Variants in Scavenger Receptor Class B Type I Gene Are Associated with HDL Cholesterol Levels in Younger Women

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Abstract

Objective: Variants within the scavenger receptor class B type I (SCARB1) receptor gene have been previously associated with lipid levels, especially in women, with some studies reporting the association to be stronger in the presence of diabetes or post-menopausal estrogen use. Based on the reported gender-specific association and modification effect of estrogen on lipid levels according to SCARB1 variants, we explored the relationship between SCARBI single nucleotide polymorphisms (SNPs) and lipid levels in an Amish population to assess sex and age differences. **Methods:** Eight SCARB1 SNPs, identified from public databases, were genotyped in 919 subjects. **Results:** Rs5888 and rs3782287 were in high linkage disequilibrium (LD), with $r^2 > 0.8$. None of the SNPs were significantly associated with lipid levels in men; however in women, rs5888 (p = 0.04) and rs5891 (p < 0.001) were significantly associated with higher HDL-C levels. Rs5891 had an allele frequency of 3% and predicts a mis- sense mutation (Ile135Val), which may be functional. Moreover, rs3782287 (p = 0.023) and rs5888 (p = 0.003) were significantly associated with higher HDL-C levels in women younger than 50 years but not in women aged 50 years or older (p for interaction between age and rs5888 = 0.045). None of the SNP effects on HDL-C were modified in the presence of diabetes, in either men or women. **Conclusions:** SCARB1 SNPs influence HDL-C levels in women, particularly in those less than 50 years old. **Condensed Abstract:** We assessed associations between SCARB1 SNPs and lipid traits in 919 Amish men and women. Two SNPs, rs3782287 and rs5888, were significantly associated with higher HDL-C levels in women younger than 50 years but not in women aged 50 years or older, supporting an interaction between common sequence variants in SCARB1 and estrogen on HDL-C.

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The metabolism of high-density lipoprotein (HDL) is complex, with many factors influencing its circulating plasma levels. One such factor is the scavenger receptor, class B, type I (SR-BI), first characterized by Acton et al. [1], and subsequently shown to be a physiologically relevant receptor that exerts a major influence on HDL cho-

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lesterol (HDL-C) levels in rodents [2]. It is highly expressed in the liver and in steroidogenic tissues [1, 3], where its primary function is to mediate the uptake of cholesteryl esters from the HDL particle core.

Much less is known regarding the role of SR-BI in humans, although recent studies have shown some variants in the *SR-BI gene (SCARB1)* to be associated with lipid levels, lipoprotein particle size and body mass index (BMI) [4–8]. *SCARB1* variants have also been studied in populations characterized with type 2 diabetes mellitus (T2DM) [5, 9] and, interestingly, a *SCARB1* variant in exon 1 was found to associate with differences in insulin sensitivity in healthy people during the consumption of an olive oil-rich diet [10]. In addition, a SCARB1 variant was studied in older women using hormone replacement therapy [11]. Osgood et al. [9] examined *SCARB1* gene variants in the participants of the Framingham Study, and reported that three common single nucleotide polymorphisms (SNPs) rs4238001 (exon 1, +4 G → A), rs5888 (exon 8, +63519 C → T) and an intron 5 (+51905 C → T) SNP were associated with variation in plasma lipoprotein concentrations and particle size, particularly in subjects with T2DM. While there was no association of SNPs with T2DM, the G allele in rs4238001 was significantly associated with lower HDL-C levels and smaller HDL particle sizes, although only among subjects with T2DM. No interaction was observed for the rs5888 SNP with T2DM, whereas the interaction between the intron 5 SNP and T2DM on HDL metabolism was seen only in men.

Gender-specific associations have been reported between some of the *SCARB1* variants and lipid levels, an observation that led Richard et al. [11] to examine the effect of exogenous estrogen on HDL-C levels by *SCARB1* variants in elderly women in the Rancho Bernardo Study. These investigators found no significant associations between either the intron 5 SNP or rs5888 and HDL-C levels after adjusting for age, BMI, alcohol, smoking, triglycerides, fasting plasma glucose and estrogen use. However, they did report significant estrogen interaction with the rs5888 SNP on HDL-C levels, with HDL-C levels significantly higher in estrogen users than non-users [11].

Motivated by these previous observations, we assessed the relationship of eight different *SCARB1* variants, including the previously studied rs4238001, intron 5 (+51905 C → T), and rs5888 variants, with lipid levels in adult men and women participants of the Amish Family Diabetes Study (AFDS) and to determine if associations were modified by the effects of age, gender and/or T2DM.

Methods

Study Population

The Old Order Amish (OOA) is a genetically homogenous population of Central European ancestry. The original founders of this population immigrated to eastern Pennsylvania (now Lancaster County, Pa., USA) in the mid to late 1700s, with the Lancaster County population now consisting of approximately 30,000 individuals [12]. The OOA today are still characterized by their social cohesiveness and rural lifestyle [13]. In 1995, AFDS was begun to identify susceptibility genes for T2DM and related traits. Probands with T2DM onset between 35 and 65 years of age were identified and all willing first-degree family members 18 years of age and older were invited to participate. First-degree relatives of any family members subsequently diagnosed with T2DM were also invited to participate. A total of 919 subjects with lipid data and DNA available for genotyping were included in this study.

Participating subjects were examined at the Amish Research Clinic in Strasburg, PA following a 12-hour overnight fast. Height and weight were measured and body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared (kg/m^2). Fasting lipid profiles [total cholesterol (TC), HDL-C, triglycerides (TG)] were performed by Quest Diagnostics (Baltimore, MD). Intra-assays CVs of duplicate samples ranged between 0.1 and 3.0%, and inter-assay CVs ranged between 0.2 and 5%. Low-density lipoprotein cholesterol (LDL-C) concentrations were estimated using Friedewald's formula [14].

A 75 g oral glucose tolerance test (OGTT) was administered, and diabetes mellitus was classified based on the American Diabetes Association plasma glucose criteria [15] of a fasting plasma glucose level (≥ 7 mmol/l) or a 2-hour OGTT plasma glucose level (≥ 11.1 mmol/l). Subjects were also considered to have diabetes if they reported current use of insulin or oral glucose-lowering agents. Subjects were excluded from these analyses if they had a diagnosis of diabetes before the age of 35 years and reported current use of insulin (n = 1). Impaired glucose tolerance was diagnosed based on OGTT plasma glucose levels (2-hour OGTT plasma glucose level ≥ 7.8 but <11.1 mmol/l). Normal glucose tolerance was defined as fasting plasma glucose level (<6.1 mmol/l) and 2-hour OGTT plasma glucose level (<7.8 mmol/l).

Informed consent was obtained from all AFDS participants, and the study protocol was approved by the Institutional Review Board at the University of Maryland School of Medicine. Details of the study design have been previously described [13].

Genotyping

The *SCARB1* gene is 93.2 kb in length and consists of 13 exons. We selected SNPs for genotyping within the coding and intronic regions of *SCARB1* from public databases (NIH dbSNP, Wellcome Trust Sanger Institute and Applied Biosystems) that were validated by frequency (minor allele frequency $\geq 1\%$) as well as SNPs reported in published articles [4–8, 16]. SNPs were genotyped using the SNPstream UHT Genotyping System (Beckman Coulter, Fullerton, CA) [17]. Eight of the eighteen SNPs genotyped were polymorphic in this sample: rs3782287, rs5888, rs5890, rs5891, rs838884, rs10846760, rs10846745, rs11057851 and the intron 5 (+51905 C → T) SNP. Error rates based upon blind replicates were 0–4.7%.

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Table 1. Characteristics (mean ± SD, %) of the Amish Family Diabetes Study cohort

	Men	Women	Age-adjusted p
n	428	491	
Age	46.8 ± 15.8	47.1 ± 16.2	0.29
BMI, kg/m^2	26.3 ± 4.0	27.9 ± 5.6	<0.0001
Diabetes	9.8%	14.2%	0.043
Impaired glucose tolerance	10.5%	19.4%	0.0002
Total cholesterol, mg/dl	213.2 ± 48.6	214.2 ± 48.9	0.86
LDL-C, mg/dl	149.6 ± 44.0	143.0 ± 43.7	0.013
HDL-C, mg/dl	46.9 ± 12.7	53.9 ± 13.0	<0.0001
Triglycerides, mg/dl	84.4 ± 57.5	86.5 ± 59.2	0.66
Lipid-lowering medications, %	1.9%	1.2%	0.42
Estrogen users, %	NA	0.4%	NA
Oral hypoglycemic agents, %	1.9%	3.0%	0.25
Insulin therapy, %	0.9%	1.0%	0.90

NA = not applicable.

Statistical Analysis

Genotypes were checked for Mendelian consistency using the PedCheck software program in the extended Amish pedigree [18]. A small number of Mendelian errors were resolved or removed prior to analysis. The distribution of all SNP genotypes conformed to those expected under Hardy-Weinberg equilibrium using the χ^2 test.

Association analyses were performed using a variance components methodology implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software program, which allowed us to account for the relatedness of study subjects [19]. Briefly, we evaluated the effects of SNP genotype on HDL levels, while simultaneously adjusting for the effects of age, age^2, sex, BMI, and diabetes status. We accounted for the non-independence among family members by modeling the residual correlations in HDL levels between relative pairs explicitly as a function of the kinship matrix. Our primary analysis was based on an additive genetic model, which implies a dose-response relationship between number of 'risk' alleles and HDL level. Genotypes were thus assigned values of 0, 1, or 2, corresponding to the number of risk alleles. Subjects currently taking lipid-lowering medications (n = 14) were excluded from the analysis. The significance of the genotype effects was assessed using the likelihood ratio test, in which we compared the likelihood of the data under a model in which the genotype effect was estimated against the likelihood of a nested model in which the genotype effect was constrained to zero.

Based upon our a priori hypothesis regarding an interaction between *SCARB1* genotype and age or sex, genotype-phenotype associations were estimated in men and women (and older and younger women) separately. Interaction terms (e.g., genotype by sex, genotype by diabetes status, and genotype by age) were included in the model to assess whether the magnitude of the genotype effect on HDL levels differed by group.

Given negligible LD between SNPs (except rs5888 and rs3782287), haplotypes could not be reliably assigned, and thus haplotype association analysis was not performed.

Results

The 919 study subjects included 103 T2DM and 816 non-T2DM individuals from 226 families, ranging in size from 1 to 169. The characteristics of the study cohort are summarized in table 1. Overall, there were slightly more women than men (53 vs. 47%). Women had higher BMI (27.9 vs. 26.3 kg/m^2, p < 0.0001), higher prevalence of T2DM (14.2 vs. 9.8%, p < 0.043), higher mean HDL-C levels (53.9 vs. 46.9 mg/dl, p < 0.0001) and lower mean LDL-C levels (143.0 vs. 149.6 mg/dl, p < 0.013). The use of prescription medications such as lipid-lowering agents and hormone replacement therapy is low in the Amish. The frequency of cigarette smoking is also low in the Amish. Physical activity levels in the Old Order Amish are typically high, especially compared to non-Amish Caucasians. The Amish diet is generally high in calories and fat.

We next examined the associations between *SCARB1* SNPs and lipid levels stratified by sex. In these analyses, we adjusted for age, age^2, BMI, diabetes status, family structure and excluded those using lipid-lowering medications. None of the *SCARB1* SNPs were associated with TC, HDL-C, LDL-C, or TG levels in men (data not shown). However, in women, rs5888 (p = 0.04) and rs5891 (p < 0.001) were significantly associated with higher HDL-C levels (fig. 1). The genotype × gender interaction was significant for rs5888 using a dominant model (p < 0.005), whereas no significant interaction was found for rs5891. As the Rancho Bernardo study [11] suggested an interaction between estrogen use and rs5888 SNP genotypes on HDL-C and because estrogen levels are related to age, we examined as-
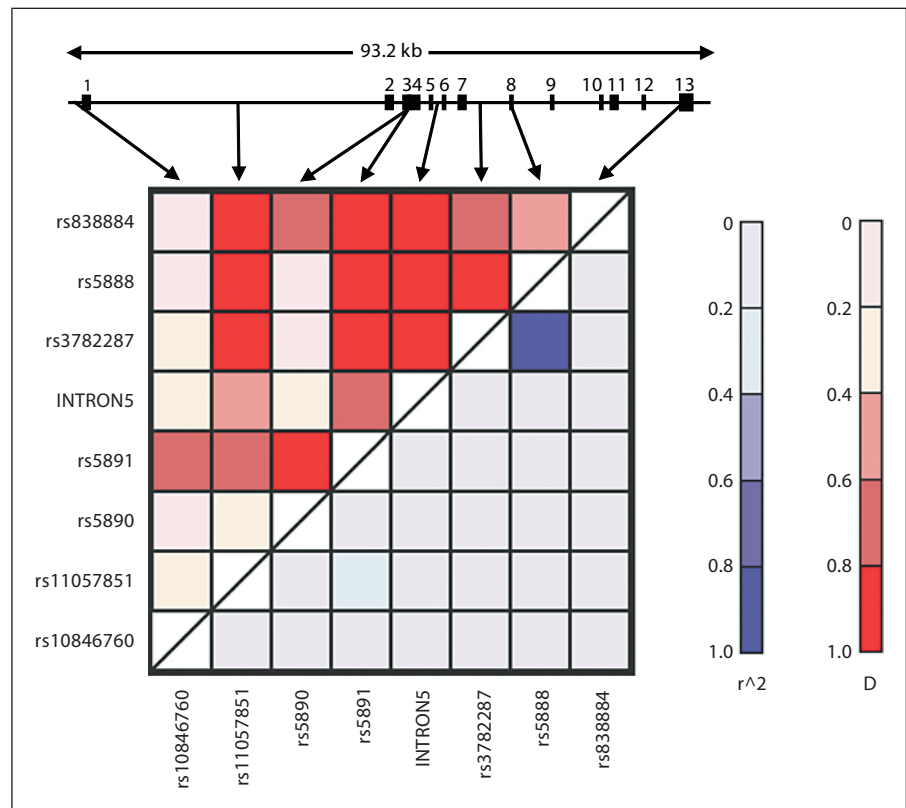
Fig. 1. Gene structure and pairwise LD among SNPs in *SCARB1*. The upper portion of the figure shows the gene structure and location of polymorphisms. The lower portion of the figure shows a schematic pairwise LD, calculated as D′ and r² among the polymorphic SNPs in the Amish. The lines connect each SNP name and the position with the corresponding cell in the LD matrix. Magnitude and significance of the D′ and r² is illustrated by shading with a red to white or a blue to white gradient reflecting higher to lower LD values.

Table 2. Mean HDL-C levels (SE) according to SCARB1 SNP genotype in women ≥50 and <50 years of age

SNP name	MAF	Women ≥50 years (n = 192): Genotype				Women <50 years (n = 293): Genotype			
		11	12	22	p*	11	12	22	p*
rs10846760 (C/T)	0.25	54.4 (6.4)	55.6 (6.4)	60.9 (6.4)	0.34	53.6 (4.4)	53.7 (4.4)	62.4 (4.4)	0.33
rs11057851 (G/A)	0.08	55.1 (1.5)	59.4 (2.7)	45.2 (12.3)	0.25	53.5 (1.2)	53.3 (2.2)		0.92
rs5890 (C/T)	0.003	54.9 (12.5)	63.4 (12.4)		0.50	53.5 (7.0)	44.1 (7.1)		0.19
rs5891 (G/A)	0.03	54.6 (1.4)	65.5 (4.2)	64.8 (12.1)	**0.01**	53.0 (1.2)	61.1 (3.1)		**0.01**
Intron5 (T/C)	0.05	55.5 (1.4)	54.2 (2.9)		0.66	54.4 (1.2)	54.1 (2.7)		0.89
rs3782287 (A/G)	0.41	54.3 (2.8)	57.1 (2.6)	52.5 (2.6)	0.84	50.2 (2.4)	54.2 (2.2)	54.9 (2.1)	**0.02**
rs5888 (C/T)	0.41	53.3 (2.7)	56.9 (2.5)	52.5 (2.5)	0.89	49.6 (2.4)	54.4 (2.1)	56.1 (2.0)	**0.003**
rs838884 (T/C)	0.23	56.2 (1.6)	54.7 (1.9)	55.0 (4.3)	0.86	54.7 (1.3)	55.8 (1.6)	45.8 (3.7)	0.72

   * p is for additive genetic model, adjusted for age, BMI, family structure, diabetes, and excluded lipid medications. Significant p values are printed in bold.

sociations between *SCARB1* SNPs and HDL-C levels in women older and younger than age 50 years. As shown in table 2, women less than 50 years of age who carried the rs5888 (p < 0.003) or rs3782287 (p < 0.023) minor allele had significantly higher HDL-C levels compared with women homozygous for the major allele (HDL-C levels ap-

proximately 13 and 9% higher, respectively). In women greater than 50 years of age, these two SNPs were not associated with HDL-C levels. The genotype × age group interaction term was statistically significant for rs5888 genotypes (p = 0.045). By contrast, rs5891, which encodes a missense mutation (Ile135Val), was associated with HDL-

C levels in women from both age groups, although this SNP was uncommon (3%) in this population. None of the SNPs were associated with diabetes, in either men or women, nor were the effects of any SNP on HDL-C levels modified in the presence of diabetes. Excluding subjects with diabetes did not appreciably change the HDL-C association results (data not shown).

A description of the gene structure showing SNP locations and pairwise LD between SNPs is provided as figure 2. Rs5888 (exon 8; silent) and rs3782287 (intron 7), both of which were associated with HDL-C levels in women younger than 50 years old, are located 4.5 kb from each other and are in high LD with each other. By contrast, rs5891 (exon 3; Ile135Val), which was also associated with HDL-C levels, is rarer and not linked to the other two HDL-C-associated SNPs, suggesting that its association with HDL-C levels is independent of the other two SNPs. This was supported by the fact that the association of rs5891 with HDL-C levels remained statistically significant even after adjusting for the effects of the other two SNPs in either total women or women of each age group. The opposite analyses were done on rs5888 or rs3782287 while adjusting for rs5891. Significant association between rs5888 or rs3782287 and HDL-C remained in total women and women younger than 50 years old (data not shown). Because of the relative absence of LD between all pairs of SNPs ($r^2 \leq 0.20$), with the exception of SNPs rs5888 and rs3782287, which were in very high LD ($r^2 = 0.86$), no haplotype association analyses were performed.

Fig. 2. Adjusted HDL-C levels in men and women according to rs5888 and rs5891 genotype. Mean HDL-C levels for men and women according to rs5888 and rs5891 genotype are shown. The p value for women is statistically significant for trend while the p value for men is not significant. There is a significant gene × gender interaction for rs5888 ($p < 0.005$).

Discussion

We have observed several *SCARB1* polymorphisms to be associated with HDL-C levels in Amish women, but not in men. Furthermore, the associations were stronger in younger as compared to older women. Because Amish women, at least in Lancaster County, rarely take estrogen (0.41% in our cohort), the stratification of women according to those younger and older than age 50 years is a proxy for examining the interaction of estrogen on lipid levels according to *SCARB1* genotype. Our results are consistent with Richard et al. [11] in that HDL-C levels were significantly higher in Amish women younger than 50 years of age (presumably pre-menopausal and having adequate endogenous estrogen levels) who carried the rs5888 variant, suggesting an important interaction between estrogen and SR-BI. In addition, we found significantly higher HDL-C in younger female carriers of the minor allele of rs3782287. However, unlike the Rancho
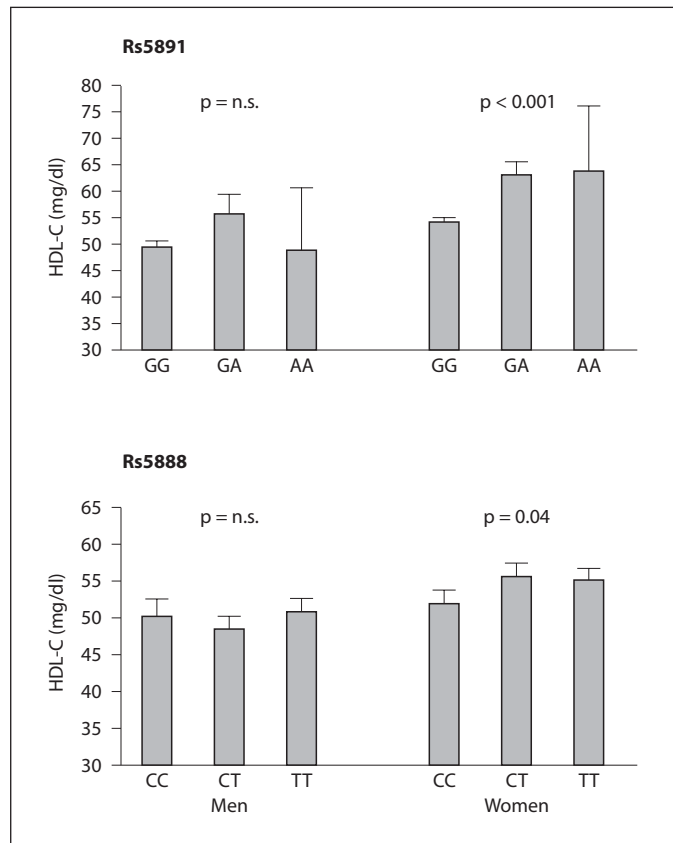
Bernardo population, we did not find a difference in LDL-C levels in the Old Order Amish women according to age and *SCARB1* genotype.

Osgood et al. [9] did not show a sex difference between lipid levels and the rs5888 SNP. After multivariate adjustments, they showed that men homozygous for the minor allele had significantly higher total HDL-C, higher HDL$_3$-C levels and larger HDL particle size compared with men homozygous for the major allele. However, in women, while there were no apparent differences in HDL-C levels between minor allele homozygotes and major allele homozygotes, LDL-C levels were lower and HDL particle size was larger in the minor allele homozygotes [9]. Acton et al. [4] also did not find significant as-

sociations between lipid levels and the rs5888 SNP in men in a population sampled from Zaragoza, Spain. While they also did not find significant associations between HDL-C levels and rs5888 variant in women, they reported that women carrying the minor allele had significantly lower LDL-C levels compared to women homozygous for the major allele. Other groups examining the rs5888 SNP have also reported association of this SNP with lipid levels [5, 6]. Thus, our findings, coupled with reports in the literature, provide evidence for replicated association of rs5888 with lipid levels, although there appear to be inconsistencies with respect to which lipid traits.

In our population we did not find associations between SCARB1 SNPs and triglycerides (p ranges 0.08–0.8). Only one SCARB1 SNP was significantly associated with BMI (rs5891, p = 0.04). Relationships between BMI, triglycerides, and SCARB1 SNPs have been found in a Spanish population [4], and relationships between triglycerides and the SCARB1 intron 5 SNP have been described in an obese population undergoing gastric bypass surgery [20]. These differences may be due to the different populations studied.

The relationship between SCARB1 and estrogen may have biological relevance given that in animals estrogen regulates the expression of SR-BI and its isoform SR-BII [21, 22], and that estrogen response elements are found within the SCARB1 promoter [23, 24]. In humans, we have shown that women undergoing oocyte retrieval for in vitro fertilization who have low estradiol levels are also low expressors of SR-BI mRNA [25]. It is possible that rs5888 or rs3782287 may be in linkage disequilibrium with a functional variant in estrogen response element in SCARB1 such that carriers of variant alleles for SCARB1 may have altered expression of SR-BI that becomes apparent only in the setting of estrogen. Interestingly, rs5891, which encodes a missense mutation (Ile135Val), is associated with HDL-C levels in both age groups. Although rare, it is possible that the effect of this potentially functional variant is strong enough to be independent of estrogen status.

The limitations of our study merit further discussion. The OOA are a closed founder population with relatively homogeneous environmental factors and fewer confounders of disease and related phenotypes (e.g., low rates of smoking, alcohol consumption, and medication usage) [13]. Although this homogenous lifestyle might enhance our ability to discern genetic influences on disease and related traits, the ability to generalize our findings will require further study in the general population. In addition, the high fat diet of the OOA (approximately 36%

saturated fat (Shuldiner, in preparation)) may also contribute to an interaction between diet, genotype and lipid levels. Perez-Martinez explored the effect of dietary fat content (saturated fat diet, carbohydrate-rich diet and a monounsaturated olive oil diet) on lipid levels in subjects stratified by the SCARB1 exon 1 SNP genotype. Subjects consuming a saturated fat diet and expressing the minor allele in exon 1 had significantly higher LDL-C levels compared to subjects homozygous for the major allele [26]. This suggests the possibility that dietary fat might also have an important interaction with other SCARB1 variants. Furthermore, Spady et al. [27] have previously shown that polyunsaturated fatty acids up-regulate SR-BI in the hamster.

It should be noted that we reported nominal p values in our statistical analysis instead of p values adjusted for multiple comparisons. However, since a major motivation of our study was to determine if observed associations between SCARB1 SNPs and HDL-C levels were influenced by age and gender given prior speculations that genetic effects at this locus might be estrogen sensitive, the comparisons we considered were not wholly independent. Nonetheless, we could not exclude the possibility of a false-positive association and we need to be cautious in our interpretation of the results.

In summary, we have shown that some SCARB1 variants are significantly associated with HDL-C levels in participants of the AFDS. One of these variants encodes a relatively rare conservative missense mutation and thus may be functional. Interestingly, there was a significant age or estrogen interaction with two more common noncoding SCARB1 variants. The clinical relevance of these associations and the mechanism by which these variants influence HDL-C levels, especially in younger women, warrants further study.

Acknowledgments

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