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***LIPC* variants in the promoter and intron 1 modify HDL-C levels in a sex-specific fashion**

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Abstract

We previously reported linkage for plasma levels of high-density lipoprotein cholesterol (HDL-C) on 15q21 in Caucasian families from the National Heart, Lung, and Blood Institute Family Heart Study (NHLBI FHS). Hepatic lipase gene (*LIPC*), which has a major role in lipoprotein metabolism, resides within the linkage region and constitutes an obvious candidate gene. While hepatic lipase is a known player in HDL metabolism, the relationship between common *LIPC* variants and HDL-C levels remains unclear. In the current study, we employed population-based and family-based tests of association with both quantitative HDL-C levels and a dichotomous dyslipidemia trait (affected men: HDL < 40 mg/dL and women: HDL < 50 mg/dL, denoted as low HDL). We genotyped 19 tag-SNPs spanning 139.9 kb around the *LIPC* in the 591 families (2238 subjects). Strong association in a proxy-promoter 5' SNP (rs261342) and HDL-C levels was detected in women, but not in men. The less common allele was associated with an increase of ~14% in HDL-C levels, and a decrease of ~30% in risk of low HDL. In addition, strong association in women of an intron 1 SNP (rs12593008) and low HDL and moderate association in men (rs8028759) with both HDL-C levels and low HDL phenotype were found and may represent either functional single nucleotide polymorphisms (SNPs), or more likely, SNPs in linkage disequilibrium with functional variants. Because of the association of lipid abnormalities with diabetes, and other lifestyle parameters, we also performed association analyses using different covariate adjustments as well as strategically selected sub-samples. The sex-specific association of rs261342, rs12593008 or rs8028759 remained substantially the same through these analyses. Finally, we found that a common haplotype was overtransmitted to off-spring with low HDL-C. The sex-specific associations found in our study could be due to the interactions with the endogenous hormonal environment, lifestyle and/or genetic factors, although the underlying physiologic mechanisms are not understood.

Keywords

Lipids; Cardiovascular disease; Diabetes; BMI; Polymorphism; Hepatic lipase

1. Introduction

Epidemiologic studies have shown a strong inverse association between high-density lipoprotein cholesterol (HDL-C) levels and cardiovascular mortality [1]. Low plasma levels of HDL-C belong to a clustering of cardiovascular risk factors involved in the metabolic syndrome [2] as defined by a dichotomous dyslipidemia trait with sex-specific thresholds (affected men: HDL < 40 mg/dL and women: HDL < 50 mg/dL, [3]). It is likely the genetic and environmental effects, as well as their interactions, influence the variation of HDL-C levels. Evidence of quantitative trait loci have been reported for HDL-C levels in several chromosome regions [4]. Our previous study showed strong evidence for linkage (LOD > 4.5) on 15q21 for HDL-C in non-diabetic Caucasian families from the NHLBI FHS [5]. A prominent candidate gene residing within the linkage region on 15q21 is hepatic lipase (*LIPC*), which has a known major role in lipoprotein metabolism. *LIPC* spans 136.9 kb and is composed of nine exons and eight introns (NC_000015.8, [6]).

The hepatic lipase enzyme (HL) is synthesized and secreted by the liver and has several metabolic functions including the hydrolysis of triglycerides, the lypolysis of phospholipids, the modeling of small, dense atherogenic LDL particles, and the catabolism of HDL, which are all implicated in atherosclerosis [7]. Studies of common variation in the *LIPC* promoter have demonstrated four polymorphisms in complete linkage disequilibrium [8,9]. The less common allele (T of -514C/T) has been shown to be associated with reduced HL activity and elevated HDL-C levels [10–12]. The T allele has been reported to account for up to 25% of the variability in HDL-C levels [13].

Several studies have suggested interactions between the *LIPC* -514 polymorphism and lifestyle and/or genetic factors in determining HDL-C levels. Some of these factors include: sex [14–17], smoking [16], abdominal fat and obesity [6,15,17,18], diabetes [7], physical activity [19,20], and dietary fat intake [18,21,22]. Nevertheless, how these factors influence plasma HDL-C levels in a genotype-specific fashion remains unclear.

The association of HDL-C and *LIPC* gene has been studied with regard to four promoter (-514C/T, -710T/C, -250G/A, -763A/G) polymorphisms ([7,10,18,22] among others) and a few intronic single nucleotide polymorphisms [12]. However, a systematic study of the common variation across the gene and its effect on HDL-C levels has not been reported. Here, we selected “tag” SNPs that mark haplotype blocks within *LIPC* and explore their association with HDL-C and their modification by other measured environmental exposures in a subset of Caucasian families from the NHLBI FHS.

2. Material and methods

The NHLBI FHS is a multi-center, population-based genetic epidemiologic study whose aims are to investigate factors influencing risk for coronary heart disease. The NHLBI FHS data is comprised of families selected through individuals who had participated in the Framingham Heart Study (Framingham, MA), the Utah Health Family Tree Study (Salt Lake City, UT), or the Atherosclerosis Risk in Communities Study (Minneapolis, MN, and Forsyth County, NC). A detailed description of NHLBI FHS has been reported elsewhere [23]. We studied a NHLBI FHS sub-sample of 591 white families (2238 subjects), which were previously selected with the highest pedigree-specific LOD scores supporting linkage on chromosomes 7 and 13 for body mass index (BMI) [24,25] and chromosome 4 for pulmonary function [26]. Thus, this sub-sample of families is not ascertained on HDL-C levels. The multipoint LOD for age-sex-adjusted HDL-C on chromosome 15q21 was 2.2 as compared with 1.8 in the entire sample of families.

Fasting plasma HDL-C levels were measured after precipitation of other lipoprotein fractions by dextran sulfate [27]. Body mass index was calculated as weight (in kilograms) divided by the square of height (in meters). Information on current smoking cigarettes (0 = No, 1 = Yes) and on habitual alcohol intake (0 = No, 1 = Yes) were obtained from questionnaire. The covariate adjustments were carried out separately by sex, using stepwise multiple regression and retaining the terms that were significant at the 5% level [5]. HDLC levels were adjusted for the effects of a polynomial in age in third degree (age, age2, age3) and field centers in the first adjustment. Self reported use of cholesterol-lowering medications was added to covariate list in a second regression model. We also analyzed HDL-C with a progressive adjustment, adding BMI, smoking and alcohol intake together with age and field center, within sex groups, in the third adjustment. We also studied a dichotomous trait, denominated as “low HDL”, which was based on the National Cholesterol Education Program Adult Treatment Panel III [3]. The cutoff to define affected subjects for lowHDL was HDL-C < 40 mg/dL in men and HDL-C < 50 mg/dL in women.

SNP information was extracted from the International HapMap Project and Illumina Inc., San Diego, CA, USA. Twenty-one *LIPC* tag SNPs, covering 139.9 kb (from 56,508,466 bp to 56,648,363 bp, that included 3 kbp in the 3' upstream of *LIPC*), were chosen from the CEU Hapmap population (Phase II, build 35) by using a pair-wise linkage disequilibrium (r^2) threshold of 0.50 and minimum minor allelic frequencies (maf) of 0.20, using the Tagger algorithm as implemented in HapMap. Four of 21 SNPs presented poor success rates (<50%) in Illumina platform and were replaced by two SNPs that showed high linkage disequilibrium (LD) with them (4 SNPs). Genomic DNA samples were prepared from peripheral blood using the PureGene kit (Gentra, Minneapolis, Minnesota) and purified using QIAEX II kits (Qiagen). The 19 tag SNPs were genotyped using the Illumina GoldenGate assay method, through the Illumina Fast-Track Genotyping service. Quality control of the genotype data generated on Illumina panel was assessed with checks on Mendelian inconsistencies and Hardy–Weinberg equilibrium.

We used both linear models and family-based association analyses. For the regression approach, we tested the association of dichotomous low HDL and quantitative HDL-C levels with the *LIPC* genotypes by use of additive and dominant models in generalized estimating equations (GEE, using SAS package) to account for correlations among family members. In the family-based analysis, we performed a transmission/disequilibrium test (TDT) of association with the low HDL phenotype by using TRANSMIT version 2.5.4 [28]. Empirical p values are reported from 1000 bootstrap replicates. Another TDT that allows for founder heterogeneity using sequential peeling was also used to assess haplotypes overtransmission (SP-TDT, [29]). SP-TDT has been shown to have higher power compared with other haplotype similarity based TDT methods (WT-TDT and HS-TDT, *cf.* [29]). The global p value represents the overall significance when the observed versus expected transmissions of the haplotypes are compared and has the correct type I error rate in a stratified population. Family-based association tests for quantitative HDL-C levels were carried out under additive and dominant models, using FBAT version 1.7.2. The null hypothesis of linkage but no association was tested by using flag “-e”, which estimates the empirical variance. We also used Benjamini-Hochberg false discovery rate (FDR, [30]) to control for multiple hypothesis testing. p Values corrected by FDR are denoted as q value, and were estimated separately by each phenotype (HDL-C or low HDL), model (additive or dominant) and association analysis (family-based or population-based). We also carried out sub-sample analyses in subjects not using cholesterol-lowering medications and subjects without diabetes. Diabetes was defined by either a fasting glucose ≥ 126 mg/dl or self-reported use of hypoglycemic medication.

Haploview software (version 4.1, [31]) was used for computation of linkage disequilibrium statistics, LD block structure and haplotype maps. The Haploview haplotype frequencies shown for HDL-C were estimated using the Expectation–Maximization (EM) algorithm.

Further, we investigated association of HDL-C levels in a subset of 872 unrelated subjects from the NHLBI FHS data that was typed with the Illumina 550K chip. A total of 110 tag-SNPs across 264 kb of *LIPC* (from 56,389,096 to 56,653,448 bp) was used in the association analysis (PLINK).

3. Results

Plasma HDL-C mean levels (S.D.) were 49.6 (14.9) mg/dL, 42.5 (11.0) mg/dL, and 55.6 (15.2) mg/dL, for total, male and female samples, respectively, while the proportions of low HDL phenotype were 43%, 46% and 41%, respectively (Table 1). We genotyped 19 tag intronic *LIPC* SNPs in 2,238 subjects distributed in 591 families. Genotype frequencies did not deviate from Hardy–Weinberg equilibrium. The minor allele frequencies are shown in Table 2. The 19 intronic SNPs are distributed in eight LD blocks (Fig. 1, Table 2 and Table 3). The haplotype blocks and the values for r^2 between SNP blocks from Haploview are shown in Fig. 1.

Table 2 summarizes family-based tests of association with both a dichotomous low HDL phenotype and quantitative HDL-C levels adjusted for age, sex and field center. The strongest association was observed using an additive FBAT model for SNPs in haplotype block 1 with HDL-C levels in women (rs261342: $p = 0.00019$, $q = 0.0036$, Fig. 2; rs261338: $p = 0.00071$; $q = 0.0068$), but not in men. To verify whether these *LIPC* SNPs accounted for the linkage signal observed on chromosome 15q21, we did the linkage analysis before and after including rs261342 (or rs261338) effect in the age–sex–centers–adjusted HDL-C using the same subset sample. The maximum LOD dropped from 2.31 to 2.00 at the same chromosome location on 15q21 (at 41 cM) after accounting for rs261342 effect in the linkage model. The modest decrease in LOD is probably reflecting the significant but small rs261342 variability (1%) in HDL-C levels found in women only ($p < 0.001$).

Also, analysis using TRANSMIT of the dichotomous low HDL phenotype indicated a suggestive association in women with rs261342 ($p = 0.01035$). Another strong association was found for the SNP in haplotype block 4 (rs12593008) and low HDL in women ($p = 0.00217$, $q = 0.0412$), and was supported by suggestive association from FBAT ($p = 0.02461$). By contrast, an association in haplotype block 6 (rs8028759) with both a dichotomous low HDL ($p = 0.01108$) and quantitative HDL-C levels ($p = 0.03534$) was suggested in men only. The FBAT association results based on dominant models were similar to the additive models but with large p values (results not shown).

The GEE analyses also showed suggestive association between the SNPs in haplotype block 1 with both HDL-C levels and the low HDL trait, in women (Table 3). The minor alleles of *LIPC* Trs261342 ($p = 0.0055$) and C-rs261338 ($p = 0.0071$) were associated with higher mean plasma HDL-C levels in women, but not in men ($p > 0.05$). We also tested whether age–field centers adjusted–HDL-C levels were influenced by SNP and sex interaction. Significant interaction between sex and rs261342 or rs261338 was found affecting the variation of HDL-C levels ($p < 0.001$, for both association analyses). For the low HDL trait, the TT-rs261342 and CC-rs261338 genotypes had a decrease in the dyslipidemia risk of ~30% (low HDL estimate = -0.75 ± 0.30 , 95% CI = -1.38 to -0.15 , $p = 0.0181$ and, low HDL estimate = -1.33 ± 0.50 , 95% CI = -2.33 to -0.32 , $p = 0.0071$, respectively). Additional evidence of association was found for low HDL with rs2099190, rs12593008, rs1869137, and rs11071387, ($0.0047 < p < 0.04$) in women, and for HDL-C with rs4775051 ($p = 0.036$) in men.

The family-based and population-based association analyses did not change appreciably after exclusion of subjects taking cholesterol-lowering medications (23 men and 11 women) or when we added cholesterol-lowering medications in the covariate adjustment (results not shown). Owing to the perturbations in lipid profiles related to diabetes, we repeated the analyses excluding diabetic subjects from the sample. The association remained quite similar for both HDL-C levels and low HDL trait in the non-diabetic sub-sample. Because of known effects of metabolic variables and lifestyle interacting with HDL metabolism, we also performed association tests with a progressive adjustment, adding BMI, smoking and habitual alcohol together with age, sex and, field center in the covariate adjustment. Associations were materially unchanged with further adjustment (results not shown).

To determine whether the *LIPC* SNP association with low HDL-C levels could be better captured by haplotypes, we employed the SP-TDT methodology. There was evidence that SNPs in intron 1 were overtransmitted from parents to affected offspring (low HDL: global $p = 0.0004$). Also, we constructed a haplotype based on the significant intron 1 SNPs (rs261342–rs261338–rs2099190–rs12593008–rs1869137–rs11071387; $0.0395 < p < 0.007$ in females, Table 3). The common 6-SNP haplotype was overtransmitted and associated with an increased prevalence of 21% and 7% in low HDL, in women and men (global p value = 0.00033 and 0.00035), respectively.

Finally, we have genotyped a subset of 872 unrelated subjects using 110 tag-SNPs across 264 kb of *LIPC* and found strong association between a promoter SNP (rs1077834, $p = 0.000051$, FDR- $p = 0.0057$, by using PLINK) with HDL-C levels. Additionally, significant association of HDL-C with five 1-intronic *LIPC* SNPs (rs261341, FDR- $p = 0.0057$; rs261336, FDR- $p = 0.0136$; rs473224, FDR- $p = 0.0136$; rs633695, FDR- $p = 0.0158$; rs573922, FDR- $p = 0.0173$) in LD with the promoter-*LIPC* (rs1077834, $0.85 \leq r^2 \leq 0.94$), was found. To construct a proper replication sample, we excluded from this unrelated subset all subjects in the pedigree discovery sample, leaving $N = 350$ subjects. The association of HDL-C levels with the five *LIPC* markers remained ($0.00048 \leq p \leq 0.00661$), despite a 60% reduction in the sample size. Therefore, these results reinforced the evidence of association between HDL-C levels and *LIPC* polymorphisms in NHLBI FHS data.

4. Discussion

This study shows evidence of association between common *LIPC* intronic variants on different haplotypic backgrounds, with sex-specific effects on both HDL-C variation and the risk of dyslipidemia (*i.e.*, low HDL). The strongest association of SNPs (rs261342 and rs261338) in haplotype block 1 and HDL-C levels was found in women only. These SNPs may serve as LD markers for the markers in the promoter and exon regions in the *LIPC* gene or other genes related to HDL metabolism. Specifically, rs261342 is proxy to the four *LIPC* markers in the 5'-flanking region: rs1800588 (–514C/T, $r^2 = 0.875$), rs1077834 (–710T/C, $r^2 = 0.83$), rs2070895 (–250G/A, $r^2 = 0.826$), and rs1077835 ($r^2 = 0.875$), according to CEU Hapmap population. *LIPC* polymorphisms (–514C/T, –710T/C, –250G/A, –763A/G) are in complete LD and together define two haplotypes [8,9]. These haplotypes together were designated as the –514 C and T alleles [8,32]. Also, we found strong evidence of association between –514C/T with HDL-C levels in a sub-sample of unrelated subjects from the NHLBI FHS follow-up study. The T variant allele frequency of this variant (0.24) is in agreement with those reported in previous studies for Caucasians [8,9,13,14,18,20,22]. The –514 site is at the center of a CAC*GGC sequence, almost analogous to E-box (CACCGTG) which can bind the upstream stimulatory factors USF1/2, involved in the regulation of glucose and lipid metabolism in the liver [33]. It was also reported that the –514 C→T substitution interrupts the upstream stimulatory factor 1 binding site present in the proximal promoter region of the *LIPC* and decreases transcriptional activity of the promoter in vitro [34]. However, the underlying

physiologic mechanism for the associations between these SNPs and HDL-C levels remain unclear, and merit follow up studies.

The findings of association between *LIPC* -514C/T and HDL-C levels have been inconsistent [35]. However, meta-analysis and genome-wide association studies have demonstrated significant association between carriers of the -514 TT [10,11] and a proxy-promoter GG-rs261332 ($r^2 = 0.915$ and 0.876 with rs1800588 and rs261342, respectively; [12]) with elevated HDL-C levels. We have seen some reported discussions [6,14,16,17] of sex-interaction -514C/T association influencing HDL-C variation that could be due to physiological characteristics, HDL metabolism interacting with other pathways (e.g., diabetes and obesity), and/or lifestyle sex differences. In our data, obesity, diabetes, smoking cigarette and/or habitual alcohol intake seem not to be the causes of sex differences because after adjusting for these covariates (together with age and sex), the association results were similar. Endogenous hormonal environment may play an important role in the variation of HDL-C levels in families from the NHLBI FHS and other studies, however, the underlying physiologic mechanisms are unclear.

We also found strong association between SNP in haplotype block 4 (rs12593008, $r^2 = 0.23$ with rs261342 and rs261338) and the risk of low HDL in both family-based and population-based analyses (TRANSMIT: $p = 0.00217$, $q = 0.0412$, GEE additive model: $p = 0.0080$) in women. The genotypes AC or AA of rs12593008 were associated with a decreased risk of low HDL (estimate = -0.35 ± 0.12 , 95% CI = -0.59 to -0.10). To our knowledge, rs12593008 *LIPC* association has never been reported and we speculate that this intronic SNP may have a physiologic function or be in LD with undiscovered promoter or exon variants in *LIPC* or even other gene involved in HDL metabolism. There is a possibility that the present intronic *LIPC* variants are functional, but further investigation will be necessary to validate this hypothesis. Resequencing *LIPC* gene could also help to identify whether our variants or another variants have functional role in HDL metabolism.

This study also shows association between haplotype block 6 (rs8028759) with both HDL-C levels and low HDL trait, in men only. Despite association of rs8028759 was not reported for lipid levels, it was found for fat mass ($p = 0.03492$, after correction by genomic control) in the Whole Genome Scan for Type 2 Diabetes in a Scandinavian Cohort. Adiposity is associated with the variation in lipid levels, and both characteristics are influenced by sex steroids [36]. Hormonal sex-differences may be playing a major confounder role for detecting association of lipid profile and *LIPC* variants, and sex-specific analysis should be considered in further investigations.

One limitation of our study is the level of coverage achieved by our selection of tags, which is an issue for any criterion of coverage. Some variation in the *LIPC* gene may be not marked by the selected tags, leading to no power to detect any such functional mutations. This approach of probing genetic variation with tag SNPs is geared to finding common variant with impact on phenotype; in general, rare variants will not be found using this approach, and would likely require sequencing data on a large number of subjects, appropriate sampling strategies and robust analytic approaches. Nonetheless, we have detected several associated SNPs with our strategy, suggesting that there are common variants influencing HDL-C levels. Follow up strategies might include dense typing of these associated regions to finally characterize the functional haplotype for experimental studies.

In conclusion, we found that *LIPC* variants influenced HDL-C levels and the risk of low HDL-C levels with sex-specific effects. Evidence of association at the *LIPC* intron 1 resides on different haplotypic backgrounds. The associations between rs12593008 and low HDL trait in women and between rs8028759 with both HDL-C levels and low HDL phenotypes in men are novel, and should be validated by other independent studies. Other strong association occurred

with rs261342, which is proxy to *LIPC* markers in the 5'-flanking region and association was reported in other studies. Moreover, there is evidence that the common alleles of 6-SNP haplotype was overtransmitted to offspring with low HDL-C.

5. Electronic-database information

Ensembl Project: <http://www.ensembl.org>

Haploview software: <http://www.broad.mit.edu/mpg/haploview>

Illumina Inc., San Diego, CA, USA: www.illumina.com

Scandinavian Cohort: <http://www.broad.mit.edu/diabetes/scandinavs/index.html>

PLINK: <http://pngu.mgh.harvard.edu/~purcell/plink/>

The Single Nucleotide Polymorphism database (dbSNP): <http://www.ncbi.nlm.nih.gov/SNP/>

The International HapMap Project: <http://www.ncbi.nlm.nih.gov/SNP/hapmap.org>

Wellcome Trust Case Control Consortium:

http://www.wtccc.org.uk/info/summary_stats.shtml GeneID 3990:

[http://www.ncbi.nlm.nih.gov/sites/entrez?](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=3990)

[Db=gene&Cmd=ShowDetailView&TermToSearch=3990](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=3990)

NC 000015.8:

http://www.ncbi.nlm.nih.gov/projects/sviewer/?id=NC_000015.8&v=56504623.56655208

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Fig. 1. r^2 and haplotype blocks of *LIPC*. (a) Light to dark color scheme represents the increasing strength of r^2 . Values for r^2 ($\times 100$) are shown. (b) SNPs numbers correspond to (a). Haplotype blocks and their frequencies were estimated using an accelerated EM algorithm implemented in Haploview. Recombination rates from one block to the next are defined by a multi-allelic value of r^2 . Haplotypes in adjacent blocks are connected by a thick line if they occur together with a frequency $>10\%$ and by a thin line if they occur together with a frequency $>1\%$.

Fig. 2.

Plasma HDL-C levels by rs261342 genotypes by sex. Standard errors (in bars) are represented. The sample sizes are: 630 (CC), 309 (CT), 58 (TT) and 727 (CC), 373 (CT), 53 (TT), for males and females, respectively. The additive GEE p values for HDL-C are 0.30 and 0.0055, for males and females, respectively, while the sex-rs261342 interaction p value is lower than 0.001.

Table 1

Characteristics of NHLBI FHS sub-sample.

Variables	Male			Female		
	N	Mean	S.D.	N	Mean	S.D.
Age (years)	1052	52.4	14.2	1233	53.2	13.9
BMI (kg/m ²)	1007	28.2	4.5	1174	27.5	6.2
Triglycerides (mg/dL)	1029	167.4	117.2	1209	142.1	96.4
Total cholesterol (mg/dL)	1029	203.7	36.8	1209	209.3	42.0
Low-density cholesterol (mg/dL)	1029	127.8	33.5	1209	125.5	36.8
HDL-C (mg/dL)	1029	42.5	11.0	1209	55.6	15.2
Low HDL (%)	1029	46%		1209	41%	
Current smoking cigarette (%)	975	16.5%		1142	15.0%	
Current habitual alcohol intake (%)	974	54.8%		1142	50.9%	
Self-reported use of high cholesterol medication (%)	905	2.5%		1083	1.0%	

Note: low HDL (affected): male: HDL-C < 40 mg/dL and female: HDL-C < 50 mg/dL.

Table 2
Family-based association results for low HDL trait using TRANSMIT and plasma HDL-C levels using FBAT.

Intron	Block	SNP number and names	Illumina location (bp)	Alleles and minor Frequency	TRANSMIT <i>p</i> values (low HDL)			FBAT <i>p</i> values (HDL-C)		
					Total	Male	Female	Total	Male	Female
1	1	1 rs261342	56518444	C/T	0.21	0.66	0.01	0.00067*	0.20	0.00019*
		2 rs261338	56522296	G/C	0.17	0.07	0.47	0.00796	0.61	0.00071*
	2	3 rs634744	56524674	G/T	0.30	0.15	0.77	0.72	0.39	0.70
		4 rs17269264	56526051	G/A	0.48	0.53	0.21	0.06	0.65	0.04
	3	5 rs4775051	56536446	T/A	0.30	0.83	0.36	0.58	0.88	0.56
		6 rs2099190	56540181	C/A	0.50	0.13	0.01	0.88	0.15	0.16
	4	7 rs12593008	56548266	C/A	0.29	0.71	0.00217*	0.53	0.15	0.02
		8 rs1869137	56565703	G/C	0.44	0.15	0.05	0.84	0.11	0.09
4	5	9 rs1973028	56578645	A/G	0.36	0.55	0.34	0.81	0.63	0.90
		10 rs16940379	56583536	C/G	0.28	0.87	0.87	0.95	0.93	0.99
	6	11 rs12324517	56588057	C/T	0.48	0.09	0.69	0.29	0.20	0.85
		12 rs4775065	56589234	G/A	0.24	0.13	0.70	0.19	0.15	0.73
	7	13 rs2899632	56599160	G/A	0.19	0.88	0.21	0.52	0.92	0.38
		14 rs8028759	56599490	T/C	0.33	0.17	0.45	0.16	0.03	0.97
	8	15 rs11071387	56615875	A/T	0.49	0.37	0.09	0.46	0.28	0.95
		16 rs11852861	56623599	C/T	0.43	0.52	0.11	0.24	0.66	0.20
5	6	17 rs7165654	56627330	G/A	0.22	0.94	0.31	0.64	0.96	0.60
		18 rs17190678	56634663	G/C	0.47	0.79	0.66	0.93	0.83	0.69
6	8	19 rs3829460	56645236	A/T	0.30	0.23	0.67	0.15	0.20	0.55

Note: (1) The numbers of introns, block structures, and the 19-typed SNPs and their respective names are represented in the first four columns. The physical SNP locations (in bp) from Illumina, and their structures in blocks (in blue and green shades) and in non-blocks (in white lines) from Haploview are represented. Allele codes and the minor allele frequencies for the second allele are shown. (2) Affected low HDL: male: HDL-C < 40mg/dL and female: HDL-C < 50mg/dL. (3) The exon locations (in bp) are: exon_1: 56,511,467–56,511,611; exon_2: 56,617,824–56,618,008; exon_3: 56,621,276–56,621,458; exon_4: 56,622,025–56,625,466; exon_5: 56,625,233–56,625,466; exon_6: 56,627,821–56,628,063; exon_7: 56,640,355–56,640,472; exon_8: 56,642,996–56,643,214; exon_9: 56,648,207–56,648,364 (Ensembl Project). (4) Significant *q* values are noted by an asterisk (*). The means (standard errors) plasma HDL-C levels by genotypes and by sex for significant associated SNPs are: 41.9 (0.4), 43.2 (0.6), 42.6 (1.3) and 54.5 (0.5), 56.0 (0.8) and 60.7 (2.0) for CC, CT and TT rs261342, in males and females; 42.1 (0.4), 43.1 (0.6), 40.9 (2.0) and 54.5 (0.5), 56.6 (0.9) and 62.7 (2.7) for GG, GC and CC rs261338, in males and females; and 42.4 (0.4), 42.2 (0.5), 42.4 (1.25) and 54.8 (0.6), 55.9 (0.7) and 56.3 (1.6) for CC, CA and AA rs12593008, in males and females, respectively. (5) The sample sizes to rs261342 (rs261338 and rs12593008) are for total: 788 (796 and 795) affected offspring in 393 (392, in both) families with transmissions to affected offspring; male: 395 (405 and 392) affected offspring in 264 (263 and 266) families with transmissions to affected offspring; and female: 400 (391 and 403) affected offspring in 262 (265 and 262) families with transmissions to affected offspring.

Table 3
Association results for low HDL trait and plasma HDL-C levels using GEE additive model.

Intron	Block	SNP number and names	Illumina location (bp)	Alleles and minor Frequency	TRANSMIT <i>p</i> values(low HDL)			FBAT <i>p</i> values(HDL-C)		
					Total	Male	Female	Total	Male	Female
1	1	1 rs261342	56518444	C/T	0.02	0.27	0.0181	0.0073	0.30	0.0055
		2 rs261338	56522296	G/T	0.05	0.65	0.071	0.03	0.56	0.0071
	2	3 rs634744	56524674	G/T	0.93	0.26	0.28	0.99	0.25	0.20
		4 rs17269264	56526051	G/A	0.04	0.13	0.46	0.14	0.10	0.35
	3	5 rs4775051	56536446	T/A	0.03	0.27	0.09	0.16	0.03	0.47
		6 rs2099190	56540181	C/A	0.35	0.39	0.01	0.67	0.93	0.43
	4	7 rs12593008	56548266	C/A	0.24	0.66	0.02	0.66	0.95	0.37
		8 rs1869137	56565703	G/C	0.19	0.75	0.01	0.50	0.93	0.16
		9 rs1973028	56578645	A/G	0.26	0.97	0.08	0.69	0.88	0.65
		10 rs16940379	56583536	C/G	0.81	0.93	0.86	0.86	0.81	0.87
	5	11 rs12324517	56588057	C/T	0.82	0.46	0.98	0.67	0.59	0.79
		12 rs4775065	56589234	G/A	0.89	0.47	0.64	0.90	0.93	0.55
	6	13 rs2899632	56599160	G/A	0.43	0.23	0.53	0.76	0.70	0.87
		14 rs8028759	56599490	T/C	0.25	0.51	0.56	0.85	0.93	0.29
		15 rs11071387	56615875	A/T	0.10	0.84	0.03	0.21	0.80	0.14
		16 rs11852861	56623599	C/T	0.68	0.46	0.22	0.35	0.43	0.25
	4									
	5									
	6									
	7									
	8									

Note (1) The numbers of introns, block structures, and the 19-typed SNPs and their respective names are represented in the first four columns. The physical SNP locations (in bp) from Illumina, and their structures in blocks (in blue and green shades) and in non-blocks (in white lines) from Haploview are represented. Allele codes and the minor allele frequencies for the second allele are shown. (2) The GEE results from additive and dominant (not shown) models presented similar scenarios for both low HDL trait and HDL-C levels. However, the significance levels improve using GEE dominant model for dyslipidemia with SNPs #6 ($p = 0.0047$), #7 ($p = 0.0080$), #8 ($p = 0.0065$) in women, and for HDL-C with SNPs #4 ($p = 0.04$) and #19 ($p = 0.03$) in men. There was no significant q value.