hormonal regulation, as demonstrated in animal models, could also be relevant in humans. However, with this sample size, we cannot reject the possibility that some of the sex differences observed could be due to type II errors. We do not know whether the polymorphisms and frequencies reported will be similar in other geographic areas and ethnic back-grounds. Moreover, the associations observed in this study were derived from a normal population in southern Europe. It remains to be determined whether these associations can be replicated in other populations with different environmental factors.

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