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The HMG-CoA reductase gene and lipid and lipoprotein levels: the Multi-Ethnic Study of Atherosclerosis

Yi-Chun Chen^{1,2}, Yii-Der I. Chen¹, Xiaohui Li¹, Wendy Post³, David Herrington⁴, Joseph F. Polak⁵, Jerome I. Rotter¹, and Kent D. Taylor¹

¹Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, USA

²Department of Neurology, Chang Gung Memorial Hospital and College of Medicine, Chang-Gung University, Taipei, Taiwan

³Department of Medicine, Division of Cardiology, and Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, USA

⁴Department of Internal Medicine/Cardiology, Wake Forest University Health Sciences, Winston-Salem, USA

⁵Department of Radiology, Tufts-New England Medical Center, Boston, USA

Abstract

HMG-CoA reductase (HMGCR) is an enzyme involved in cholesterol synthesis. To investigate the contribution of the *HMGCR* gene to lipids and lipoprotein subfraction in different ethnicities, we performed an association study in the Multi-Ethnic Study of Atherosclerosis (MESA). Totally, 2444 MESA subjects (597 African-Americans (AA), 627 Chinese-Americans (CHA), 612 European-Americans (EA), and 608 Hispanic-Americans (HA)) without statin use were included. Participants had measurements of blood pressure, anthropometry, and fasting blood samples. Subjects were genotyped for 10 single nucleotide polymorphisms (SNPs). After excluding SNPs with minor allele frequency <5%, a single block was constructed. The most frequent haplotype was H1 (41-56%) in all ethnic groups except AA (H2a, 44.9%). Lower triglyceride level was associated with the H2a haplotype in AA and H2 in HA. In HA, H4 carriers had higher levels of triglyceride and small low-density lipoprotein (s-LDL), and lower high-density lipoprotein cholesterol (HDL-c), while carriers with H7 or H8 had associations with these traits in the opposite direction. No significant association was discovered in both CHA and EA. The total variation for triglyceride that could be explained by H2 alone was 2.6% in HA and 1.4% in AA. In conclusion, *HMGCR* gene variation is associated with multiple lipid/lipoprotein traits, especially with triglyceride, s-LDL, and HDL-c. The impact of the genetic variance is modest and differs greatly between ethnicities.

Keywords

hydroxymethylglutaryl CoA reductases; association study; cholesterol; triglyceride; low density lipoprotein size

Introduction

Elevated triglyceride (TG), low high-density lipoprotein cholesterol (HDL-c), and elevated small, dense low-density lipoprotein particles (s-LDL) have been established as important risk factors for cardiovascular diseases (CVD) [1]. Several studies have revealed ethnic difference in the variation in lipid/lipoprotein traits [2,3]. Compared to other ethnic groups, despite a higher prevalence of hyperglycemia, hypertension, and obesity, the prevalence of hypertriglyceridemia and low HDL-c is lower in blacks [2-4]. In contrast, Asians had higher TG and lower HDL-c than whites despite the lower body mass index (BMI) [5]. The interethnic disparity in lipid/lipoprotein profile could not be solely explained by the differences in environmental or nutritional factors [2].

Genetic effects on lipid/lipoprotein phenotypes have been shown in numerous studies. Atherogenic lipoprotein phenotype was demonstrated to segregate in Caucasian families [6], and heritability of LDL particles was estimated to range from 0.34 to 0.60 in Caucasian and Hispanic families [7-10]. Linkage studies have reported several chromosome loci with lipid/lipoprotein traits in multiple ethnicities [11-15]. Two independent groups [11,15] have reported a promising locus on 5q13-14, which suggests as a potential candidate gene, the HMG-CoA reductase gene (*HMGCR*). This locus was linked to TG and total cholesterol (TC) levels in Turkish/Mediterranean families [15], and to LDL peak particle diameter and TG levels in Quebec families [11].

HMGCR is the rate-limiting enzyme in cholesterol biosynthesis and is the target of statin therapy [16]. Pharmacokinetic studies have identified interethnic differences in the statin responses [17,18]. Previous studies [19,20] have shown that the *HMGCR* gene contributed to the difference in response to statin. A major role of *HMGCR* alternative splicing in influencing lipids response to statin therapy [21] and LDL-c variation [22] has been shown in the previous reports. Although the *HMGCR* gene was associated with the baseline LDL-c, it has not been clarified to what extent *HMGCR* gene contributes to lipids and lipoprotein subfraction in multiple ethnic groups. To investigate the contribution of the *HMGCR* gene to the lipid/lipoprotein traits in different ethnicities, we utilized the Multi-Ethnic Study of Atherosclerosis study (MESA) to test the association between the *HMGCR* gene and a comprehensive profile of lipid and lipoprotein subfraction in AA (African-Americans), CHA (Chinese-Americans), EA (European-Americans), and HA (Hispanic-Americans). We used a tagged single nucleotide polymorphisms (SNPs) approach to comprehensively assess gene variation.

Methods

Subjects

MESA is a prospective cohort study designed to study the progression of subclinical cardiovascular disease, consisting of 6814 subjects who were free of clinical cardiovascular disease at entry. The participants were recruited from six communities (Baltimore, Chicago, Forsyth County, Los Angeles County, Northern Manhattan, and St. Paul). The sampling procedures have been described in detail [23] and are available at www.mesa-nhlbi.org. A subcohort of 2880 MESA subjects (720 in each ethnic group with matched age and gender) were randomly selected from subjects who gave informed consent for genetic studies. After excluding subjects with statin use, we included 2444 subjects in the current study (597 AA, 627 CHA, 612 EA, and 608 HA).

Phenotyping

All data were collected at the first MESA examination (2000-2002), when participants had measurements of blood pressure (BP), anthropometry, and fasting blood samples. Three resting

BP readings were recorded, and hypertension was defined as a mean systolic BP ≥ 140 or diastolic BP ≥ 90 mmHg, or use of antihypertensive medication. Diabetes mellitus (DM) was defined as a fasting glucose >6.88 mmol/L or use of antidiabetic medication.

Using CDC-standardized methods, lipid levels were measured on samples obtained after an overnight fast. LDL-c was calculated in plasma specimens having a TG value <400 mg/dL by the Friedewald equation. Measurements for lipoprotein particles have been previously described [24].

Genotyping

Genomic DNA was extracted from peripheral leukocytes isolated from packed cells of anticoagulated blood by use of a commercially available DNA isolation kit (Puregene; Gentra Systems, Minneapolis). The DNA was quantified by determination of absorbance at 260 nm followed by PicoGreen analysis (Molecular Probes, Inc., Eugene).

Because of linkage disequilibrium (LD) in the human genome, genotyping chosen SNPs will provide sufficient information to assess the remainder of the common SNPs and to construct each of the common haplotypes in specific region [25]. Using International HapMap data on NCBI Build 35 assembly for whites, Asian, and Yoruban populations, we selected 10 SNPs to tag major haplotypes by Haploview v4 [26]. Selection of single-nucleotide polymorphisms (tagSNPs) in candidate gene loci were according to the following criteria: (1) within the proximal and distal 10 k base regions 5' and 3' to the given candidate gene (NCBI Build 35); (2) compatibility with the Illumina GoldenGate technology [27,28] as determined by the Assay Design Tool (TechSupport, Illumina, San Diego, CA); (3) minor allele frequency (MAF) >0.05 or a tag (r^2 value >0.8) for another SNP with MAF >0.05 as determined by applying the multilocus or "aggressive" "Tagger" option of Haploview v3 [26,29] using International HapMap project data for CEPH and Yoruban populations (release 19), (International HapMap Consortium 2003). All the ten SNPs were used for haplotyping, except for the SNP rs5908, since its MAF was <0.05 in all MESA ethnic groups, and thus providing little information in association study. The 10 SNPs were rs3761738 (G/A, mRNA untranslated region, UTR), rs3761739 (G/A, UTR), rs4704209 (A/G, intron), rs10038095 (A/T, intron), rs2303152 (G/A, intron), rs3846662 (G/A, intron), rs5908 (reference allele A/G, I637V), rs17238540 (A/C, intron), rs3846663 (G/A, intron), and rs5909 (G/A, UTR). As is well-known, G/A is the most common change in the human genome due to a methylation of the C on the opposite strand followed by deamination to a T. Although the MAF of the SNP rs5908 in CEPH shown in the International HapMap was 0.34, which is borderline lower than 0.05 it was still considered to be genotyped in this study because of its function of a non-synonymous polymorphism (reference allele A, I637V). Genotyping was performed by Illumina genotyping services (Illumina Inc., San Diego, GoldenGate assay). All 10 SNPs were successfully typed with a genotype calling rate of 100%. Each SNP was in Hardy-Weinberg equilibrium (HWE, significance level of 0.005) in each ethnic group. We utilized the SNPs with minor allele frequency (MAF) ≥ 0.05 to evaluate LD in each ethnic group and in the combined group by Haploview v4 [26]. Because the MAF of rs5908 was <0.05 in all ethnic groups, it was not included in haplotype construction. In each ethnic group, there was a single block structure, composed of a portion of the remaining 9 SNPs. Because the MAF was different for each SNP between ethnicities, different ethnic groups had different portions of the 9 SNPs for the same haplotype (Appendix 1). For example, the H2 haplotype was separated into H2a and H2b in AA based on the variation of rs17238540, in which the MAF was 0.08 in AA but <0.05 in the others. Haplotypes were reconstructed based on the LD results in each ethnic group by PHASE 2.0 [30]. Haplotypes with frequency $<1\%$ were excluded, and were numbered in the order of their frequency in the combined sample.

Statistical Analysis

Interethnic differences of the demographic/phenotypic characteristics and haplotypes distribution were performed by ANOVA or the Pearson's χ^2 -test where appropriate. We used haplotypes to examine the possible associations because it is expected that haplotype analyses will provide greater power than single SNPs since certain untyped SNPs may be in LD with a combination of typed SNPs [31]. Association tests for individual SNPs were only applied to those which differentiate the haplotypes with significant association results. We use general linear model (GLM) to estimate association in each ethnic group. Covariates included age, gender, BMI, and DM. Dominant models for SNP analyses were used because the majority of the SNPs had minor allele homozygotes <10. Parameter estimates were derived from the same models. Permutation testing of 10,000 replicates was performed for empirical estimates to account for the multiple testing. To identify the interaction between significant haplotypes and ethnic groups, the multiplicative term of significant haplotypes and ethnic groups was examined by the GLM. R squared was calculated to estimate the proportion of explained variation of the outcome variable as a function of independence in the GLM.

Under the sample of 600 independent individuals and *a priori* TC data in MESA, we tested the ability to detect an association between a SNP and TC. At 0.005 significance level, for detectable effect size >0.4, we had sufficient power (>0.80) to identify the association under a dominant model with disease allele frequency >0.10.

Results

Except for age and gender, the prevalence of DM and hypertension, and the quantitative traits of BMI, waist circumference, and lipid/lipoprotein levels, significantly differed between ethnicities (Table 1). The prevalence of hypertension was higher in AA (52%) than the others (35-40%). BMI and waist circumference were higher in AA and HA, and lower in CHA. Compared to the other groups, AA had a more protective lipid/lipoprotein phenotype.

Haplotype structure and frequencies of the *HMGCR* gene in each ethnic group are shown in Table 2 and Appendix 1. The frequency of each haplotype differed significantly between ethnic groups (χ^2 -test, $p<0.0001$). The H1 haplotype was the most frequent haplotype in every ethnic group except AA, in which 44.9% had H2a.

There was no significant association between any of the lipids traits and the *HMGCR* gene in both EA and CHA. However, lower TG was associated with H2a in AA ($p=0.005$) and H2 in HA ($p=0.021$) (Table 3). TG/HDL-c ratio was also lower in the H2a carriers in AA ($p=0.023$). In HA, the H4 haplotype was associated with the lipid/lipoprotein traits, specifically, higher TG ($p=0.012$), TG/HDL-c ratio ($p=0.003$), s-LDL ($p=0.016$), l-VLDL ($p=0.012$), m-VLDL ($p=0.002$), and lower HDL-c ($p=0.004$) and l-HDL ($p=0.0004$) (Fig. 1); in contrast, H8 was associated with these traits in the opposite direction, including lower TG ($p=0.002$), TG/HDL-c ratio ($p=0.0005$), s-LDL ($p=0.009$), l-VLDL ($p=0.021$), s-VLDL ($p=0.039$), and higher HDL-c ($p=0.002$) and l-HDL ($p=0.002$). In HA, the H7 haplotype was also associated with lower TG ($p=0.013$), lower TG/HDL-c ratio ($p=0.032$), and lower s-VLDL ($p=0.017$), but to a lesser magnitude compared with H8; in addition, H7 was associated with lower TC ($p=0.026$) and m-VLDL ($p=0.012$), but not significantly associated with the subclasses of LDL and HDL. LDL-c was not associated with any of the examined haplotypes in any ethnic group. In HA, when combining the subjects carrying H7 or H8, carriers had lower TC ($p=0.04$), lower TG ($p<0.0001$), higher HDL-c ($p=0.01$), lower l-VLDL ($p=0.003$), and higher l-HDL ($p=0.001$) than non-carriers (Fig. 1), and had lower TC ($p=0.03$), TG ($p<0.0001$), s-LDL ($p=0.02$), l-VLDL ($p=0.0004$), and higher HDL-c ($p=0.003$) and l-HDL ($p=0.0003$) levels than the H4 carriers.

Examination of the interaction effect of H2 and the ethnicity of AA and HA on TG levels was performed to determine if the higher frequency of H2 in AA could explain their lower TG levels. The H2 haplotype was associated with lower TG in both AA and HA. However, compared to HA, both H2 carriers and non-carriers in AA had lower TG level (Fig. 2). The difference in TG level between H2 carriers and non-carriers were similar in AA and HA ($p=0.70$). The interethnic difference in TG thus could not be solely explained by the H2 effect only. The total variation for TG that could be explained by H2 alone was 2.6% in HA and 1.4% in AA when controlling for covariates.

Associations were examined for the SNPs that differentiated significant haplotypes, including rs3846662 (separating H2a and H1), rs17238540 (H2a and H2b), and rs10038095 (H2a and H7) in AA, and rs3846662 (H2 and H1), rs3846663 (H7 and H3), rs3761739 (H8 and H3), and rs4704209 (H4 and H8) in HA. All the selected 3 SNPs in AA and rs3846662 and rs3761739 in HA showed no contribution toward the associations of the relevant haplotypes (data not shown). In contrast, the minor allele of rs3846663 was associated with higher TG ($p=0.001$), higher TG/HDL-c ratio ($p=0.006$), and higher m-VLDL ($p=0.002$) in HA, which was consistent with the association and the direction of H7. The association with rs4704209 was entirely equivalent to that with the H4 haplotype in HA.

Discussion

To our knowledge, this is the first report regarding the interethnic differences in the distribution of *HMGCR* haplotypes and the differential associations of *HMGCR* gene with lipid/lipoprotein levels. The significant associations occurred mainly in HA. In HA, the H4 haplotype was associated with higher TG, s-LDL, VLDL and lower HDL-c, and the haplotypes H2, H7, and H8 were associated with lower TG, s-LDL, VLDL and higher HDL-c. The H2 haplotype was associated with lower TG and lower VLDL in AA as well. The absence of H2 in CHA and the lack of the distinct, separable H4 and H8 haplotypes in AA and EA could well have accounted for ethnic differences in the associations. In HA, SNP analyses suggested that the association of H7 and H4 could be attributed to rs3846663 and rs4704209, respectively (or any SNP in complete LD with the two SNPs). This study supports that AA have less proportion of hypertriglyceridemia and low HDL-c than the other ethnic groups. Although AA had a remarkably high frequency (44.9%) of H2a, this study revealed that the *HMGCR* H2a haplotype had only a modest effect on TG level, and did not have significant effect on the interethnic difference of TG levels. In summary, the *HMGCR* gene variation is associated with multiple lipid/lipoprotein traits. The impact of the genetic variance is modest and differs greatly between ethnicities.

The first published MESA genetic study [32] utilized a MESA sample ($n=969$, white=448, Chinese =97, AA=205, HA:219) from the overall baseline MESA cohort who were randomly selected from the 5030 MESA participants enrolled prior to February 2002, before the completion of the overall recruitment. That study found ethnic differences regarding the association between ABCA1 gene SNPs and HDL-c as well as subclinical atherosclerosis. The multi-ethnic composition of the MESA cohort is both a strength (analyses in multiple ethnicities) and a potential limitation (relatively small sample sizes) [32].

The limitation of the present study included moderate sample sizes of each population group, which could limit the capacity to reveal valid genetic information for the complex phenotype of lipoprotein levels. Independent confirmation or further replication is needed to verify these associations. The other potential limitation of this study was that the LDL-c level was calculated by the Friedewald equation in stead of direct tests; therefore, LDL-c of subjects with TG value greater than 400 mg/dL was not included in the genetic analysis. However, there were only 1.4 % (34 subjects) with elevated TG were excluded from analyses. This study can not discover

any association between *HMGCR* gene and lipids traits in EA and CHA. It is possible that lipids were associated with other SNPs and haplotypes in the *HMGCR* gene which have more potent associations with lipids/lipoproteins traits. In addition, the MESA subjects consisted of only subjects free from known cardiovascular diseases at baseline, and this study has excluded subjects who took statin; both of the above selection criteria may lead to somewhat different results. The strengths of this study included the multiple samples of different ethnicities, extensive LD mapping and haplotyping of the locus, comparisons of haplotype frequency and genetic associations across ethnic groups, and the consistent associations between lower triglyceride level and the *HMGCR* haplotype 2 in two ethnic groups, AA and HA.

The *HMGCR* gene map locus at 5q13.3-q14. Previous studies showed linkage evidence of this locus with several lipid/lipoprotein traits, mainly in whites [11,15]. In AA, CHA, or HA, there was no promising data supporting this linkage [12-14]. In contrast to the linkage reports, the associations between *HMGCR* gene and the lipid/lipoprotein traits in our study were most striking in HA and AA, but not in EA. In EA, the absence of association between the *HMGCR* gene and lipid/lipoprotein traits was consistent with the finding in the previous studies [19,20]. The Pravastatin Inflammation/CRP Evaluation (PRINCE) study and the Cholesterol and Pharmacogenetics (CAP) study have demonstrated that the *HMGCR* gene contributed to the difference in response to statin. [19,20]. The PRINCE study did not find association between the *HMGCR* gene and the baseline LDL-c level in the studied population which consisted of mainly whites (88.7%). The CAP study showed an association of their Hap2 (Haplotype 2) and/or Hap7 (Haplotype 7) with lower baseline LDL-c level in blacks, but not in whites. The CAP Hap2 and Hap7 are analogous to our H2a and H2b in AA, respectively; the combination of the Hap2 and Hap7 is analogous to our H2 in the other ethnic groups. The study herein supports the association of the H2 with lower TG in both AA and HA. In addition, the percentage of the designated Hap2 in the CAP study was higher in AA (32%) than EA (2%), which was similar to our results (H2a, 44.9% in AA and H2, 5.5% in EA). In AA, we observed the association of the H2 haplotype with lower TG instead of LDL-c level shown in the previous study [20]. In HA, this is the first association data that showed significant associations between *HMGCR* haplotypes with multiple lipid/lipoprotein traits, especially with TG, s-LDL, and HDL-c. In CHA, we did not observe any association, although an association between 8302A/C variation of the *HMGCR* gene and lipids was reported in one Chinese study [33]. It should be noticed that the absence of H2 in CHA and the lack of the distinct, separable H4 and H8 haplotypes in AA and EA could well have accounted for the ethnic differences in the associations. Because the associations of the *HMGCR* gene with lipid/lipoprotein traits were mainly in AA and HA, and the haplotype distribution was remarkably different between ethnicities, future studies of statin therapy will need to include adequate representation from several ethnic groups, such as AA and HA.

Recently, a genome-wide association study (GWAS) [34] using variance-weighted meta-analysis from up to four GWAS revealed an association between SNP rs12654264 of *HMGCR* and LDL-c level in the European population ($p=1 \times 10^{-20}$). A simultaneous GWAS [35] using similar study cohorts did not report this association (threshold $p=5 \times 10^{-7}$). Based on the CEU data in HapMap, SNP rs12654264 is in LD with rs3846662 ($r^2=0.84$) and rs3846663 ($r^2=0.97$); however, in our white group, both SNPs did not exhibit any significant association with lipids level in EA ($p>0.05$). The SNP rs12654264 is in complete LD with rs3846662 and rs3846663 in the CHB and JPT data. In our CHA group, neither of the two SNPs was associated with lipids traits. The SNP rs12654264 is not in LD with any of our tagSNPs in the AA group, and both the two SNPs rs3846662 and rs3846663 showed no association with lipids traits in AA. The only association discovered in our study between lipids and the two SNPs was rs3846663 with TG levels and l-VLDL and m-VLDL in HA. We had no LD information between rs3846663 and rs12654264 in our Hispanic groups. The inconsistency between the two GWAS and/or our reports might be caused by the different

sample size and study/analysis methods. Independent confirmation for this inconsistency is necessary. Further studies addressing the function related to the SNP and the intermediate phenotypes such as mevalonate and HMG-CoA reductase would increase the evidence supporting a functional biochemical evidence for the association.

It is somewhat surprising that the *HMGCR* gene was associated with TG and HDL-c because *HMGCR* does not play a direct role in TG synthesis and hydrolysis. The association may be indirectly caused by the requirement of hepatic cholesterol for VLDL assembly, [36] regulation of LDL receptor number, and the conversion of s-LDL from large TG-rich VLDL particles [36]. Several studies have also shown the effect of statin on lowering TG and increasing HDL-c [16]. The *HMGCR* gene is thus a potential contributor, at least to some extent, for the differences in statin response of TG and HDL-c.

In summary, *HMGCR* gene variations were associated with multiple lipid/lipoprotein traits in African-Americans and Hispanic-Americans. The impact of the *HMGCR* gene on lipid/lipoprotein levels appears modest, and differs greatly between ethnicities.

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Figure 1. Comparisons of lipid/lipoprotein levels between the carriers and non-carriers of the *HMGR* H4 and the combination of H7 and/or H8 in Hispanic-Americans

Data are presented as mean of the levels. Subjects carrying the H4 haplotype (H4 (+)) have significantly higher TG, lower HDL-c, higher s-LDL, higher l-VLDL, and lower l-HDL than non-carriers. In contrast, H7 or H8 carriers (H7/H8 (+)) have lower total cholesterol (TC) and TG, higher HDL-c, lower l-VLDL, and higher l-HDL than non-carriers. Except for LDL-c, H4 carriers have higher TC, TG, s-LDL, l-VLDL, and lower HDL-c and l-HDL levels than H7/H8 carriers. To convert mg/dL to mmol/L, multiply LDL-c, HDL-c, and total cholesterol values by 0.02586 and TG by 0.011.

	β coefficient	P-value
H2 haplotype	-0.13	0.005
Ethnicity	0.35	<0.0001
Interaction of H2 and ethnicity	0.02	0.70

Figure 2. Triglyceride levels in H2 carriers and non-carriers in African-Americans (AA) and Hispanic-Americans (HA)

The H2 haplotype had lower TG level in both AA and HA. Both H2 carriers and non-carriers in HA had higher TG level (142 ± 81 and 162 ± 90 mg/dL, respectively) than African-Americans (101 ± 55 and 115 ± 75 mg/dL, respectively). The difference in TG level between H2 carriers and non-carriers was similar in African-Americans and Hispanic-Americans (14 and 20 mg/dL, respectively), without interaction between the H2 haplotype and ethnicity ($p=0.70$).

Table 1

Mean Values with Standard Deviations or Percentage Distributions of Clinical, Lipid, and Lipoprotein Variables in each MESA Ethnic Group

	AA (N=597)	CHA (N=627)	EA (N=612)	HA (N=608)	ALL (N=2444)
Age, y	60.9±10.0	61.4±10.3	60.9±10.6	60.5±10.2	60.9±10.3
Male gender	45.7%	49.8%	45.6%	45.9%	46.8%
Diabetes mellitus (DM) *	43.0%	44.2%	28.2%	46.9%	40.5%
Hypertension *	52.4%	34.9%	35.8%	39.8%	40.6%
BMI,kg/m ² *	30.0±5.7	23.9±3.3	27.6±5.3	29.6±5.1	27.7±5.5
Waist circumference,cm *	100.6±14.3	86.8±9.9	97.2±15.3	100.3±13.0	96.1±14.4
Total Cholesterol,mg/dl *	192±37	195±31	199±34	201±37	196±35
LDL-c,mg/dl †	117±33	118±28	120±31	122±31	119±31
l-LDL,nmol/L *	421±204	345±190	434±203	372±215	392±206
s-LDL,nmol/L *	837±459	947±441	855±422	1024±472	916±455
Triglyceride,mg/dl *	104±60	141±82	130±82	158±88	133±81
l-VLDL,nmol/L *	2.3±3.7	3.5±5.5	4.1±5.3	6.0±7.1	4.0±5.7
m-VLDL,nmol/L *	24±22	45±29	31±23	37±26	34±26
s-VLDL,nmol/L *	36±20	40±19	40±21	43±19	40±20
HDL-c,mg/dl *	53±15	49±12	53±16	47±12	50±14
l-HDL,μmol/L *	7.6±4.0	6.9±3.6	7.1±4.2	6.0±3.5	6.9±3.9
m-HDL,μmol/L *	3.4±3.7	3.2±3.5	5.7±4.8	5.3±4.2	4.4±4.2
s-HDL,μmol/L *	20±5	21±5	19±5	19±4	20±5
TG/HDL-c ratio *	2.3±1.9	3.3±2.6	2.9±2.7	3.8±2.8	3.1±2.5

l-HDL: large HDL; m-HDL: medium HDL; s-HDL: small HDL; l-LDL: large LDL; s-LDL: small LDL; l-VLDL: large VLDL/Chylomicron; m-VLDL: medium VLDL; s-VLDL: small VLDL.

With the exception of age and gender, the frequency of DM, hypertension and the traits of BMI, waist circumference, lipid and lipoprotein are all significantly different between race/ethnic groups

* p<0.0001,

† p=0.035.

To convert mg/dL to mmol/L, multiply LDL-C, HDL-C, and total cholesterol values by 0.02586 and triglycerides by 0.011.

Table 2
Haplotype Frequencies of the *HMGCR* Gene in each MESA Ethnic Group

Ethnic groups Haplotype	Combined n (%)	AA n (%)	CHA n (%)	EA n (%)	HA n (%)
H1 GGAAAGAAG	2024 (41.4)	157 (13.2)	610 (48.7)	681 (55.9)	576 (47.5)
H2a GGAAGGAGG	869 (17.8)	536 (44.9)	-	67 (5.5)	174 (14.3)
H2b GGAAGGCGG		96 (8.0)			
H3 GGATGGAAG	652 (13.3)	*	281 (22.4)	160 (13.1)	123 (10.1)
H4 GAGTGAAG	420 (8.6)	*	231 (18.4)	*	88 (7.3)
H5 GGATAGAAG	386 (7.9)	63 (5.3)	96 (7.7)	130 (10.7)	97 (8.0)
H6 AAATGGAAA	315 (6.4)	*	*	125 (10.3)	125 (10.3)
H7 GGATGGAGG	128 (2.6)	106 (8.9)	-	-	16 (1.3)
H8 GAATGGAAG	86 (1.8)	*	*	*	15 (1.2)
		[†] 236 (19.8)	[†] 35 (2.8)	[†] 55 (4.5)	

Haplotypes were constructed in the above SNP order. Detailed haplotype structure is in Appendix 1.

-Very rare (<1% frequency) haplotype.

* Haplotypes that were not differentiated because of certain rare SNPs (MAF <0.05).

[†]Sum of haplotypes which were marked by *

Table 3

Differences (95% confident interval) of lipid traits as a function of the presence or absence of *HMGR* haplotypes in African-Americans (AA) and Hispanic Americans (HA) in MESA

	TC (mg/dl)	TG (mg/dl)	TG/ HDL-c	LDL		VLDL (nmol/L)		HDL		
				LDL-c (mg/dl)	s-LDL (nmol/L)	l-VLDL	m-VLDL	s-VLDL	HDL-c (mg/dl)	l-HDL (nmol/L)
AA										
H2a	-3.1 (-9,3)	-10.7 [†] (-18,-3)	-0.2 [‡] (-0.4,-0.03)	-1.1 (-7,5)	1.0 (-74,76)	-0.2 (-0.5,0.1)	-3.9 [‡] (-7,-0.6)	-5.2 [†] (-9,-2)	0.4 (-2,3)	0.6 (-0.4,0.9)
H2b	4.8 (-3,13)	1.6 (-8,12)	0.01 (-0.2,0.3)	4.0 (-3,11)	56.6 (-39,152)	0.1 (-0.3,0.5)	0.8 (-3,5)	-1.8 (-6,3)	0.7 (-2,4)	0.1 (-0.7,0.9)
HA										
H2	-0.9 (-7,6)	-14.8 [‡] (-26,-2)	-0.3 (-0.6,0.1)	3.1 (-3,9)	15.5 (-69,100)	-0.6 (-1.3,0.1)	-2.9 (-7,1.5)	0.5 (-3,4)	-0.5 (-2,1.4)	0.09 (-0.5,0.7)
H4	3.2 (-5,12)	21.7 [‡] (4,6,41)	0.7 [†] (0.2,1.3)	0.1 (-7,8)	127.2 [‡] (24,231)	1.3 [‡] (0.3,2.6)	9.2 [†] (3.6,14.8)	2.7 (-2,7)	-3.3 [‡] (-5.3,-1)	-1.1 [*] (-1.8,-0.5)
H7	-19.3 [‡] (-34,-3)	-37.3 [‡] (-60,-9)	-0.9 [‡] (-1.5,-0.1)	-13.5 (-29,2)	17.2 (-208,242)	-1.6 (-2.7,0.1)	-13.3 [‡] (-24,-3)	-11.9 [‡] (-21,-2)	1.5 (-3,7)	1.0 (-0.6,2.6)
H8	-5.3 (-22,13)	-46.3 [†] (-67,-19)	-1.4 [*] (-1.8,-0.7)	-5.3 (-21,11)	-310.7 [†] (-542,-80)	-1.9 [‡] (-2.9,-0.4)	-11.0 (-22,-0.06)	-10.5 [‡] (-20,-0.6)	8.6 [†] (3,15)	2.7 [†] (1.0, 4.5)
HA										
3846662	2.1 (-4,8)	2.7 (-9,15)	0.06 (-0.3,0.4)	0.4 (-5,6)	11.9 (-67,90)	0.2 (-0.5,1.0)	0.5 (-3.6,4.5)	0.6 (-3,4)	-0.2 (-2,1.6)	0.09 (-0.4,0.6)
3846663	4.8 (-1,11)	18.2 [†] (7,30)	0.4 [†] (0.1,0.8)	0.8 (-4,6)	45.1 (-28,118)	0.8 [‡] (0.2,1.6)	5.9 [†] (2.2,9.7)	1.9 (-1,5)	-0.4 (-2,1.2)	-0.3 (-0.8,0.2)
3761739	6.7 [‡] (0.7,13)	13.3 [‡] (1,4,26)	0.3 (-0.01,0.7)	3.0 (-2,8)	45.5 (-30,122)	0.5 (-0.2,1.3)	6.5 [†] (2.5,10.5)	2.4 (-0.9,6)	-0.5 (-2,1.3)	-0.2 (-0.8,0.3)
4704209	3.2 (-5,12)	21.7 [‡] (4,6,41)	0.7 [†] (0.2,1.3)	0.1 (-7,8)	127.2 [‡] (24,231)	1.3 [‡] (0.3,2.6)	9.2 [†] (3.6,14.8)	2.7 (-2,7)	-3.3 [†] (-5.3,-1)	-1.1 [*] (-1.8,-0.5)

Parameter estimates (95% CI) derived from GLM models that included age, sex, BMI, and diabetes as independent variables show the difference in each trait in carriers (haplotype or genotype consisting at least one minor allele) compared to non-carriers. Definition for minor allele is based on the allele frequency of each SNP in entire cohort subjects.

* p<0.001,
[†] p<0.01,
[‡] p<0.05.

The negative signs mean the level of each trait in carriers is lower to that in non-carriers.

To convert mg/dL to mmol/L, multiply LDL-C, HDL-c, and total cholesterol values by 0.02586 and triglycerides by 0.011.

Appendix 1
Haplotype structure and frequencies of the *HMGCR* gene in each MESA ethnic group

SNP MAF (%) in All		rs3761738 (G/A, UTR) (6.5)	rs3761739 (G/A, UTR) (16.9)	rs4704209 (A/G, intron) (8.6)	rs10038095 (A/T, intron) (41.0)	rs2303152 (G/A, intron) (8.2)	rs3846662 (G/A, intron) (40.9)	rs17238540 (A/C, intron) (3.6)	rs3846663 (G/A, intron) (38.4)	rs5909 (G/A, UTR) A (6.5)
Haplotype	n (%)									
AA	H1:	157 (13.2)	*	*	*	A	G	A	G	*
	H2a:	536 (44.9)	*	*	*	A	G	A	G	*
	H2b:	96 (8.0)	*	*	*	A	G	C	G	*
	H3,4,6,8:	236 (19.8)	*	*	*	T	G	A	A	*
	H5:	63 (5.3)	*	*	*	T	A	A	A	*
	H7:	106 (8.9)	*	*	*	T	G	A	G	*
	H7:	106 (8.9)	*	*	*	T	G	A	G	*
CHA	H1:	610 (48.7)	*	G	A	A	G	A	*	G
	H3:	281 (22.4)	*	G	A	T	G	*	A	*
	H4:	231 (18.4)	*	A	G	T	G	*	A	*
	H5:	96 (7.7)	*	G	A	T	A	*	A	*
	H6,8:	35 (2.8)	*	A	A	T	G	*	A	*
	H2, H7:	0 (0)	-	-	-	-	-	-	-	-
	H2, H7:	0 (0)	-	-	-	-	-	-	-	-
EA	H1:	681 (55.9)	G	G	*	A	G	A	*	G
	H2:	67 (5.5)	G	G	*	A	G	*	G	G
	H3:	160 (13.1)	G	G	*	T	G	*	A	G
	H5:	130 (10.7)	G	G	*	T	A	*	A	G
	H6:	125 (10.3)	A	A	*	T	G	*	A	A
	H4,8:	55 (4.5)	G	A	*	T	G	*	A	G
	H7:	0 (0)	-	-	-	-	-	-	-	-
HA	H1:	576 (47.5)	G	G	A	A	G	A	*	G
	H2:	174 (14.3)	G	G	A	A	G	G	*	G
	H3:	123 (10.1)	G	G	A	T	G	G	*	A
	H4:	88 (7.3)	G	A	G	T	G	G	*	A
	H5:	97 (8.0)	G	G	A	T	A	G	*	A
	H6:	125 (10.3)	A	A	A	T	G	G	*	A
	H7:	16 (1.3)	G	G	A	T	G	G	*	G
	H8:	15 (1.2)	G	A	A	T	G	G	*	A
ALL	H1:	2024 (41.4)	G	G	A	A	G	A	*	G
	H2:	869 (17.8)	G	G	A	A	G	G	*	G
	H3:	652 (13.3)	G	G	A	T	G	G	*	A
	H4:	420 (8.6)	G	A	G	T	G	G	*	A
	H5:	386 (7.9)	G	G	A	T	A	G	*	A
	H6:	315 (6.4)	A	A	A	T	G	G	*	A
	H7:	128 (2.6)	G	G	A	T	G	G	*	G
	H8:	86 (1.8)	G	A	A	T	G	G	*	A

Haplotypes were constructed in the above SNP order. Haplotype frequency (%) shows the percentage for each haplotype in each race/ethnic group. The distribution of haplotypes was significant different among the ethnic groups (χ^2 -test, $p < 0.0001$).

-Absent or very rare (<1% frequency) haplotype.

* SNP with minor allele frequency (MAF) <5% was not included in haplotype construction.