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Multiple genetic determinants of plasma lipid levels in Caribbean Hispanics

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Abstract

Objectives: To identify candidate genes in relation to plasma lipid levels in Caribbean Hispanics.

Design and methods: A total of 114 single nucleotide polymorphisms (SNPs) at 17 lipid-related genes were genotyped in 477 Caribbean Hispanics from the Northern Manhattan Study (NOMAS). Analyses for each SNP and haplotype were performed to evaluate the associations with four lipid traits: high- and low-density lipoprotein cholesterol (HDL-C, LDL-C), triglyceride (TG) and total cholesterol (TC).

Results: We identified 19 SNPs at 10 genes that were significantly related to lipids ($p < 0.01$), including nine involved in the reverse cholesterol transport pathway, and one involved in bile acid synthesis. Three genes, namely the apolipoprotein A5, apolipoprotein B and cytochrome p450 polypeptide 7A1 genes, accounted for the largest proportion of variation in HDL-C/TG, TC and LDL-C respectively.

Conclusions: The cumulative effects of multiple genetic variants led to a substantially better prediction of inter-individual variations in lipid levels.

Keywords

Gene; Single nucleotide polymorphisms; Reverses cholesterol transport pathway; Lipids; Haplotype

Introduction

Cardiovascular disease (CVD) and stroke remain the leading cause of death in the United States. Since lipid levels are major predictors of CVD, genes that influence lipid metabolism may also contribute to CVD risks. Approximately half of the variability in plasma lipid levels

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is determined by genetic factors [1]. Several genes, such as the apolipoprotein A5 (*APOA5*), apolipoprotein B (*APOB*), apolipoprotein E (*APOE*), and cholesterol ester transfer protein (*CETP*) genes, have been reported to be related to the lipid profiles in whites and blacks [2-5]. However, only few of them (*APOA5*, *APOE*, and *CETP*) have been investigated in Hispanics [6-8].

With a 400% population growth each year, Hispanics will constitute up to a quarter of the US population by year 2050 [9]. Compared with other populations, Hispanics have a higher prevalence of diabetes, incidence of stroke, and premature cardiovascular death [10,11]. Certain population-specific genes may alter disease susceptibility of Hispanics by influencing lipid profiles or changing host response to detrimental lipid profiles. For example, the $\epsilon 4$ allele of *APOE* is associated with an increase of low density lipoprotein cholesterol (LDL-C) levels by 2.6 mg/dL in Caucasian men but 8.5 mg/dL in Hispanic men [4,12]. Furthermore, an elevated LDL-C level leads to a more prominent progression in carotid atherosclerosis in Hispanics than other ethnic groups [13], suggesting that race-ethnicity may influence the relationship between genes, lipids and CVD risk.

Complex traits are likely to be regulated by multiple genes. Hence, assessing a group of genes simultaneously may provide a more comprehensive view to characterize the contributions from susceptible genes. Lipid metabolism is modulated by several interacting networks through numerous enzymes, lipid transporters, cellular receptors and apolipoproteins. The combined effect of multiple genes involved in reverse cholesterol transport (RCT) pathway explained the lipid variation considerably better than a single gene approach [14,15].

Accordingly, the primary aim of the present study was to test the relationship between 17 candidate genes and lipid profiles in a population of Caribbean Hispanics. In addition, we evaluated the combined effects of multiple genes on the variation of plasma lipid levels.

Methods

Subjects and cardiovascular risk assessments

The study participants were drawn from the Caribbean Hispanics recruited in the prospective community-based Northern Manhattan Study (NOMAS). NOMAS consists of about 60% of Caribbean Hispanics and therefore provides a unique opportunity to evaluate the genetic effects in this racial-ethnic group. The details of recruitment criteria and ethnic identification could be found elsewhere [16]. A total of 477 Caribbean Hispanic subjects were selected for the present study based on two criteria: (1) available DNA samples and (2) carotid imaging study performed. Blood samples were drawn after an overnight fast. Fasting glucose was measured with a Hitachi 747 automated spectrometer (Boehringer, Indianapolis, IN, USA). Plasma levels of total cholesterol (TC) and triglyceride (TG) were measured using standardized enzymatic procedures with a Hitachi 705 automated spectrophotometer (Boehringer Mannheim, Mannheim, Germany). High-density lipoprotein cholesterol (HDL-C) was measured after precipitation of apoB lipoprotein with phosphotungstic acid. LDL-C levels were calculated using the Friedewald et al. equation [17]. The interassay coefficients of variation in our laboratory were 2% for TC, 4% for TG and 3% for HDL-C [18]. Diabetes was defined by the patient's self-report of such a history, use of insulin or hypoglycemic agent, or fasting glucose ≥ 126 mg/dL. Hypertension was defined as a blood pressure $\geq 140/90$ mm Hg, the patient's self-report of hypertension, or antihypertensive medication use. Body mass index (BMI) was calculated as weight (kg) divided by height square (m^2). Current smoking was defined as smoking within the past year. The study was approved by the Columbia University Medical Center Institutional Review Board.

SNPs selection criteria and genotyping

Seventeen candidate genes were chosen because of their biological links to lipid metabolism (Table 1). Single nucleotide polymorphisms (SNPs) were selected based on the public information on the dbSNP website (NCBI genome build 35). An SNP with the minor allele frequency (MAF) >0.05 and submitted to the dbSNP database by more than one source were mandatory requirements. Candidate SNPs that are most likely to be biologically relevant were selected using the following criteria: (1) non-synonymous SNPs, (2) had been examined in previous genetic studies, (3) with known functional relevance, or (4) located at evolutionarily conserved sequence homology (Vista website, <http://genome.lbl.gov/vista/index.shtml>). We also selected tagging SNPs across different human populations from the SeattleSNPs website (http://pga.gs.washington.edu/genes_genotyped.html). When tagging SNPs were not available, we chose common SNPs (MAF >0.05) in addition to the functional SNPs to have a more comprehensive coverage of the candidate gene. If two investigated SNPs were within 3 kb of each other, the functional SNP was compulsively kept but the other SNP was not included. SNPs were then screened by the Illumina (Illumina®, San Diego, USA) proprietary algorithm to predict their performance on the Illumina platform.

Blood was drawn into the EDTA tube (K3-EDTA 1 mg/mL) and centrifuged at 3000×g at 4 °C for 20 min. DNA was extracted from whole blood or buffy coats using salting out protocols [19]. Genotyping was performed by the Illumina BeadArray technology [20-22]. In brief, this genotyping method uses arrays coated with large-scale oligonucleotide beads (Sentrix® Array Matrix). Each oligonucleotide (bead type) corresponds to a specific SNP locus and can hybridize to its complementary strand of DNA sequence. Array-based hybridization takes place after DNA amplification and genotyping is achieved by detecting Cy-3 and Cy-5 labeled primers. Of the 114 attempted SNPs in or near the 17 candidate genes, 103 were successfully genotyped (Table 1). Among the 20664 duplicated genotyping (i.e., 41328 genotypes), there was no inconsistent genotyping data.

Statistical analysis

Statistic analysis was performed by the SAS 9.0 software (SAS®, North Carolina, USA). The mean and standard deviation (SD) of each lipid trait was calculated. Plasma levels of TG and HDL-C were natural log-transformed to approximate a normal distribution. Each lipid trait was adjusted for age, sex, diabetes, BMI and current smoking by multivariate linear regression. Age was treated as discrete variable (≤65 year or >65 year), and hypertension was not included because of lack of significance. Residuals were then used for the subsequent genetic statistic analysis.

Hardy–Weinberg equilibrium (HWE) was examined by the χ^2 goodness of fit test. Because 103 SNPs were tested, we reduced the significant p value to 0.01 as an indication of a deviation from HWE. We used three models (assuming the rare allele had an additive, dominant or recessive effect) to evaluate the genetic effects in the regression analysis. A threshold of nominal $p < 0.01$ was used to identify candidate genes related to lipids. The Haploview software was used to calculate linkage disequilibrium (LD) and to define haplotype blocks. The associations between haplotypes and phenotypes were evaluated by the Hap-Clustering program [23], which provided an empirical p value using a regression-based approach.

To test for the combined effects of multiple genes, we fitted a series of regression models and selected the best fitting model by comparing each model with a full model where all the significant genes were included. For the genes having more than one SNP related to lipids, we only kept the most significant SNP or haplotype in the regression model to avoid collinearity. The best fitting model was selected according to the Akaike information criterion (AIC) for the non-hierarchical models or the likelihood ratio test (LRT) for the hierarchical models. To

avoid over-fitting, we calculated the heuristic shrinkage estimator (C_{heur}) and the ratio between number of observations and degree of freedom. Gene-gene interactions were evaluated by including interaction terms into the regression models. The absolute values of the regression coefficient (β) derived from individual genes were added to estimate the phenotype difference contributed by the most deleterious genetic combinations.

Results

Data on 477 persons were initially included in the study. Seventeen subjects were excluded because of having plasma lipid levels beyond mean $\pm$ 3 SD or no gender information. Table 2 summarizes the demographic characteristics of the participants. The mean of candidate SNPs at each gene was 6.1 (range 3–15, Table 1). The genomic region covered by the candidate SNPs was 847 kb with a mean of 49.8 kb (SD = 43.0) per gene. The average inter-SNP distance within a gene was 9.9 kb (ranged 3.8–21.5 kb). Among the 103 qualified SNPs, 23.3% were non-synonymous (average MAF = 0.18), 30.1% were located at the 5' or 3' region, and 46.6% were at the intron (average MAF = 0.29). Four SNPs were not in HWE and were excluded. The final 99 SNPs were used in the subsequent analysis.

Nineteen SNPs at 10 genes were significantly associated with at least one of the four lipid traits (TC, LDL-C, HDL-C, and TG) with a nominal $p < 0.01$ (Table 3). Seven genes were associated with more than one lipid trait. Nine of the 10 significant genes are involved in the RCT pathway, and one gene (cytochrome p450 polypeptide 7A1, *CYP7A1*) controls the rate-limiting step of cholesterol excretion. Among the nine RCT genes, two (*APOA5*, *APOB*) encode apolipoproteins and two (the microsomal triglyceride transfer protein (*MTP*) and the ATP-binding cassette 1 (*ABCA1*) genes) serve as cellular transporters/receptors. One gene (*CETP*) is a lipid transfer protein and the rest four are members of lipase family, i.e., the lipoprotein lipase (*LPL*), hepatic lipase (*LIPC*), hormone-sensitive lipase (*LIPE*), and endothelial lipase (*LIPG*) genes.

The most significant SNP related to TG was rs662799 at *APOA5* (nominal $p = 0.00009$) and the same SNP was also the most significant one related to HDL-C ($p = 0.00045$). The most significant SNP associated with TC was rs10957057 at *CYP7A1*, which was also the most significant SNP in relation to LDL-C ($p = 0.00025$ and 0.00042 , respectively). Among the 10 genes, five had more than one SNP significantly related to lipid traits. Several SNPs within a gene demonstrated strong LD (Table 3). Haplotype analysis yielded similar results as single SNP analysis (Fig. 1). All the genes identified in single SNP analyses were also significantly related to lipids in haplotype analysis except for *CETP*. All the other nine genes remained significant while adding 17 excluded subjects into the analyses (data not shown).

In the initial regression models, covariates explained 13.4%, 6.9%, 3.5% and 2.2% of variation in HDL-C, TC, LDL-C and TG levels respectively. In regard to the multiple gene effects, the best fitting model for HDL-C contained seven genetic variants explaining 10.5% of variation in the residual of HDL-C values (Table 4). On the contrary, each model including only one genetic variant explained less than 3% of the variation. The combination of seven risky genotypes caused a reduction of plasma HDL-C values by 22.39 mg/dL (Table 4). For TG, the best fitting model containing six genetic variants explained 8.6% of variation in TG and contributed to an elevation of plasma TG values by 99.03 mg/dL. SNP rs662799 at *APOA5* was the most significant genetic variant for TG, which explained 3.5% of the phenotypic variation. For TC and LDL-C, the best fitting model contained seven genetic variants and explained 8.6% of variation in TC and 8.7% in LDL-C (Table 4). These genetic variants contributed to a similar extent of elevation in TC and LDL-C values (58.27 and 57.68 mg/dL). *CYP7A1* and *APOB* were the most significant genes for TC and LDL-C. The genetic variants at these two genes accounted for 2.6% and 2.9% variation in TC; 2.5% and 2.4% variation in

LDL-C respectively. The variation contributed by other individual genetic variant was less than 2%. For these 10 significant genes, there were 45 possible interactions from pairs of SNPs, but none of the interactions had a p value <0.01 .

Discussion

Among the 17 candidate genes, 10 genes were associated with lipid levels in our Caribbean Hispanic subjects. These 10 significant genes, which were previously related to the lipid variation in whites and blacks, displayed similar biological effects in this Hispanic population. Previous genetic studies in the Hispanic population mostly focused on Mexican Americans rather than Caribbean Hispanics [7,8,24]. Our study presented valuable information for this under-investigated, but rapidly growing population. Similar to the previous reports, each genetic variant contributed a small to moderate effect; however, the combined effect of all significant variants explained a modest extent of lipid variation (about 10%). This finding supported that multiple gene approach might provide a better prediction to the lipid variation in a general population [15].

The genetic variants on three genes, *APOA5*, *APOB* and *CYP7A1*, accounted for the largest proportion of the variation in the lipid values in this study. *APOA5* was the most significant gene related to TG and HDL-C, and it also accounted for the largest proportion of variation in the residual TG and HDL-C values. For the most significant SNP rs662799 (−1131 T>C) of *APOA5*, the rare allele was associated with an elevated TG level by 28.25 mg/dL in the present study, 23.04 mg/dL in white men of the Coronary Artery Risk Development in Young Adults (CARDIA) study and 17.1 mg/dL in both men and women of the Framingham Offspring Study (FOS) [25,26]. Moreover, SNP rs662799 at *APOA5* had a stronger effect ($r^2=3.5\%$ variation of TG) in Hispanics than in the white subjects from the CARDIA study (1.57%) or the FOS ($<1\%$) [25,26]. The discrepancy of the genetic effect across different studies can be due to different ethnic background, variation in other important covariates such as age, and potential gene–gene and gene–environment interactions.

Genetic variants on *APOB* and *CYP7A1* accounted for the largest proportion of variation in the residual TC and LDL-C values. Four SNPs at *APOB* were significantly related to lipids in our population: promoter SNP rs934197, non-synonymous SNPs rs1367117, rs1042031 and rs1801702. The most significant SNP rs1367117 is 2875 bp away from the extensively studied Ins/Del polymorphism [3]; therefore, it might tag the effect of the functional Ins/Del polymorphism. For SNP rs1042031 (aka *EcoRI*), the mean differences between the rare and common homozygous genotypes in our study (12.65 mg/dL) were similar to a meta-analysis that included 33 publications (15.46 mg/dL) [3], suggesting that the *APOB* gene exerted a comparable effect across ethnic groups. The genetic effect of *CYP7A1* was more prominent in Hispanics than Caucasians (13.09 mg/dL in NOMAS vs. 6 mg/dL in FOS) [27]. However, a direct comparison of the genetic effects may not be valid as these two studies analyzed different SNPs. Although the $\epsilon 2/\epsilon 3/\epsilon 4$ variant of APOE gene was reported to account for 5.6% of the phenotypic variance for TC in Hispanic females [8], this polymorphism was not accessible by the Illumina technology.

Lipase facilitates TG transfer among different lipoproteins. All the selected lipase genes (*LPL*, *LIPC*, *LIPG*, and *LIPE*) showed significant associations with lipid levels in our population. Besides, the regression model clearly implied a cumulative effect of multiple lipase genes, which was significantly superior to the model including only one individual lipase gene according to AIC or LRT. For example, the combined effect of *LPL*, *LIPE* and *LIPC* accounted for 4.4% variation in HDL-C levels and had a better ability to predict the HDL-C levels than any single lipase gene. Similarly, the cumulative genetic effect of the lipase genes was previously reported [28]. Since we did not find any interactions between the lipase genes, the

overall impact of multiple lipase genes is likely due to an additive rather than multiplicative effect.

In the present study, the combined effects of significant SNPs predicted approximately one tenth of the lipid variation in the general population. Although our result explained only a moderate proportion of the overall genetic influence on the lipid variation, the extent was similar to the previous studies which included multiple genes. For example, Boekholdt et al. reported that 12.5% of HDL-C variation can be accounted by eight genes and Hegele et al. showed 3.2–7.8% variation of four lipid traits can be explained by nine genes [14,29]. In fact, the effect size of the significant variants in our study was estimated to be 58.27 mg/dL for TC and 57.68 mg/dL for LDL-C, which is comparable to an average effect of current statin treatment in dyslipidemia [30].

Our study has several limitations. We acknowledged that the sparse spacing of SNPs might attenuate the power to detect a true association. The average inter-SNP distance in the present study was 9.9 kb, which may not be sufficient in capturing all the genetic variants. Some of the functional SNPs like the $\epsilon 2/\epsilon 3/\epsilon 4$ variant of *APOE* could not be genotyped by the Illumina platform. Consequently, we could have overlooked some of the gene–phenotype associations. To be noticed, the phenotypic variation estimated by currently investigated SNPs might not represent the overall effects of candidate genes. Another limitation was a relatively small sample size, which might have raised the concern of insufficient power, especially when the genetic effect was quite small. In addition, the current sample size may restrict the detection of multiplicative gene–gene interaction.

A large number of SNPs tested in this study raised an issue of a proper correction for multiple testing. We used nominal $p < 0.01$ as a threshold of significance for at least two reasons. First, we examined highly correlated phenotypes and some of the SNPs were not independent. Second, we selected the candidate genes based on the *priori* hypothesis and biological plausibility. Using a stringent threshold for statistical significance but without considering biological information may falsely reject an important biological finding. Since the associations observed in the 10 genes had been reported previously in the white and black populations [3,5,6,27,31–36], our findings were less likely to be false positive.

In summary, we reported 10 genes affecting lipid levels in Hispanics. The spectrum of plasma lipid levels was resulted from various combinations of detrimental genetic variants. The combined effect of multiple variants can explain larger variation in the lipid levels than the effect of individual gene, offering a better insight into the role of common genetic variants in dyslipidemia. Although the extent of genetic effect in this population is comparable to the effect in other populations, future studies in Hispanic populations are warranted to replicate our results.

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References

1. Heller DA, de Faire U, Pedersen NL, Dahlen G, McClearn GE. Genetic and environmental influences on serum lipid levels in twins. *N Engl J Med* 1993;328:1150–6. [PubMed: 8455681]
2. Pennacchio LA, Rubin EM. Apolipoprotein A5, a newly identified gene that affects plasma triglyceride levels in humans and mice. *Arterioscler Thromb Vasc Biol* 2003;23:529–34. [PubMed: 12615678]

3. Boekholdt SM, Peters RJ, Fountoulaki K, Kastelein JJ, Sijbrands EJ. Molecular variation at the apolipoprotein B gene locus in relation to lipids and cardiovascular disease: a systematic meta-analysis. *Hum Genet* 2003;113:417–25. [PubMed: 12942366]
4. Pablos-Mendez A, Mayeux R, Ngai C, Shea S, Berglund L. Association of apo E polymorphism with plasma lipid levels in a multiethnic elderly population. *Arterioscler Thromb Vasc Biol* 1997;17:3534–41. [PubMed: 9437203]
5. Boekholdt SM, Sacks FM, Jukema JW, et al. Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. *Circulation* 2005;111:278–87. [PubMed: 15655129]
6. Pennacchio LA, Olivier M, Hubacek JA, Krauss RM, Rubin EM, Cohen JC. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. *Hum Mol Genet* 2002;11:3031–8. [PubMed: 12417524]
7. Mahaney MC, Almasy L, Rainwater DL, et al. A quantitative trait locus on chromosome 16q influences variation in plasma HDL-C levels in Mexican Americans. *Arterioscler Thromb Vasc Biol* 2003;23:339–45. [PubMed: 12588781]
8. Kamboh MI, Aston CE, Hamman RF. The relationship of APOE polymorphism and cholesterol levels in normoglycemic and diabetic subjects in a biethnic population from the San Luis Valley, Colorado. *Atherosclerosis* 1995;112:145–59. [PubMed: 7772075]
9. Ayala C, Greenlund KJ, Croft JB, et al. Racial/Ethnic disparities in mortality by stroke subtype in the United States, 1995–1998. *Am J Epidemiol* 2001;154:1057–63. [PubMed: 11724723]
10. LaRosa JC, Brown CD. Cardiovascular risk factors in minorities. *Am J Med* 2005;118:1314–22. [PubMed: 16378771]
11. Sacco RL, Boden-Albala B, Gan R, et al. Stroke incidence among white, black, and Hispanic residents of an urban community: the Northern Manhattan Stroke Study. *Am J Epidemiol* 1998;147:259–68. [PubMed: 9482500]
12. Schaefer EJ, Lamon-Fava S, Johnson S. Effects of gender and menopausal status on the association of apolipoprotein E phenotype with plasma lipoprotein levels. Results from the Framingham Offspring Study. *Arterioscler Thromb* 1994;14:1105–13. [PubMed: 8018666]
13. Sacco RL, Roberts JK, Boden-Albala B, et al. Race–ethnicity and determinants of carotid atherosclerosis in a multiethnic population. The Northern Manhattan Stroke Study. *Stroke* 1997;28:929–35. [PubMed: 9158627]
14. Boekholdt SM, Souverein OW, Tanck MW, et al. Common variants of multiple genes that control reverse cholesterol transport together explain only a minor part of the variation of HDL cholesterol levels. *Clin Genet* 2006;69:263–70. [PubMed: 16542392]
15. Knoblauch H, Bauerfeind A, Toliat MR, et al. Haplotypes and SNPs in 13 lipid-relevant genes explain most of the genetic variance in high-density lipoprotein and low-density lipoprotein cholesterol. *Hum Mol Genet* 2004;13:993–1004. [PubMed: 15044381]
16. Sacco RL, Anand K, Lee HS, et al. Homocysteine and the risk of ischemic stroke in a triethnic cohort: the Northern Manhattan Study. *Stroke* 2004;35:2263–9. [PubMed: 15345803]
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuges. *Clin Chem* 1972;18:499–502. [PubMed: 4337382]
18. Paultre F, Tuck CH, Boden-Albala B, et al. Relation of Apo(a) size to carotid atherosclerosis in an elderly multiethnic population. *Arterioscler Thromb Vasc Biol* 2002;22:141–6. [PubMed: 11788474]
19. Medrano JF, Aasen E, Sharrow L. DNA extraction from nucleated red blood cells. *Biotechniques* 1990;8:43. [PubMed: 2182076]
20. Shen R, Fan JB, Campbell D, et al. High-throughput SNP genotyping on universal bead arrays. *Mutat Res* 2005;573:70–82. [PubMed: 15829238]
21. McMahon FJ, Buervenich S, Charney D, et al. Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *Am J Hum Genet* 2006;78:804–14. [PubMed: 16642436]

22. Ruigrok YM, Rinkel GJ, van't Slot R, Wolfs M, Tang S, Wijmenga C. Evidence in favor of the contribution of genes involved in the maintenance of the extracellular matrix of the arterial wall to the development of intracranial aneurysms. *Hum Mol Genet* 2006;15:3361–8. [PubMed: 17038484]
23. Tzeng JY, Wang CH, Kao JT, Hsiao CK. Regression-based association analysis with clustered haplotypes through use of genotypes. *Am J Hum Genet* 2006;78:231–42. [PubMed: 16365833]
24. Mahaney MC, Blangero J, Rainwater DL, et al. A major locus influencing plasma high-density lipoprotein cholesterol levels in the San Antonio Family Heart Study. Segregation and linkage analyses. *Arterioscler Thromb Vasc Biol* 1995;15:1730–9. [PubMed: 7583550]
25. Klos KL, Hamon S, Clark AG, Boerwinkle E, Liu K, Sing CF. APOA5 polymorphisms influence plasma triglycerides in young, healthy African Americans and whites of the CARDIA Study. *J Lipid Res* 2005;46:564–71. [PubMed: 15604515]
26. Lai CQ, Demissie S, Cupples LA, et al. Influence of the APOA5 locus on plasma triglyceride, lipoprotein subclasses, and CVD risk in the Framingham Heart Study. *J Lipid Res* 2004;45:2096–105. [PubMed: 15342688]
27. Couture P, Otvos JD, Cupples LA, Wilson PW, Schaefer EJ, Ordovas JM. Association of the A-204C polymorphism in the cholesterol 7 α -hydroxylase gene with variations in plasma low density lipoprotein cholesterol levels in the Framingham Offspring Study. *J Lipid Res* 1999;40:1883–9. [PubMed: 10508208]
28. Reilly MP, Foulkes AS, Wolfe ML, Rader DJ. Higher order lipase gene association with plasma triglycerides. *J Lipid Res* 2005;46:1914–22. [PubMed: 15961789]
29. Hegele RA, Brunt JH, Connelly PW. Multiple genetic determinants of variation of plasma lipoproteins in Alberta Hutterites. *Arterioscler Thromb Vasc Biol* 1995;15:861–71. [PubMed: 7600118]
30. Vaughan CJ, Gotto AM Jr. Update on statins: 2003. *Circulation* 2004;110:886–92. [PubMed: 15313959]
31. Frikke-Schmidt R, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Genetic variation in ABC transporter A1 contributes to HDL cholesterol in the general population. *J Clin Invest* 2004;114:1343–53. [PubMed: 15520867]
32. Juo SH, Han Z, Smith JD, Colangelo L, Liu K. Common polymorphism in promoter of microsomal triglyceride transfer protein gene influences cholesterol, ApoB, and triglyceride levels in young African American men: results from the coronary artery risk development in young adults (CARDIA) study. *Arterioscler Thromb Vasc Biol* 2000;20:1316–22. [PubMed: 10807748]
33. Qi L, Shen H, Larson I, Barnard JR, Schaefer EJ, Ordovas JM. Genetic variation at the hormone sensitive lipase: gender-specific association with plasma lipid and glucose concentrations. *Clin Genet* 2004;65:93–100. [PubMed: 14984467]
34. Paradis ME, Couture P, Bosse Y, et al. The T111I mutation in the EL gene modulates the impact of dietary fat on the HDL profile in women. *J Lipid Res* 2003;44:1902–8. [PubMed: 12867537]
35. Isaacs A, Sayed-Tabatabaei FA, Njajou OT, Witteman JC, van Duijn CM. The –514 C->T hepatic lipase promoter region polymorphism and plasma lipids: a meta-analysis. *J Clin Endocrinol Metab* 2004;89:3858–63. [PubMed: 15292318]
36. Hu Y, Liu W, Huang R, Zhang X. A systematic review and meta-analysis of the relationship between lipoprotein lipase Asn291Ser variant and diseases. *J Lipid Res* 2006;47:1908–14. [PubMed: 16741292]

Fig. 1. Haplotypes found to be significantly associated with lipids (empirical $p < 0.01$). The X-axis demonstrated all the SNPs at the nine significant genes (*CETP* was not shown because of insignificant in haplotype analysis). The horizontal bar indicated LD blocks of each gene.

Table 1

Characteristics of 103 tested SNPs at 17 candidate genes

Gene symbol	Gene official name	Inter-SNP ^a distance (kb)	Number of tested SNPs			
			Total	5'SNPs	3'SNPs	Non-syn SNPs ^b
<i>ABCA1</i>	ATP-binding cassette, sub-family A, member 1	11.1	15	1	1	3
<i>ALOX5</i>	Arachidonate 5-lipoxygenase	16.4	5	0	0	0
<i>ALOX5AP</i>	Arachidonate 5-lipoxygenase activating protein	9.3	5	0	1	0
<i>APOA5</i>	Apolipoprotein A5	3.8	4	2	1	1
<i>APOB</i>	Apolipoprotein B	3.9	12	2	0	7
<i>APOE</i>	Apolipoprotein E	6.2	4	2	0	1
<i>CETP</i>	Cholesterol ester transfer protein	8.1	4	0	2	1
<i>CYP7A1</i>	Cytochrome P450 family 7 subfamily A polypeptide 1	6.3	3	1	1	0
<i>LIPC</i>	Hepatic lipase	14.2	11	2	1	2
<i>LIPE</i>	Hormone-sensitive lipase	8.8	3	1	0	1
<i>LIPG</i>	Endothelial lipase	17.4	3	1	1	1
<i>LPL</i>	Lipoprotein lipase	7.8	5	0	1	2
<i>LRP1</i>	Low-density lipoprotein-related protein 1	15.2	5	0	0	1
<i>MTP</i>	Microsomal triglyceride transfer protein	8.0	9	2	2	3
<i>OLRI</i>	Oxidized low-density lipoprotein receptor 1	5.4	6	1	2	1
<i>PLTP</i>	Phospholipids transfer protein	5.2	4	0	2	0
<i>SCARB1</i>	Scavenger receptor class B, member 1	21.5	5	1	0	0
Total		9.9 per gene	6.1 per gene	16	15	24

^aMean of inter-SNP distance within a gene.^bNon-synonymous SNPs.

Table 2
 Characteristics of NOMAS subjects (mean±SD or percent)

	Men (n=197)	Women (n=263)	<i>p</i> Value
Age (year)	64.6±8.0	66.2±8.1	<0.05
TC (mg/dL)	188.2±33.6	204.0±35.4	<0.01
LDL-C (mg/dL)	122.4±30.6	129.0±32.5	<0.05
HDL-C (mg/dL)	38.0±10.1	45.7±11.9	<0.01
TG (mg/dL)	137.7±67.3	143.2±70.0	NS
BMI (kg/m ²)	27.8±4.7	28.8 ±5.1	<0.05
Diabetes (%)	27.4	24.0	NS
Hypertension (%)	74.1	76.0	NS
Current smoker (%)	16.2	11.4	NS

p values were calculated by Chi-squared test or Student's *t* test where appropriate.

Table 3
Associations between ten significant genes and four lipid traits (nominal *p* value)

Gene	SNP	TC	LDL-C	HDL-C	TG
ABCA1	rs2472384	...	...	0.0047	0.0068
	rs651821 ^a	...	...	0.0005	0.0006
	rs662799 ^a	...	...	0.0005	0.00009
APOA5	rs1042031	...	...	0.0035	...
	rs1367117 ^b	0.0018	0.0048	...	...
	rs1801702	0.0041	0.0088	...	...
APOB	rs934197 ^b	0.0046	0.0094	...	...
	rs1566439	...	0.0049	...	...
	rs10957057	0.0003	0.0004	...	...
CETP	rs3825776	...	...	0.0051	...
CYP7A1	rs16975750 ^c	0.0081	...	...	...
LIPC	rs7248439 ^c	0.0015	0.0076	...	...
LIPE	rs2000813	...	...	...	0.0059
LIPG	rs10096633	...	...	0.0016	...
LPL	rs1534649	...	...	...	0.0035
MTP	rs10516446 ^d	...	...	0.0073	...
	rs7659550 ^d	...	...	0.0071	...
	rs7698798 ^d	...	...	0.0060	...
	rs881980 ^d	...	...	0.0024	0.0025

Only SNPs with at least one nominal *p* value <0.01 are shown.

a, b, c, d Indicate SNPs in strong LD.

^a Include rs662799 and rs651821 at APOA5 ($r^2=0.76$, $D'=97\%$).

^b Include rs1367117 and rs934197 at APOB ($r^2=0.96$, $D'=100\%$).

^c Include rs7248439 and rs16975750 at LIPE ($r^2=0.97$, $D'=100\%$).

^d Include rs881980 and rs10516446 at MTP ($r^2=0.62$, $D'=97\%$), rs7698798 and rs7659550 at MTP ($r^2=0.71$, $D'=100\%$).

Table 4

Best fitting model of each lipid trait

Trait	Gene (r^2) in the besting fitting model	Overall r^2 (%)	Overall β	C heur	Ratio
TC	APOB (2.9%), CYP7A1 (2.6%), CETP (1.3%), LIPE (1.3%), APOA5 (0.6%), LIPG (0.4%), LPL (0.3%)	8.6	58.27	0.82	69.8
LDL-C	CYP7A1 (2.5%), APOB (2.4%), CETP (1.6%), LIPE (0.8%), LIPG (1.3%), APOA5 (0.4%), LPL (0.3%)	8.7	57.68	0.82	68.8
HDL-C	APOA5 (2.9%), LPL (1.9%), LIPC (1.8%), ABCA1 (1.7%), MTP (1.6%), LIPE (0.5%), CYP7A1 (0.2%), APOA5 (3.5%), LIPG (1.8%), ABCA1 (1.2%), MTP (1.4%), APOB (0.5%), LPL (0.4%)	10.5	22.39	0.86	69.8
TG		8.6	99.03	0.85	84.2

Overall r^2 denotes proportion of phenotypic variation contributed by multiple genes in conjunction.
Overall β is the summation of the absolute values of β derived from individual gene in the best fitting model.
C_{heur} (the ratio of adjusted r^2 and r^2) close to one denotes no over-fitting.
Ratio (number of observations/degree of freedom) should be at last 10 to avoid over-fitting.