REPLICATION STUDY OF PLASMA LIPOPROTEIN LEVELS-ASSOCIATED POLYMORPHISMS IDENTIFIED IN RECENT GENOME-WIDE ASSOCIATION STUDIES

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REPLICATION STUDY OF PLASMA LIPOPROTEIN LEVELS-ASSOCIATED POLYMORPHISMS IDENTIFIED IN RECENT GENOME-WIDE ASSOCIATION STUDIES

Emily Kate Bryant, M.S.

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Cardiovascular disease (CVD) is a major public health concern in the U.S., and is the leading cause of death for both men and women. Abnormal plasma lipoprotein levels, especially low high-density lipoprotein cholesterol (HDL-C) levels, are among major factors that influence the CVD risk. Functional and candidate gene association studies and recent genome-wide association studies (GWAS) have identified several genes as being potentially significant for HDL-C and other lipid (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], and triglycerides [TG]) levels. Our group has been comprehensively investigating several HDL-C levels-associated genes using sequencing and genotyping methods to test both common and rare variant hypotheses. In this study, we sought to replicate the GWAS signals from other genes that have not been targeted by our sequencing effort in three epidemiological samples, U.S. non-Hispanic Whites, U.S. Hispanics, and African Blacks. We selected 40 SNPs (primarily those influencing the HDL-C levels) for analysis and genotyped each SNP in all populations when present at sufficient frequency (6 SNPs were not analyzed in Blacks). For 25 SNPs, we were able to replicate the genome-wide significant associations with the same lipid trait in the same direction in at least one ethnic group studied: at nominal significance (p<0.05) for 14 SNPs, with marginal p-values (0.05-0.10) for 3 SNPs, and with trend for association with p-values between 0.10-0.20 for 8 SNPs. Similarly, we were able to replicate 18 of 37 SNPs for HDL-C, 5 of 6 SNPs

for TC, 5 of 7 SNPs for TG, and 2 SNPs for LDL-C levels. Two SNPs showed significant (p<0.05) but discordant results for association with the HDL-C levels as compared to those reported in the original GWAS. For 10 SNPs, we observed significant associations with lipid traits other than those reported as genome-wide significant in the original GWAS. Identification and replication of genetic associations with plasma lipid levels is relevant to public health as it may lead to improvements in prevention and treatment of dyslipidemia, which is a major risk factor for heart disease.

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1.0 BACKGROUND AND SIGNIFICANCE

1.1 CARDIOVASCULAR DISEASE AND CHOLESTEROL LEVELS

Cardiovascular disease (CVD), including coronary heart disease (CHD), stroke, and heart failure is a serious health concern in the United States today. According to the American Heart Association, more than 1 in 3 American adults, or more than 81,000,000 people, are estimated to have one or more types of CVD. Including congenital heart defects, CVD was listed as the underlying cause in 34.3% of all deaths in 2006 and more than 630,000 total deaths in the United States were due to heart disease (*Centers for Disease Control and Prevention, 2011*). CVD is the leading cause of death in the United States for both men and women. While percentages vary slightly, CVD, specifically coronary heart disease, is the leading cause of death in most ethnic groups in the United States. In 2004, heart disease was the cause of 27.5% of all deaths in Non-Hispanic Whites, 22.7% in Hispanics, and 25.8% in African Americans (*CDC, 2011*).

There are a number of conditions and behavioral factors that can increase a person's risk of CHD. Excessive alcohol consumption and cigarette smoking can increase the risk of all forms of cardiovascular disease by promoting atherosclerosis and raising blood pressure. Diets high in cholesterol and saturated fats, as well as physical inactivity, can lead to obesity, which is

also linked to dyslipidemia, diabetes and high blood pressure, which are all risk factors for heart disease (*CDC, 2011*). Cholesterol levels, specifically low high-density lipoprotein (HDL) cholesterol (HDL-C) levels, have been linked to CHD and have been used by clinicians since the 1980s to assess cardiovascular risk (Weissglas-Volkov et al., 2010).

While high levels of low-density lipoprotein cholesterol (LDL-C; "bad" cholesterol) and high levels of triglycerides, are believed to increase atherosclerosis risk, the HDL-C ("good" cholesterol) which makes up approximately one-fourth to one-third of all blood cholesterol is believed to protect against heart disease. The optimal level for LDL-C is less than 100 mg/dL, and the total blood cholesterol should remain below 200 mg/dL. The ideal level for triglycerides is less than 150 mg/dL. The average LDL-C and triglyceride levels for American adults (20 years and older) are 115.0 mg/dL and 144.2 mg/dL, respectively (*American Heart Association, 2011*).

For HDL-C, 60 mg/dL and above is considered to be protective against heart disease. Low HDL-C levels (below 40 mg/dL in males and below 50 mg/dL in females) can increase a person's risk of heart disease. HDL-C levels range between 40 and 50 mg/dL and between 50 and 60 mg/dL in average men and women, respectively, with an average of 54.3 mg/dL in American adults 20 years and older (*American Heart Association, 2011*). Studies have shown that an increase in HDL-C of 1 mg/dL is associated with a decreased risk of coronary artery disease of 2% in men and 3% in women (Boes et al., 2009).

1.2 CHOLESTEROL

The common form of the HDL particle is composed of a hydrophobic core, which is made up of cholesteryl esters (CEs) and small amounts of triglycerides, surrounded by a monolayer of phospholipids, unesterified cholesterol, and apolipoproteins (Weissglas-Volkov et al., 2010). The most predominant apolipoproteins (apos) found in HDL particles are apoAI (approximately 70%) and apoAII, although the particles contain also minor apolipoproteins (apoAIV, apoCI, apoCII, apoCIII, apoD, apoE, apoJ, apoL, and apoM) as well as antioxidants and lipid metabolism-related enzymes, such as PON1, LCAT, and CETP (Weissglas-Volkov et al., 2010, Kontush et al., 2006).

The highly heterogeneous HDL particles differ in shape, size, density, composition, and surface charge (Weissglas-Volkov et al., 2010). The discoidal form of HDL consists of apoAI/AII molecules enclosed in a monolayer of phospholipids and free cholesterol. By esterification of the free cholesterol, the discoidal form converts into the larger spherical form that has a hydrophobic core containing CEs and small amounts of triglycerides (Kontush et al., 2006).

The LDL particles are composed of many molecules including apoB100, cholesterol, CEs, phospholipids and low amounts of triglycerides. Previous studies have shown a correlation between oxidized LDL particles and increased risk of CVD. Elevated oxidized LDL concentrations were shown to be associated with the severity of acute coronary syndromes (Park et al., 2011, Ehara et al., 2001) and aerobically trained individuals were found to exhibit lower concentrations of oxidized LDL than sedentary individuals, indicating that an active lifestyle can

reduce the risk of CVD by lowering the concentrations of oxidized LDL (Park et al., 2011, Vinagre et al., 2007).

Triglycerides (TG) are often found in lipoprotein particles and it is believed that triglyceride-rich lipoproteins (TRLs) are associated with an increased risk of CVD. TRLs include chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and some remnant particles. It is believed that these particles may be associated with an increased risk of CVD; however elevated TRLs are also often associated with low HDL-C (Haynes, 2003). Although the exact relationship is unclear due to the strong relationship between HDL-C and TG, according to meta-analyses, a 1-mmol/L (89 mg/dL) elevation in TG levels may be associated with a 14-37% increase in CVD incidence (Haynes, 2003).

1.3 HDL AND REVERSE CHOLESTEROL TRANSPORT

An important role of HDL-C is reverse cholesterol transport (Dastani et al., 2006). Reverse cholesterol transport (RCT) is the process in which cholesterol molecules are taken from peripheral tissues and transported to the liver, where they are then removed via the bililary excretion system. RCT is a multi-step process, involving a number of forms of HDL-C. In the initial step, cholesterol is removed from peripheral cells by poorly-lipidated apoAI in preβ-HDL (Bencharif et al., 2010). Preβ-HDL is able to take up the free cholesterol and phospholipids from the peripheral cells due to the presence of apoAI and its interaction with the ATP binding cassette transporter A1 (ABCA1) (Bencharif et al., 2010).

After taking up the cholesterol, the particles become enriched in esterified cholesterol, by lecithin cholesterol acyl transferase (LCAT), and phospholipids, forming HDL3 particles, which then go on to form HDL2 particles (Bencharif et al., 2010). The CEs in HDL2 particles can be taken up into the liver through the selective scavenger receptor B1 (SR-B1). The apoE-enriched HDL particles can be taken up into the liver via the apoB/apoE LDL receptors (LDLR) (Bencharif et al., 2010).

In addition to RCT, HDL-C also may prevent atherosclerosis through a number of other processes, including by reducing oxidized acyl chains in LDL, inhibiting endothelial cell adhesion molecule expression, stimulating vasodilation and endothelial nitric oxide production, promoting endothelial progenitor cell migration and repair, inhibiting endothelial apoptosis, and inhibiting platelet activation (Toth, 2009). While other forms of cholesterol can induce atherosclerosis when present in elevated levels, these functions of HDL-C help to reduce the amount of lipid disposition in the arteries (Toth, 2009).

1.4 HERITABILITY AND CHOLESTEROL

A number of factors, including age, gender, body mass index (BMI), smoking, alcohol consumption, diet, physical activity level, certain drugs, and metabolic disorders such as insulin resistance and liver disease, have been shown to affect the HDL-C levels (Weissglas-Volkov et al., 2010). Of these factors, the BMI (specifically obesity, which is characterized as a BMI of 30 or higher) has been shown to have the strongest correlation with decreased HDL-C levels (Weissglas-Volkov et al., 2010).

Another major factor shown to influence cholesterol levels is heritability. Based on family and twin studies, HDL-C has an estimated heritability of 40-60% (Weissglas-Volkov et al., 2010), with some studies showing a heritability of up to 80% (Boes et al., 2009). The heritability estimates are also relatively high for other lipid traits, with estimates of 40-65% for LDL-C and 35-50% for TG (Basu et al., 2009).

1.5 GENETICS OF HDL-CHOLESTEROL

Prior to genome-wide association studies (GWAS), genome-wide linkage scans and candidate gene (positional and/or biological) association studies were the main approaches used to unravel the genetic determinants of complex traits such as plasma HDL-C levels. These studies have implicated a number of genes and variants as determinants of plasma HDL-C levels, of which some were more consistently replicated (**Table 1**) while several others yielded inconsistent results.

With the availability of GWAS platforms, it became possible to identify susceptibility variants and genes for complex traits without making *a priori* assumptions. Several GWAS investigating plasma lipid traits primarily in subjects with European ancestry have been published to date (Kathiresan et al., 2007; Willer et al., 2008; Kathiresan et al., 2008b; Kooner et al., 2008; Wallace et al., 2008; Heid et al., 2008; Chasman et al., 2008; Sabatti et al., 2009; Aulchenko et al., 2009; Kathiresan et al., 2009; Teslovich et al., 2010). These GWAS confirmed a number of genes previously implicated in HDL-C metabolism based on functional and/or candidate gene association studies (**Table 1**) as well as identified several new loci and genes

(**Table 2**). A very recent GWAS (Teslovich et al., 2010) that examined >100,000 individuals has further expanded the list of HDL-C levels-associated genes by implicating more than 40 loci, of which 31 were novel.

Table 1. Established HDL-C levels-associated genes that have been implicated prior to GWAS and further confirmed by subsequent GWAS

Gene	Chr	Functional involvement	References
LPL	8p22	Indirect effect on HDL metabolism through hydrolysis of TG-rich lipoproteins, receptor-mediated lipoprotein uptake	Boes et al., 2009; Weissglas-Volkov et al., 2010; NCBI-Gene
ABCA1	9q31	Initial step of HDL formation and RCT	Boes et al., 2009; Teo et al., 2010; Weissglas-Volkov et al., 2010
APOA1-C3-A4-A5	11q23-q24	Components of the HDL particle, cholesterol efflux from tissues, catabolism of TG-rich particles	Boes et al., 2009; Weissglas-Volkov et al., 2010; NCBI-Gene
LIPC	15q21-q23	Hydrolysis of TG and phospholipids, selective uptake of cholesterol ester from HDL	Boes et al., 2009; Weissglas-Volkov et al., 2010
CETP	16q21	Transfer of esterified cholesterol from HDL to apoB-containing particles in exchange for TG	Boes et al., 2009; Teo et al., 2010; Weissglas-Volkov et al., 2010
LCAT	16q22	Catalysis of synthesis of the major portion of cholesteryl esters	Boes et al., 2009; Teo et al., 2010; Weissglas-Volkov et al., 2010
LIPG	18q21	Turnover of HDL components	Boes et al., 2009; Weissglas-Volkov et al., 2010

Table 2. Novel loci associated with HDL-C levels in published GWAS

Genes implicated	Chr	Functional involvement	References
PABPC4	1p34	Regulation of stability of mRNA in activated T cells or regulation of protein translation in platelets	Teslovich et al., 2010; NCBI-Gene
ZNF648	1q25	Transcriptional regulation	Teslovich et al., 2010; NCBI-Gene
GALNT2	1q41-q42	Enzymatic glycosylation of proteins involved in HDL and TG metabolism	Teslovich et al., 2010; Willer et al., NCBI-Gene
APOB	2p24-p23	Main apolipoprotein of chylomicrons and low density lipoproteins	Teslovich et al., 2010; NCBI-Gene
COBLL1	2q24	Unknown	Teslovich et al., 2010
IRS1	2q36	Encodes a protein which is phosphorylated by insulin receptor tyrosine kinase	Teslovich et al., 2010; NCBI-Gene
SLC39A8	4q22-q24	A member of SLC39 family of solute-carrier genes which show structural characteristics of zinc transporters	Teslovich et al., 2010; NCBI-Gene
ARL15	5p15	Unknown	Teslovich et al., 2010
C6orf106	6p21	Unknown	Teslovich et al., 2010
CITED2	6q23	Encodes a protein that inhibits transactivation of HIF1A-induced genes	Teslovich et al., 2010; NCBI-Gene
LPA	6q26	Encodes a serine proteinase that inhibits the activity of tissue-type plasminogen activator I	Teslovich et al., 2010; NCBI-Gene
MLXIPL	7q11	Glucose utilization and energy storage	Teslovich et al., 2010; NCBI-Gene
KLF14	7q32	A member of the Kruppel-like family of transcription factors	Teslovich et al., 2010; NCBI-Gene

Table 2. Continued

Genes implicated	Chr	Functional involvement	References
PPP1R3B	8p23	Encodes a protein that is expressed in liver and skeletal muscle tissue and may be involved in regulating glycogen synthesis in these tissues	Teslovich et al., 2010; NCBI-Gene
TRIB1	8q24	Encodes tribbles-1, which acts as secondary messenger in MAPK-related signaling cascades	Teslovich et al., 2010; NCBI-Gene
TRPS1	8q24	Encodes a transcription factor that represses GATA-regulated genes	Teslovich et al., 2010; NCBI-Gene
TTC39B	9p22	Unknown	Teslovich et al., 2010; Kathiresan et al., 2009
LRP4	11p11	A member of low-density lipoprotein receptor-related protein family	Teslovich et al., 2010; NCBI-Gene
MADD-FOLH1	11p11	MADD encodes a protein involved in apoptosis through interaction with TNF-alpha 1; *FOLH1* encodes a protein that acts as a glutamate carboxypeptidase on alternative substrates	Aulchenko et al., 2009; NCBI-Gene
AMPD3	11p15	A member of AMP deaminase gene family, which acts in the adenylate catabolic pathway	Teslovich et al., 2010; NCBI-Gene
FADS1/2/3	11q12-q13	Encode proteins regulating desaturation of fatty acids	Teslovich et al., 2010; Kathiresan et al., 2009; NCBI-Gene
UBASH3B	11q24	Encodes a protein that inhibits endoctyosis of epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor	Teslovich et al., 2010; NCBI-Gene
PDE3A	12p12	Encodes an enzyme with high affinity for cAMP and cGMP and competitive inhibition of cAMP hydrolytic activity	Teslovich et al., 2010; NCBI-Gene

Table 2. Continued

Genes implicated	Chr	Functional involvement	References
LRP1	12q13-q14	An endocytic receptor involved in many cellular processes, including lipid homeostasis	Teslovich et al., 2010; NCBI-Gene
MVK/MMAB	12q24	Encode enzymes involved in cholesterol synthesis and degradation	Kathiresan et al., 2009; Willer et al., 2008; NCBI-Gene
SCARB1	12q24	Transfer of cholesterol between cells and HDL	Teslovich et al., 2010; NCBI-Gene
ZNF664	12q24	Transcriptional regulation	Teslovich et al., 2010; NCBI-Gene
SBNO1	12q24	Unknown	Teslovich et al., 2010
LACTB	15q22	Encodes a protein from 39S subunit of mitochondrial ribosome	Teslovich et al., 2010; NCBI-Gene
CTCF-PRMT8	16q21-q22	CTCF encodes a transcriptional regulator protein; PRMT8 is involved in arginine methylation, which is necessary for processes such as DNA repair and RNA transcription	Aulchenko et al., 2009; NCBI-Gene
CMIP	16q23	T-cell signaling pathway	Teslovich et al., 2010; NCBI-Gene
STARD3	17q11-q12	A member of subfamily of lipid-trafficking proteins	Teslovich et al., 2010; NCBI-Gene
ABCA8	17q24	A member of the superfamily of ATP-binding cassette transporters	Teslovich et al., 2010; NCBI-Gene
PGS1	17q25	Biosynthesis of the anionic phospholipids phosphatidylglycerol and cardiolipin	Teslovich et al., 2010; NCBI-Gene
MC4R	18q22	Encodes a membrane-bound receptor and member of melanocortin receptor family	Teslovich et al., 2010; NCBI-Gene
ANGPTL4	19p13	Regulation of glucose homeostasis, lipid metabolism	Teslovich et al., 2010; Kathiresan et al.,2009; NCBI-Gene
LOC55908	19p13	Unknown	Teslovich et al., 2010

Table 2. Continued

Genes implicated	Chr	Functional involvement	References
LILRA3	19q13	Encodes a leukocyte Ig-like receptor	Teslovich et al., 2010; NCBI-Gene
HNF4A	20q13	Gluconeogenesis, regulation of glucose metabolism, insulin expression and secretion	Teslovich et al., 2010; Kathiresan et al., 2009; NCBI-Gene
PLTP	20q13	Transfers phospholipids from TG-rich lipoproteins to HDL	Teslovich et al., 2010; NCBI-Gene
UBE2L3	22q11	Targeting of abnormal or short-lived proteins for degradation	Teslovich et al., 2010; NCBI-Gene

1.6 STUDY OBJECTIVES

Genome-wide association studies and their meta-analyses have confirmed several previously implicated genes as well as identified a number of new genes and loci associated with plasma TC, LDL-C, HDL-C, and TG levels. Several established HDL-C-associated genes that have been confirmed also by GWAS have been comprehensively investigated by our group using sequencing and genotyping to test both common and rare variant hypotheses. In this study, we sought to replicate the GWAS signals (primarily those influencing the HDL-C levels) from other genes that have not been targeted by our sequencing effort in three epidemiological samples, U.S. non-Hispanic Whites, U.S. Hispanics, and African Blacks. In each sample, we performed association analyses with all lipid levels (TC, LDL-C, HDL-C, and TG) regardless of previously reported specific associations for selected SNPs.

2.0 SUBJECTS AND METHODS

2.1 SUBJECTS AND LABORATORY MEASUREMENTS

2.1.1 Sample populations

The study consisted of 621 NHW and 413 Hispanic non-diabetic subjects drawn from the San Luis Valley Diabetes Study, a population-based case-control study in the San Luis Valley in Southern Colorado, and 787 African Blacks drawn from a study on CHD-related risk factors in Benin City, Nigeria. Detailed information on these studies and samples can be found elsewhere (Hamman et al., 1989, Rewers et al., 1993, Harris et al., 1998, Bunker et al., 1995 & 1996). The ages of participants ranged from 24 to 75 in NHWs, 21 to 75 in Hispanics, and 19 to 70 in African Blacks. **Table 3** summarizes the demographic and phenotypic characteristics of the subjects included in this study, which was approved by the University of Pittsburgh IRB.

2.1.2 Laboratory methods

Enzymatic methods were used to measure plasma lipoprotein levels and the Friedewald equation was used to calculate LDL cholesterol levels when triglyceride levels were less than 400 mg/dl.

Table 3. Biometric and quantitative data (unadjusted mean ± S.E.) of study samples

Variable	NHWs (*n* = 621)	Hispanics (*n* = 413)	African Blacks (*n* = 787)
Gender (Female/Male)	328/293	209/204	292/495
Age (years)	52.82 ± 0.46	51.17 ± 0.62	40.96 ± 0.30
BMI (kg/m^2)	25.48 ± 0.16	25.76 ± 0.22	22.84 ± 0.14
Total cholesterol (mg/dl)	216.20 ± 1.68	212.79 ± 2.15	171.98 ± 1.39
LDL-C (mg/dl)	138.12 ± 1.53	134.31 ± 1.98	109.23 ± 1.24
HDL-C (mg/dl)	50.79 ± 0.58	48.62 ± 0.65	47.97 ± 0.46
Triglycerides (mg/dl)	139.26 ± 2.80	149.34 ± 3.46	71.80 ± 1.24

2.2 SNP SELECTION AND GENOTYPING

2.2.1 SNP selection

Our group is comprehensively investigating (using deep sequencing and genotyping) several HDL-C levels-associated genes that have been initially implicated by candidate gene association

studies and subsequently confirmed by GWAS. The purpose of this study was to replicate the HDL-C-related GWAS signals from the genes that have not been targeted by our sequencing effort. We analyzed a total of 40 SNPs selected from following publications; Willer et al., 2008, Aulchenko et al., 2009, Kathiresan et al., 2009, and Teslovich et al., 2010. The SNPs chosen for this study are listed in **Table 4**: the primarily implicated genes are shown in **bold** and the associated alleles and related traits are shown in color (red: increasing effect, blue: decreasing effect). Alleles (whether on forward or reverse strands) reflect those stated in the original GWAS. Given our group's focus on genetics of plasma HDL-C levels, we primarily targeted those SNPs (n=36) that reached genome-wide level of significance for association with HDL-C levels. From two studies (Willer et al., 2008, Aulchenko et al., 2009), we also included 4 SNPs (highlighted in gray) that did not reach genome-wide level of significance for HDL-C but they were either highly significant for HDL-C or modestly significant for HDL-C but genome-wide level significant for other lipids. Whenever there were more than one significant SNP reported for a given locus by the same group and/or various groups, only one SNP was selected for our study. Although all these GWAS primarily investigated individuals of European ancestry (EU), one of them (Teslovich et al., 2010) also sought replication in various non-European populations including African Americans. Whenever the information was available, the effects observed in African Americans were shown as 'concordant' (AA) or 'discordant' (AA*). Of 40 SNPs selected from these GWAS in individuals of European ancestry (EU), all were analyzed in NHWs and Hispanics whereas only a subset (n=34) with sufficient frequency was analyzed in Africans.

Table 4. SNPs selected from 4 recently published GWAS in individuals of European ancestry (EU) for replication in our multiethnic sample. For HDL-C, p-values ranged from $7.7x10^{-4}$ to 0.02 for 4 SNPs (highlighted in gray) but were ≤ $5x10^{-8}$ for all other SNPs as well as for other lipid traits included in the table. When available, replication results in African Africans (AA) were shown (*=discordant).

SNP	Chr	Gene(s)	Alleles	Associated lipid trait with genome-wide level of significance	Population	Reference(s)
rs646776	1p13	*PSRC1,* ***CELSR2***	A/G	TC, LDL-C	EU	Aulchenko et al., 1/09
rs10889353	1p31	***DOCK7***	A/C	TC, TG	EU	Aulchenko et al., 1/09
rs10903129	1p36	***TMEM57***	G/A	TC	EU	Aulchenko et al., 1/09
rs4660293	1p34	***PABPC4***	A/G	HDL-C	EU, AA	Teslovich et al., 8/10
rs1689800	1q25	*LOC100130996,* ***ZNF648***	A/G	HDL-C	EU, AA*	Teslovich et al., 8/10
rs2144300	1q42	***GALNT2***	C/T	HDL-C	EU	Willer et al., 2/08
rs1042034	2p24	***APOB***	T/C	HDL-C, TG	EU, AA	Teslovich et al., 8/10
rs12328675	2q24	***COBLL1***	T/C	HDL-C	EU, AA	Teslovich et al., 8/10
rs2972146	2q36	***IRS1***	T/G	HDL-C, TG	EU, AA	Teslovich et al., 8/10
rs13107325	4q24	***SLC39A8***	C/T	HDL-C	EU, AA*	Teslovich et al., 8/10
rs6450176	5q11	***ARL15***	G/A	HDL-C	EU, AA*	Teslovich et al., 8/10
rs2814944	6p21	***C6orf106***	G/A	HDL-C	EU, AA*	Teslovich et al., 8/10
rs605066	6q24	***CITED2***	T/C	HDL-C	EU, AA	Teslovich et al., 8/10
rs17145738	7q11	*TBL2,* ***MLXIPL***	C/T	HDL-C, TG	EU, AA*	Teslovich et al., 8/10
rs4731702	7q32	***KLF14***	C/T	HDL-C	EU, AA	Teslovich et al., 8/10
rs9987289	8p23	***PPP1R3B***	G/A	HDL-C, LDL-C, TC	EU, AA	Teslovich et al., 8/10
rs2293889	8q23	***TRPS1***	G/T	HDL-C	EU, AA	Teslovich et al., 8/10
rs471364	9p22	*C9orf52,* ***TTC39B***	T/C	HDL-C	EU	Kathiresan et al., 1/09

Table 4. Continued

SNP	Chr	Gene(s)	Alleles	Associated lipid trait with genome-wide level of significance	Population	Reference(s)
rs1323432	9q31	*PPP3R2,* ***GRIN3A***	A/G	HDL-C	EU	Willer et al., 2/08
rs7395662	11p11	*OR4A47,* ***MADD-FOLH1***	G/A	HDL-C	EU	Aulchenko et al., 1/09
rs3136441	11p11	*F2,* ***LRP4***	T/C	HDL-C	EU	Teslovich et al., 8/10
rs2923084	11p15	***AMPD3***	A/G	HDL-C	EU, AA	Teslovich et al., 8/10
rs174547	11q12	***FADS1-FADS2-FADS3***	T/C	HDL-C, TG	EU	Kathiresan et al., 1/09
rs7941030	11q24	*STS-1,* ***UBASH3B***	T/C	HDL-C, TC	EU, AA	Teslovich et al., 8/10
rs7134375	12p12	***PDE3A***	C/A	HDL-C	EU, AA	Teslovich et al., 8/10
rs2338104	12q24	*KCTD10,* ***MMAB, MVK***	G/C	HDL-C	EU	Kathiresan et al., 1/09; Willer et al., 2/08
rs11613352	12q13	***LRP1***	C/T	HDL-C, TG	EU, AA	Teslovich et al., 8/10
rs2652834	15q22	***LACTB***	G/A	HDL-C	EU	Teslovich et al., 8/10
rs2271293	16q22	*NUTF2,* ***CTCF-PRMT8***	G/A	HDL-C	EU	Aulchenko et al., 1/09
rs2925979	16q23	***CMIP***	C/T	HDL-C	EU, AA	Teslovich et al., 8/10
rs11869286	17q12	***STARD3***	C/G	HDL-C	EU, AA	Teslovich et al., 8/10
rs4148008	17q24	***ABCA8***	C/G	HDL-C	EU, AA	Teslovich et al., 8/10
rs4129767	17q25	***PGS1***	A/G	HDL-C	EU, AA	Teslovich et al., 8/10
rs12967135	18q21	***MC4R***	G/A	HDL-C	EU, AA	Teslovich et al., 8/10
rs2967605	19p13	*RAB11B,* ***ANGPTL4***	C/T	HDL-C	EU	Kathiresan et al., 1/09
rs737337	19p13	***LOC55908***	T/C	HDL-C	EU, AA	Teslovich et al., 8/10
rs386000	19q13	***LILRA3****, LILRB3*	G/C	HDL-C	EU, AA*	Teslovich et al., 8/10
rs1800961	20q13	***HNF4A***	C/T	HDL-C, TC	EU, AA*	Kathiresan et al., 1/09; Teslovich et al., 8/10
rs6065906	20q13	*FLJ40606,* ***PLTP****, PCIF1*	T/C	HDL-C, TG	EU, AA	Teslovich et al., 8/10
rs181362	22q11	***UBE2L3***	C/T	HDL-C	EU, AA	Teslovich et al., 8/10

2.2.2 Genotyping

DNAs were extracted from either buffy coats (NHWs and Hispanics) or blood clots (African Blacks) using standard methods. Samples were whole-genome amplified using the GenomiPhi DNA Amplification Kit (GE Healthcare Bio-Sciences, Piscataway, NJ) prior to genotyping. Twenty SNPs were genotyped using the TaqMan method (Applied Biosystems, Foster City, CA) in 621 NHWs, 413 Hispanics, and 787 African Blacks. Another twenty were genotyped using the iPLEX Gold technology (Sequenom, San Diego, CA) in 621 NHWs, 382 Hispanics, and 787 African Blacks. Depending on the genotyping method used, about 7-10% of samples were repeated to test the consistency of genotype calls for each assay.

2.2.2.1 TaqMan SNP genotyping

TaqMan uses the 5' nuclease assay chemistry and involves two primers for amplifying the target sequence and two TaqMan® minor groove binder (MGB) probes for discriminating the alleles. The allele-specific TaqMan® MGB probes contain a reporter dye (VIC or FAM) at the 5’ end and a non-fluorescent quencher (NFQ) at the 3’ end. During the PCR, the probes bind specifically to their target sequences in between the forward and reverse primer binding sites. The genotype is determined based on the changes occurring in fluorescence of the dyes as the PCR proceeds. The allele-specific probes are cleaved by the 5’ nuclease activity of Taq DNA polymerase, allowing the reporter dye to separate from the quencher dye, which results with increasing fluorescence by the reporter dye during the PCR.

The TaqMan SNP genotyping assays (Applied Biosystems) that were used in this study are listed in **Table 5**.

Table 5. SNPs genotyped by TaqMan

Ref SNP ID	Assay Type	Assay ID	Alleles VIC/FAM	Alleles dbSNP	Genotyped Samples
rs646776	Functionally Tested	C_3160062_10	C/T	A/G	NHWs, HSPs, ABs
rs10889353	Functionally Tested	C_31145250_10	A/C	A/C	NHWs, HSPs, ABs
rs1689800	Functionally Tested	C_8859423_10	A/G	C/T	NHWs, HSPs, ABs
rs2144300	Functionally Tested	C_32309821_10	C/T	C/T	NHWs, HSPs, ABs
rs12328675	Functionally Tested	C_31900020_10	C/T	C/T	NHWs, HSPs, ABs
rs9987289	Functionally Tested	C_1614468_10	A/G	A/G	NHWs, HSPs, ABs
rs2293889	Functionally Tested	C_16185476_20	G/T	G/T	NHWs, HSPs, ABs
rs471364	Functionally Tested	C_621313_10	C/T	A/G	NHWs, HSPs, ABs
rs1323432	Functionally Tested	C_8783661_20	A/G	C/T	NHWs, HSPs
rs174547	Functionally Tested	C_2292336_10	C/T	C/T	NHWs, HSPs
rs7941030	Functionally Tested	C_147231_10	C/T	C/T	NHWs, HSPs, ABs
rs2338104	Functionally Tested	C_2877290_10	C/G	C/G	NHWs, HSPs, ABs
rs2271293	Functionally Tested	C_15959906_10	A/G	A/G	NHWs, HSPs, ABs
rs4148008	Validated	C_1174418_10	C/G	C/G	NHWs, HSPs, ABs
rs4129767	Functionally Tested	C_26076172_10	A/G	C/T	NHWs, HSPs, ABs
rs12967135	Functionally Tested	C_3058682_10	A/G	A/G	NHWs, HSPs, ABs
rs2967605	Validated	C_11513719_10	C/T	A/G	NHWs, HSPs, ABs
rs737337	Functionally Tested	C_8727006_10	C/T	C/T	NHWs, HSPs, ABs
rs1800961	Functionally Tested	C_7591528_10	C/T	C/T	NHWs, HSPs
rs181362	Validated	C_622868_20	C/T	A/G	NHWs, HSPs, ABs

NHWs: Non-Hispanic Whites, HSPs: Hispanics, ABs: African Blacks

To begin, TaqMan genotyping master mix and assay were added to 384-well plates containing dried DNA samples. The plates were then subjected to PCR on Applied Biosystems' GeneAmp 9700 instruments. The reaction and cycling conditions are provided in **Table 6**. An ABI Prism 7900HT instrument (Applied Biosystems) was used for end-point fluorescence readings. **Figure 1** shows the cluster plot from one of the TaqMan assays used in this study.

Table 6. TaqMan reaction and cycling conditions

TaqMan reaction (total volume of 5 µL)		PCR Conditions
dH_20	2.435 µL	1. 95° C for 10 minutes
TaqMan Genotyping Master Mix (2x)	2.5 µL	2. 95° C for 15 seconds
TaqMan Genotyping Assay Mix (40x)	0.065 µL	3. 60° C for 1 minute repeat steps 2-3 49x

2.2.2.2. iPLEX Gold Assay for SNP genotyping

The Sequenom iPLEX Gold genotyping was performed at the University of Pittsburgh Genomics and Proteomics Core Laboratories (GPCL). The iPLEX Gold assay uses a single-base primer extension chemistry and MALDI-TOF MS detection system and allows multiplexing for up to 36

SNPs in one rea:tion. The process results with alel speci1ic mass differences between the extension produas,which are deteaed by the da:a cnaty'sis software.

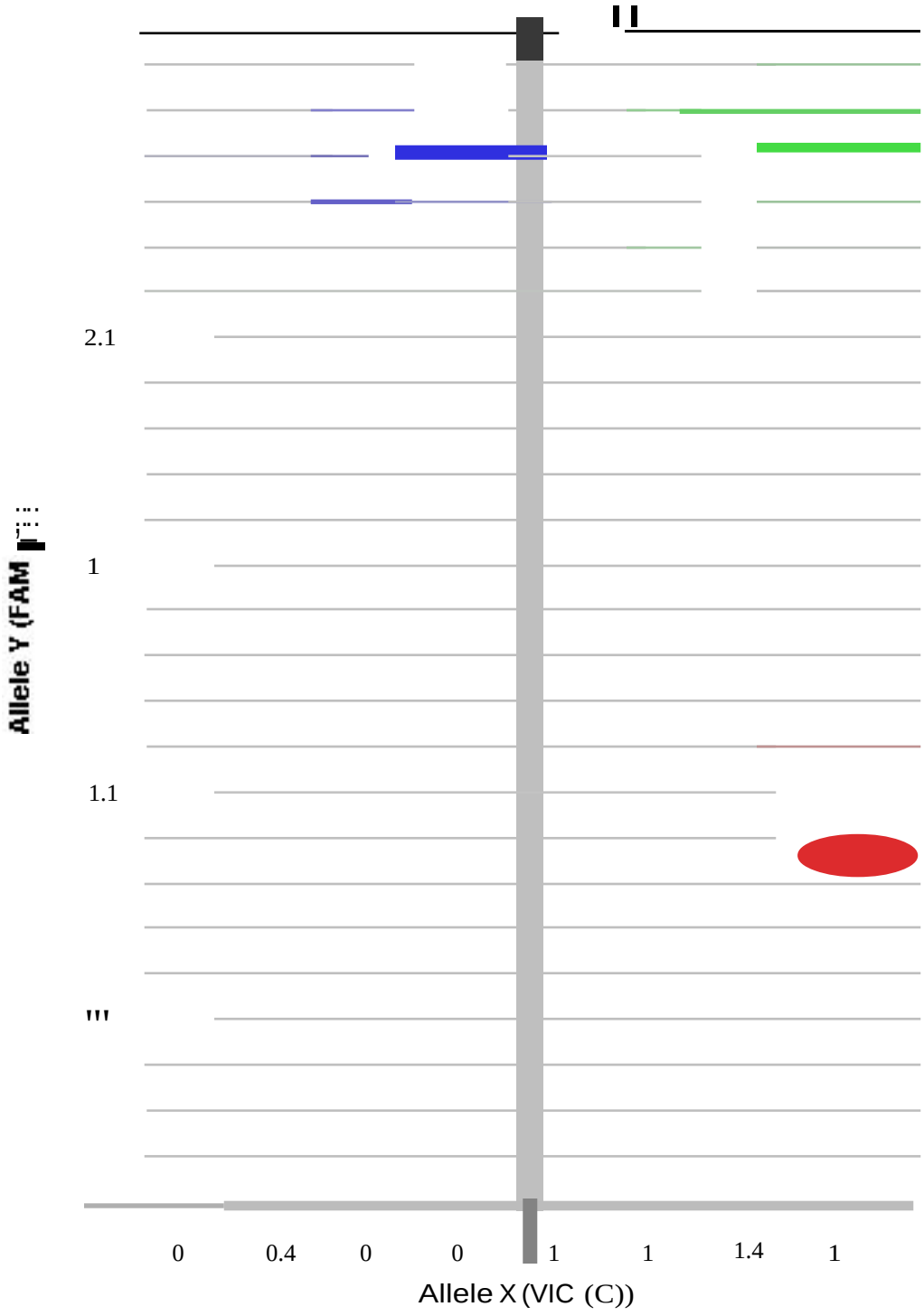

Figure 1. The duster pbt from one of the TaqMan assays used in this study

The SNPs genotyped by iPLEX Gold assay and the **PCR** and extension primers used are presented in **Table 7. Figure 2** shows the genotype clustering for one of the SNPs typed by iPLEX Gold as part of this study.

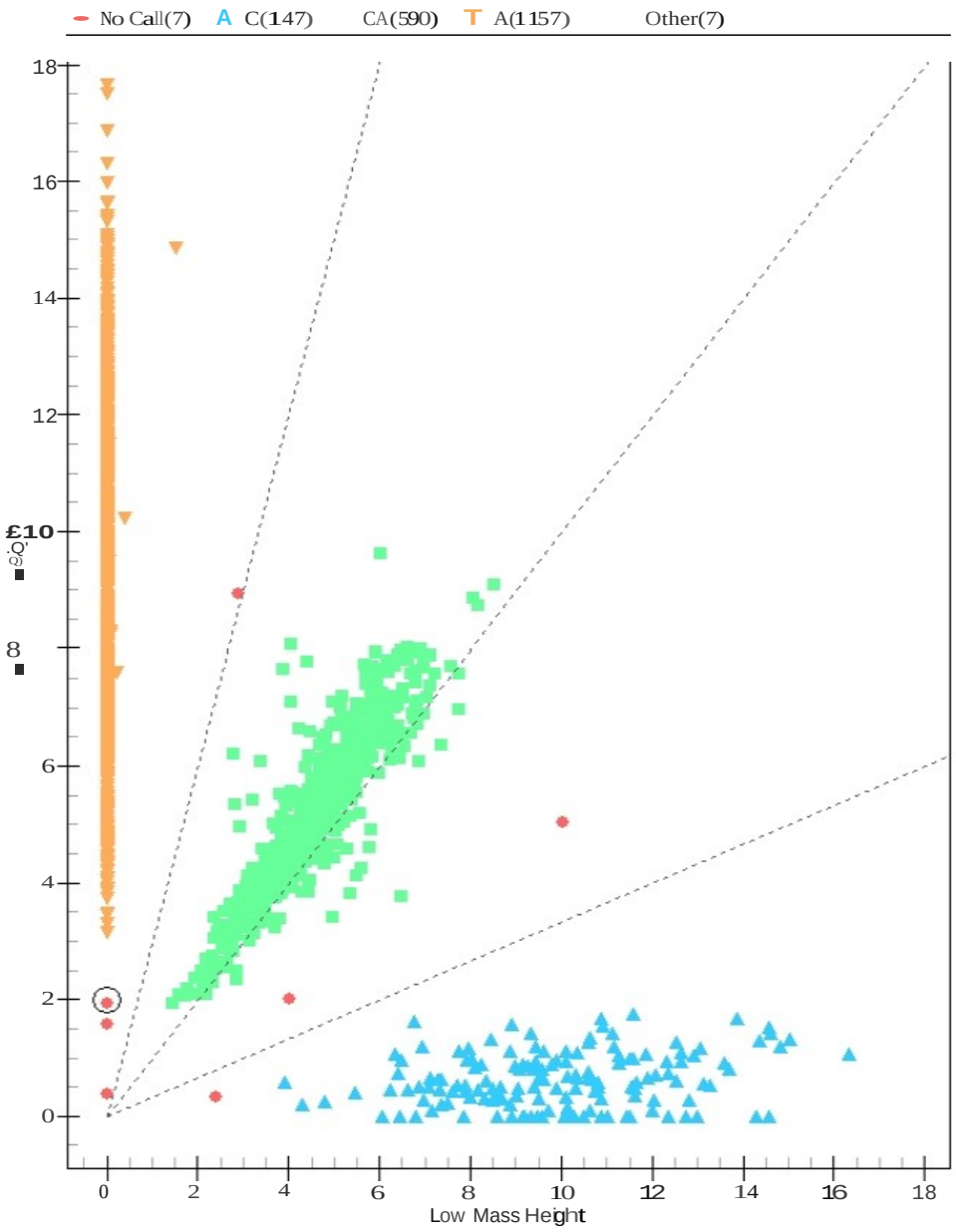

Figure 2. The cluster plot from one of the study SNPs genotyped by iPLEX Gold assay

Table 7. SNPs genotyped by iPLEX Gold

Ref SNP ID	PCR Primer 1	PCR Primer 2	Extension Primer	Genotyped Samples
rs10903129	ACGTTGGATGCCACAGACTCATTTCTGTAG	ACGTTGGATGCCTTAGTACCTGACCTTTTC	ggaAGACTAATGGCCATTTAGTTCA	NHWs, HSPs, ABs
rs4660293	ACGTTGGATGTGGTGGTTGAGTGCCTGTTC	ACGTTGGATGTTTAGAGCAAGGACAGCCAC	gggCTCCTCTCCTCTTTCACA	NHWs, HSPs
rs1042034	ACGTTGGATGGGCATAGGTTTTCTTTCAACA	ACGTTGGATGATCCAAGATGAGATCAACAC	ATGAGATCAACACAATCTTCA	NHWs, HSPs, ABs
rs2972146	ACGTTGGATGATCAGGATATGGGATATGAG	ACGTTGGATGCTCACACTAGGGCAAATATC	taaGGCAAATATCTTAACTTGTTGAT	NHWs, HSPs, ABs
rs13107325	ACGTTGGATGCTATGTTGGGCTAGCTTTTG	ACGTTGGATGCCTCCAGCAAGTGCAAATAT	AAGTGCAAATATAATATTTGGAG	NHWs, HSPs
rs6450176	ACGTTGGATGAGAAATGTGTCCTTGGCTAC	ACGTTGGATGGCTTTATTCCATGTGTGTCC	ggggATGTGTGTCCTGGATCTG	NHWs, HSPs, ABs
rs2814944	ACGTTGGATGGGATAGGAGTTCTGATGAGG	ACGTTGGATGGTCTCGGCGTGTTAGTTAAG	TGTTAGTTAAGGCTTCCCC	NHWs, HSPs, ABs
rs605066	ACGTTGGATGCAGTGAAAATGGGTCACTAA	ACGTTGGATGATTTCTCTCTCCACAGATT	ccCTCCACAGATTGCCTAT	NHWs, HSPs, ABs
rs17145738	ACGTTGGATGTCCCCCCTCTAAATGCTATG	ACGTTGGATGCTTACACCCAGGTAACTGAC	cccaTGACCCTTCACACATTTA	NHWs, HSPs, ABs
rs4731702	ACGTTGGATGAATCAAAATTAAAAAACAGC	ACGTTGGATGATCTTTTTGGTGCTAAATG	agTTTTGGTGCTAAATGGAACTG	NHWs, HSPs, ABs
rs7395662	ACGTTGGATGGATTTTCCCTGCATGCTAGT	ACGTTGGATGCTCCAGGAAAGCCATTTGTG	CCATTTGTGATTATTTAATTGCC	NHWs, HSPs, ABs
rs3136441	ACGTTGGATGCACTGTGTTCTGAAGGCACC	ACGTTGGATGGGGAGACCCTGTCTCTTATA	aaCCTGTCTCTTATAAATAAACAAAG	NHWs, HSPs
rs2923084	ACGTTGGATGTGTCCTTCCCTGCTAACTGA	ACGTTGGATGCTATCGGGTGTCATTCACAG	ACAGAAAGGCTGGACATACA	NHWs, HSPs, ABs
rs7134375	ACGTTGGATGACAGGAAAACTGACCTGACG	ACGTTGGATGCCCTCTGATATTCCTTGCTC	gaTCTTGCTAAGAGAGACATC	NHWs, HSPs, ABs
rs11613352	ACGTTGGATGAGCCACCGCACCCAGCCCT	ACGTTGGATGTGCCAGATATGACTGCCATC	ctTGTAAATTTTACCTTCTGAGAAA	NHWs, HSPs, ABs
rs2652834	ACGTTGGATGGCGGGGAGAGGATTTTCTG	ACGTTGGATGGCCTGATATGATAGGATTAG	gAGAGTTCTTATAAGAAGAGACACA	NHWs, HSPs, ABs
rs2925979	ACGTTGGATGCGCAGTAATTTGTGTTGGTG	ACGTTGGATGTCCTTAGCAAGACAAGACTG	ttAAATGCAATCTCCAACCCCATC	NHWs, HSPs, ABs
rs11869286	ACGTTGGATGTCTCTGGGACAATTTGGATG	ACGTTGGATGACAAAGCCCCCTTCAGAGAG	AGGGAAGATAAGACCCCT	NHWs, HSPs, ABs
rs386000	ACGTTGGATGGACTCACGACATAAACTAAG	ACGTTGGATGACTTGGCCTTTCTCGAATGC	cctgTCTCGAATGCTTTATCTGCATCG	NHWs, HSPs, ABs
rs6065906	ACGTTGGATGAGGAGAAAGCTTCAGAGGAG	ACGTTGGATGCCCTTTTCCTTGCCTACCTG	CCTGGAAAATCTATTTTTCCAA	NHWs, HSPs, ABs

NHWs: Non-Hispanic Whites, HSPs: Hispanics, ABs: African Blacks

2.3 STATISTICAL METHODS

Concordance of the genotype distribution to Hardy-Weinberg equilibrium (HWE) was tested for each variant using a χ^2 goodness-of-fit test. When it was necessary to reduce effects of non-normality, the dependent quantitative variables were transformed using either log or square root transformation. The significant covariates for each dependent variable were identified using both backward and forward stepwise regression. To test for the effects of genotypes on the means of the quantitative traits, a linear regression analysis was performed (under the additive model) and the results were adjusted for certain covariates. For NHWs and Hispanics, the covariates were sex, age, BMI, and smoking. For African Blacks, the covariates were sex, age, waist, jobmin (minutes walking or bicycling to work each day), and staff (staff level – junior or senior). The R statistical software package (version 2.12.2, http://www.r-project.org) was used to perform all analyses. Because this was a replication study, we considered a nominal p-value of less than 0.05 as evidence of association.

3.0 RESULTS

3.1 GENOTYPING CALL RATES AND HWE TESTING RESULTS

Minor allele frequencies (MAFs) of the genotyped SNPs are shown in **Table 8** along with the genotyping call rates and the HWE testing results.

Most SNPs differed in allele frequencies across various ethnic groups. For 10 SNPs (highlighted in gray), it was not always the same allele that was the minor allele across various ethnic groups. The SNPs with relatively less common minor alleles (MAF<5%) are shown in blue color.

The call rates for the assays were overall very high (≥95%) and only a small number of SNPs had lower call rates: 1 in NHWs (87%), 3 in Hispanics (between 92-95%), and 2 in African Blacks (between 92-95%). No SNP showed low call rates in all populations genotyped. Discrepancy was detected for only one assay (rs174547) for which the rate was 0.5%.

Five of 40 SNPs had p-values less than 0.05 for deviation from HWE in at least one population studied (*italics* in Table 8) but none of them would remain significant after correction for multiple testing. Three of these SNPs (rs4660293, rs2972146, and rs4731702) had HWE p-values ≤ 0.05 for at least one population listed in the dbSNP database.

Table 8. Allele frequencies, call rates and HWE testing results for genotyped SNPs

SNP	NHWs				Hispanics				African Blacks			
	HWE *P*	Call rate	MAF	Alleles	HWE *P*	Call rate	MAF	Alleles	HWE *P*	Call rate	MAF	Alleles
rs646776	0.223	0.989	0.212	T:C	0.585	0.973	0.258	T:C	0.887	0.994	0.348	T:C
rs10889353	0.635	0.981	0.337	A:C	0.924	0.988	0.365	A:C	0.324	0.989	0.447	A:C
rs10903129	0.819	0.995	0.439	G:A	0.175	0.995	0.492	G:A	0.228	0.982	0.222	G:A
rs4660293	*0.023*	0.997	0.212	A:G	*0.035*	0.992	0.206	A:G	NA	N/A	NA	NA
rs1689800	0.125	0.994	0.347	A:G	1.000	0.995	0.346	A:G	0.216	0.987	0.264	A:G
rs2144300	0.690	0.965	0.384	T:C	0.195	0.993	0.425	T:C	1.000	0.980	0.040	C:T
rs1042034	0.366	0.970	0.215	A:G	0.669	0.984	0.298	A:G	1.000	0.925	0.119	A:G
rs12328675	0.058	0.989	0.143	T:C	1.000	0.990	0.101	T:C	0.914	0.982	0.201	T:C
rs2972146	0.572	0.998	0.377	A:C	*0.003*	0.997	0.230	A:C	0.524	0.996	0.123	A:C
rs13107325	0.899	0.995	0.068	C:T	0.928	1.000	0.045	C:T	NA	N/A	NA	NA
rs6450176	0.599	0.989	0.240	G:A	1.000	0.995	0.296	G:A	0.870	0.977	0.317	G:A
rs2814944	0.551	1.000	0.163	G:A	0.636	0.995	0.132	G:A	0.784	0.974	0.342	G:A
rs605066	0.439	0.994	0.403	T:C	0.991	0.992	0.406	T:C	0.783	0.982	0.402	C:T
rs17145738	0.543	0.995	0.108	C:T	0.846	1.000	0.071	C:T	0.603	0.995	0.086	C:T
rs4731702	0.121	0.998	0.494	C:T	0.234	0.997	0.436	C:T	*0.006*	0.985	0.184	C:T
rs9987289	1.000	0.997	0.091	G:A	0.423	0.998	0.178	G:A	0.693	0.938	0.191	G:A
rs2293889	0.370	0.992	0.442	G:T	0.636	0.993	0.445	G:T	0.232	0.992	0.040	G:T
rs471364	0.997	0.990	0.125	T:C	0.116	0.939	0.105	T:C	0.852	0.981	0.206	T:C
rs1323432	0.307	0.987	0.115	A:G	0.731	0.988	0.087	A:G	NA	N/A	NA	NA
rs7395662	0.861	0.992	0.373	G:A	0.633	0.992	0.323	G:A	0.994	0.978	0.407	A:G

Table 8. Continued

SNP	NHWs				Hispanics				African Blacks			
	HWE *P*	Call rate	MAF	Alleles	HWE *P*	Call rate	MAF	Alleles	HWE *P*	Call rate	MAF	Alleles
rs3136441	0.470	0.989	0.137	T:C	0.522	0.987	0.268	T:C	NA	N/A	NA	NA
rs2923084	0.311	0.998	0.179	A:G	1.000	0.992	0.307	A:G	0.253	0.985	0.474	G:A
rs174547	0.184	0.973	0.345	T:C	0.318	0.922	0.441	C:T	NA	N/A	NA	NA
rs7941030	0.095	0.868	0.367	T:C	1.000	0.995	0.294	T:C	1.000	0.996	0.444	T:C
rs7134375	0.488	0.994	0.458	C:A	0.105	0.990	0.497	A:C	0.876	0.990	0.308	C:A
rs2338104	0.683	0.992	0.429	G:C	1.000	0.976	0.486	G:C	0.625	0.981	0.196	G:C
rs11613352	0.349	0.990	0.245	C:T	0.879	0.995	0.382	C:T	0.739	0.987	0.063	C:T
rs2652834	0.364	1.000	0.188	C:T	0.557	1.000	0.154	C:T	0.138	0.975	0.338	C:T
rs2271293	0.905	0.984	0.121	G:A	0.961	0.985	0.155	G:A	0.450	0.986	0.081	G:A
rs2925979	0.490	0.998	0.287	G:A	0.979	1.000	0.203	G:A	0.189	0.977	0.293	G:A
rs11869286	0.992	0.994	0.357	C:G	0.959	0.995	0.387	C:G	0.171	0.994	0.172	G:C
rs4148008	0.989	0.987	0.332	C:G	0.050	0.995	0.272	C:G	0.371	0.978	0.409	G:C
rs4129767	0.285	0.990	0.498	G:A	0.583	0.988	0.461	A:G	0.508	0.985	0.313	G:A
rs12967135	0.320	0.997	0.235	G:A	0.983	1.000	0.175	G:A	*0.010*	0.983	0.324	G:A
rs2967605	0.570	0.997	0.169	C:T	0.885	0.956	0.214	C:T	1.000	0.982	0.237	C:T
rs737337	0.355	0.994	0.079	T:C	0.689	0.993	0.267	T:C	0.269	0.981	0.483	C:T
rs386000	*0.003*	0.987	0.203	C:G	*0.026*	0.992	0.437	C:G	1.000	0.988	0.183	C:G
rs1800961	0.958	0.995	0.032	C:T	0.121	0.947	0.033	C:T	NA	N/A	NA	NA
rs6065906	0.556	0.995	0.173	T:C	1.000	0.990	0.097	T:C	0.134	0.979	0.158	T:C
rs181362	1.000	0.992	0.201	C:T	1.000	0.993	0.366	C:T	0.913	0.990	0.445	C:T

3.2 ASSOCIATION ANALYSES WITH LIPID TRAITS

A total of 40 SNPs were genotyped and analyzed in NHWs and Hispanics, of which 34 were analyzed in African Blacks. Genotype associations with lipid traits (TC, LDL-C, HDL-C, TG levels) were tested under additive model using linear regression analysis and certain covariates and transformation methods in each ethnic group. The dependent quantitative variables with skewed distributions were transformed as follows: the 'log10' transformation was used for the HDL-C and TG levels in NHWs and the HDL-C levels in Hispanics, the 'natural log' transformation was used for the TG levels in Hispanics and the TC and TG levels in African Blacks, and the 'square root' transformation was used for the LDL-C and HDL-C levels in African Blacks. The covariates were 'sex, age, BMI, and smoking' in NHWs and Hispanics and 'sex, age, waist, jobmin (minutes walking or bicycling to work each day), and staff (staff level – junior or senior)' in African Blacks.

Tables 9, 10, 11 show the genotype counts, adjusted p-values and effect size estimates (for minor alleles) in relation to 4 lipid traits analyzed in NHWs, Hispanics, and African Blacks, respectively. Significant p-values (<0.05) are shown in red and marginal ones (between 0.05-0.10) in blue. For the SNPs highlighted in gray, the other allele was the minor allele in Hispanics or African Blacks as compared to NHWs. For these SNPs, the effect size estimates were not made for the same allele across various ethnic groups since the genotypic effects were modeled as the additive effect of the minor allele (which was not the same in all populations). A summary of the association results for 4 lipid traits in all 3 ethnic groups is provided in **Table 12** for an easy cross-sample comparison.

A total of 22 SNPs showed significant association (p-values less than 0.05) with at least one lipid trait in at least one ethnic group (total 40 significant p-values), several of which had also marginal p-values (0.05-0.10) for additional lipid traits. Although our SNP selection was biased toward including primarily HDL-C-related variants, we ended up identifying similar number of significant associations with TC (n=10), LDL-C (n=12), and HDL-C (n=10), but relatively less with TG (n=6). An additional 5 SNPs had only marginal p-values (0.05-0.10) for at least one lipid trait in at least one ethnic group. Of these 27 SNPs, 9 were associated with at least one lipid trait in more than one ethnic group, although not always with the same trait across various ethnic groups.

In NHWs, 12 SNPs showed significant association (p<0.05) with at least one lipid trait. The most significant SNP for each trait was: *GRIN3A*/rs1323432 with TC (p=0.002) & LDL-C (p=0.009), *KLF14*/rs4731702 with HDL-C (p=0.003), and *DOCK7*/rs10889353 with TG (p=4.9x10^{-5}) levels. Six SNPs (*DOCK7*/rs10889353, *GALNT2*/rs2144300, *NUTF2*/rs2271293, *GRIN3A*/rs1323432, *CELSR2*/rs646776, and *PGS1*/rs4129767) were significantly associated with more than one lipid trait.

In Hispanics, 10 SNPs showed significant association (p<0.05) with at least one lipid trait. The most significant SNP for each trait was: *APOB*/rs1042034 with TC (p=2.0x10^{-4}) & LDL-C (p=8.0x10^{-5}), *PPP1R3B*/rs9987289 with HDL-C (p=0.011), and *DOCK7*/rs10889353 with TG (p=0.004) levels. Three SNPs (*APOB*/rs1042034, *OR4A47*/rs7395662, and *PGS1*/rs4129767) were significantly associated with more than one lipid trait.

In African Blacks, 7 SNPs showed significant association (p<0.05) with at least one lipid trait. The most significant SNP for each trait was: *CMIP*/rs2925979 with TC (p=0.008),

PPP1R3B/rs9987289 with LDL-C (*p*=0.010), *KCTD10*/rs2338104 with HDL-C (*p*=0.002), and *UBASH3B*/rs7941030 with TG (*p*=0.043) levels. Two SNPs (*PPP1R3B*/rs9987289, *CMIP*/rs2925979) were significantly associated with more than one lipid trait.

Of 40 SNPs analyzed (34 in African Blacks), 25 showed either significant (p-values less than 0.05) or marginal (p-values between 0.05-0.10) association or trend for association (p-values between 0.10-0.20) in at least one ethnic group that we studied, all in the same direction as that seen for the same genome-wide level significant traits in the original GWAS (please see the Discussion section and **Table 13** for more details). For 2 SNPs, we obtained discordant results for association with the HDL-C levels. For 10 SNPs, we observed significant association with lipid traits other than those reported as genome-wide level significant in the original GWAS. Three of these 10 SNPs did not show any association or trend for association with the traits identified as genome-wide level significant in the original reports.

Table 9. Genotype distributions (GT), adjusted p-values and effect size estimates (for minor alleles) for 40 SNPs in relation to 4 lipid traits in NHWs

NHWs SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log10)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs646776	TT	385	-6.801	0.012	382	-5.754	0.023	387	0.003	0.711	384	-0.005	0.722
	TC	192			192			193			193		
	CC	33			32			33			33		
rs10889353	AA	270	-5.183	0.031	268	-2.223	0.320	270	$-9.2x10^{-5}$	0.988	270	-0.046	**$4.9x10^{-5}$**
	AC	264			262			266			265		
	CC	72			72			72			71		
rs10903129	GG	189	1.767	0.448	188	1.680	0.437	192	-0.008	0.186	190	0.018	0.099
	GA	307			305			307			306		
	AA	117			116			117			117		
rs4660293	AA	392	0.796	0.765	388	0.673	0.785	393	$3.3x10^{-4}$	0.961	391	0.002	0.902
	GA	186			186			187			187		
	GG	38			38			38			38		
rs1689800	AA	251	-2.747	0.268	251	-2.481	0.279	253	-0.010	0.121	251	0.013	0.261
	AG	298			294			298			297		
	GG	64			64			65			65		
rs2144300	TT	229	4.585	0.051	227	4.498	0.040	230	-0.011	0.060	228	0.030	**0.006**
	TC	275			273			277			276		
	CC	91			91			91			91		

Table 9. Continued

NHWs SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log10)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs1042034	AA	372	-2.519	0.361	371	-2.721	0.285	374	0.006	0.398	374	-0.019	0.142
	AG	195			193			195			193		
	GG	32			32			32			32		
rs12328675	TT	454	-3.158	0.315	453	-4.021	0.169	456	0.002	0.814	455	0.009	0.522
	TC	138			135			138			137		
	CC	19			19			19			19		
rs2972146	AA	242	0.726	0.756	240	1.337	0.536	244	0.005	0.373	241	-0.004	0.710
	CA	283			280			283			283		
	CC	91			92			92			92		
rs13107325	CC	531	-1.391	0.762	527	-3.258	0.444	534	0.022	0.064	531	-0.013	0.554
	CT	80			80			80			80		
	TT	2			2			2			2		
rs6450176	GG	354	1.573	0.552	353	1.193	0.627	357	0.003	0.668	355	-0.002	0.859
	GA	218			215			218			217		
	AA	38			38			38			38		
rs2814944	GG	433	-1.491	0.623	429	-0.349	0.901	436	-0.007	0.377	433	-0.002	0.879
	AG	165			165			165			165		
	AA	19			19			19			19		
rs605066	TT	211	-4.002	0.092	209	-4.946	0.025	213	0.003	0.675	212	0.001	0.918
	TC	307			306			307			306		
	CC	95			94			95			95		

Table 9. Continued

NHWs SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log10)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs17145738	CC	489	2.133	0.558	486	4.142	0.219	492	0.011	0.219	489	-0.041	0.016
	CT	115			114			115			115		
	TT	9			9			9			9		
rs4731702	CC	147	0.120	0.960	145	-0.881	0.689	148	0.018	**0.003**	146	-0.012	0.302
	TC	328			325			329			328		
	TT	140			141			141			141		
rs9987289	GG	509	0.971	0.808	504	1.565	0.671	510	0.002	0.867	508	0.006	0.757
	GA	101			102			103			102		
	AA	5			5			5			5		
rs2293889	GG	186	-0.410	0.861	183	0.715	0.743	186	-0.005	0.399	186	-0.013	0.258
	GT	315			315			316			315		
	TT	112			111			113			112		
rs471364	TT	468	-2.177	0.532	465	2.511	0.437	470	-0.016	0.063	469	-0.016	0.344
	TC	133			132			134			132		
	CC	10			10			10			10		
rs1323432	AA	474	11.295	**0.002**	472	8.951	**0.009**	476	-0.003	0.754	473	0.026	0.141
	AG	130			128			131			131		
	GG	5			5			5			5		
rs7395662	GG	239	2.869	0.225	238	3.129	0.154	240	-2.9×10^{-6}	1.000	238	0.008	0.484
	AG	289			287			291			290		
	AA	83			82			83			83		

Table 9. Continued

NHWs SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log10)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs3136441	TT	458	-0.523	0.874	457	-0.607	0.843	460	0.006	0.469	458	0.013	0.420
	TC	138			135			139			138		
	CC	14			14			14			14		
rs2923084	AA	419	2.969	0.307	418	3.145	0.243	422	-0.015	0.048	419	0.020	0.135
	GA	173			170			173			173		
	GG	24			24			24			24		
rs174547	TT	248	-3.992	0.112	249	-2.996	0.196	250	-0.010	0.098	249	-0.011	0.371
	TC	289			285			289			288		
	CC	63			63			64			63		
rs7941030	TT	224	5.359	0.030	223	3.490	0.131	226	0.005	0.410	225	0.021	0.067
	TC	230			229			231			230		
	CC	82			80			82			81		
rs7134375	CC	186	1.439	0.525	185	0.531	0.799	186	0.011	0.049	186	-0.009	0.412
	CA	295			292			297			294		
	AA	132			132			133			133		
rs2338104	GG	204	0.357	0.877	204	0.705	0.740	204	-0.005	0.383	204	0.006	0.601
	GC	295			291			295			293		
	CC	114			114			116			116		
rs11613352	CC	343	1.291	0.635	341	0.244	0.923	345	-0.002	0.722	345	-0.003	0.815
	TC	235			233			236			233		
	TT	32			32			32			32		

Table 9. Continued

NHWs SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log10)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs2652834	CC	403	-0.576	0.847	400	-0.532	0.848	405	-0.001	0.918	404	0.006	0.682
	TC	196			195			197			195		
	TT	18			18			18			18		
rs2271293	GG	468	7.170	0.044	464	4.285	0.192	471	0.023	**0.009**	468	-0.003	0.843
	GA	131			131			131			131		
	AA	8			8			8			8		
rs2925979	GG	319	1.514	0.544	316	3.074	0.184	319	-0.007	0.273	317	-0.014	0.236
	AG	243			242			246			245		
	AA	54			54			54			54		
rs11869286	CC	252	3.194	0.182	251	2.940	0.185	254	-0.006	0.286	252	0.009	0.431
	CG	281			278			282			281		
	GG	79			79			79			79		
rs4148008	CC	273	1.800	0.458	270	2.287	0.310	273	$3.4x10^{-4}$	0.957	271	0.008	0.488
	CG	269			269			271			271		
	GG	68			67			68			68		
rs4129767	GG	148	3.112	0.183	146	3.988	0.065	148	-0.014	0.015	147	0.027	0.013
	GA	320			319			321			320		
	AA	144			143			145			145		
rs12967135	GG	364	-4.651	0.078	361	-3.709	0.130	367	-0.001	0.848	364	-0.018	0.152
	GA	213			212			213			213		
	AA	39			39			39			39		

Table 9. Continued

NHWs SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log10)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs2967605	CC	426	1.575	0.598	422	1.256	0.649	429	-0.003	0.678	426	0.007	0.625
	CT	169			169			169			169		
	TT	20			20			20			20		
rs737337	TT	521	-2.436	0.555	517	-4.911	0.198	524	0.014	0.180	521	0.012	0.527
	TC	86			86			86			86		
	CC	6			6			6			6		
rs386000	CC	401	1.725	0.524	398	3.353	0.180	402	-0.003	0.629	399	-0.005	0.703
	GC	170			169			172			172		
	GG	37			37			37			37		
rs1800961	CC	575	-0.463	0.943	571	1.910	0.748	578	-0.009	0.568	575	-0.028	0.345
	CT	38			38			38			38		
	TT	1			1			1			1		
rs6065906	TT	423	3.707	0.213	421	2.263	0.416	424	-0.001	0.921	422	0.010	0.461
	CT	171			170			172			172		
	CC	21			20			21			21		
rs181362	CC	391	0.284	0.921	388	0.948	0.721	393	-0.002	0.790	391	-0.002	0.896
	CT	196			195			197			196		
	TT	25			25			25			25		

Table 10. Genotype distributions (GT), adjusted p-values and effect size estimates (for minor alleles) for 40 SNPs in relation to 4 lipid traits in Hispanics

Hispanics SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs646776	TT	217	1.515	0.675	214	0.712	0.830	217	0.012	0.163	217	-0.044	0.218
	TC	159			159			158			159		
	CC	24			24			23			24		
rs10889353	AA	164	-4.087	0.190	162	-2.515	0.382	163	0.008	0.305	164	-0.088	**0.004**
	AC	187			186			186			187		
	CC	55			55			55			55		
rs10903129	GG	105	0.283	0.926	105	-0.553	0.842	105	0.011	0.110	105	-0.013	0.669
	GA	176			175			175			176		
	AA	99			97			99			99		
rs4660293	AA	232	1.972	0.630	230	3.258	0.386	231	-0.010	0.278	232	-0.004	0.917
	GA	138			137			138			138		
	GG	9			9			9			9		
rs1689800	AA	175	-0.820	0.798	173	-0.014	0.996	175	-0.007	0.378	175	-0.002	0.945
	AG	185			184			184			185		
	GG	49			49			48			49		
rs2144300	TT	128	1.537	0.631	126	0.636	0.830	128	-0.005	0.534	128	0.020	0.533
	TC	213			212			212			213		
	CC	67			67			66			67		

Table 10. Continued

Hispanics SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs1042034	AA	183	-12.814	$2.0x10^{-4}$	182	-12.508	$8.0x10^{-5}$	183	-0.005	0.528	183	-0.015	0.669
	AG	162			161			161			162		
	GG	31			30			31			31		
rs12328675	TT	305	4.136	0.425	302	1.913	0.686	304	0.007	0.559	305	0.054	0.289
	TC	68			68			68			68		
	CC	4			4			4			4		
rs2972146	AA	237	-1.986	0.561	235	-0.623	0.842	237	-0.005	0.562	237	-0.054	0.111
	CA	113			112			112			113		
	CC	31			31			31			31		
rs13107325	CC	348	-3.310	0.667	345	0.236	0.973	347	-0.032	0.066	348	0.018	0.812
	CT	34			34			34			34		
rs6450176	GG	188	-3.270	0.337	187	-2.020	0.517	187	-0.005	0.514	188	-0.015	0.662
	GA	159			157			159			159		
	AA	33			33			33			33		
rs2814944	GG	288	-6.484	0.156	285	-7.810	0.062	287	-0.008	0.428	288	0.060	0.181
	AG	84			84			84			84		
	AA	8			8			8			8		
rs605066	TT	134	1.230	0.699	134	1.408	0.628	134	-0.010	0.170	134	0.035	0.266
	TC	182			179			181			182		
	CC	63			63			63			63		

Table 10. Continued

Hispanics SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs17145738	CC	329	1.020	0.869	327	2.486	0.663	328	-0.001	0.940	329	-0.046	0.448
	CT	52			51			52			52		
	TT	1			1			1			1		
rs4731702	CC	115	0.638	0.845	115	1.459	0.625	114	-0.009	0.222	115	0.016	0.617
	TC	200			198			200			200		
	TT	66			65			66			66		
rs9987289	GG	275	-7.966	0.051	273	-6.099	0.106	273	-0.025	0.011	275	0.038	0.340
	GA	125			124			125			125		
	AA	10			10			10			10		
rs2293889	GG	123	1.852	0.551	123	2.085	0.467	122	0.005	0.506	123	0.003	0.914
	GT	207			205			206			207		
	TT	78			77			78			78		
rs471364	TT	306	-6.466	0.233	304	-3.277	0.515	304	-0.015	0.253	306	-0.015	0.776
	TC	79			78			79			79		
	CC	1			1			1			1		
rs1323432	AA	339	7.703	0.148	336	4.455	0.364	338	0.027	0.038	339	-0.013	0.799
	AG	63			63			62			63		
	GG	4			4			4			4		
rs7395662	GG	176	-8.075	0.014	176	-7.407	0.014	175	0.008	0.289	176	-0.042	0.204
	AG	161			159			161			161		
	AA	42			41			42			42		

Table 10. Continued

Hispanics SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs3136441	TT	199	-4.952	0.168	196	-4.207	0.202	198	-0.006	0.444	199	-0.002	0.950
	TC	154			154			154			154		
	CC	24			24			24			24		
rs2923084	AA	182	-3.556	0.289	181	-2.051	0.508	182	-0.004	0.591	182	-0.005	0.870
	GA	161			160			160			161		
	GG	36			35			36			36		
rs174547	CC	124	5.627	0.072	122	5.716	0.048	124	0.001	0.901	124	-0.012	0.711
	CT	177			176			176			177		
	TT	78			78			77			78		
rs7941030	TT	204	-0.860	0.797	202	-0.729	0.814	203	-0.005	0.511	204	0.035	0.292
	TC	170			170			169			170		
	CC	35			34			35			35		
rs7134375	AA	87	-3.594	0.269	86	-3.243	0.278	87	0.003	0.695	87	-0.013	0.687
	CA	206			205			205			206		
	CC	85			84			85			85		
rs2338104	GG	106	-5.109	0.098	104	-5.855	0.041	106	0.002	0.777	106	-0.010	0.751
	GC	201			201			200			201		
	CC	94			93			93			94		
rs11613352	CC	144	1.589	0.621	144	1.545	0.599	144	-0.004	0.569	144	0.014	0.669
	TC	182			179			181			182		
	TT	54			54			54			54		

Table 10. Continued

Hispanics SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs2652834	CC	271	-2.073	0.638	269	-3.853	0.340	271	-0.009	0.368	271	0.041	0.341
	TC	104			103			103			104		
	TT	7			7			7			7		
rs2271293	GG	288	2.092	0.626	287	3.055	0.440	287	0.006	0.549	288	-0.033	0.427
	GA	108			106			107			108		
	AA	9			9			9			9		
rs2925979	GG	242	-4.750	0.224	241	-3.317	0.355	241	-0.008	0.383	242	-0.016	0.687
	AG	125			123			125			125		
	AA	15			15			15			15		
rs11869286	CC	142	0.465	0.885	140	0.435	0.883	142	-0.003	0.732	142	0.006	0.845
	CG	182			181			181			182		
	GG	56			56			56			56		
rs4148008	CC	209	3.567	0.323	207	3.112	0.351	208	-0.003	0.694	209	0.022	0.539
	CG	178			177			177			178		
	GG	22			22			22			22		
rs4129767	AA	114	6.496	0.038	114	6.770	0.019	114	-0.007	0.385	114	0.034	0.269
	AG	209			208			207			209		
	GG	83			81			83			83		
rs12967135	GG	280	6.168	0.121	277	6.215	0.090	280	-0.010	0.295	280	0.009	0.824
	GA	118			118			116			118		
	AA	13			13			13			13		

Table 10. Continued

Hispanics SNP	GT	TC			LDL-C			HDL-C (log10)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs2967605	CC	241	-1.284	0.734	238	0.781	0.823	241	-0.012	0.189	241	-0.033	0.377
	CT	135			135			133			135		
	TT	17			17			17			17		
rs737337	TT	221	-4.401	0.195	219	-6.843	0.029	221	-0.003	0.723	221	0.041	0.220
	TC	156			155			155			156		
	CC	31			31			30			31		
rs386000	CC	109	4.067	0.224	108	2.619	0.396	109	$1.2x10^{-4}$	0.988	109	0.045	0.175
	GC	209			208			209			209		
	GG	61			60			60			61		
rs1800961	CC	365	-11.381	0.169	363	-9.706	0.211	363	0.005	0.794	365	-0.096	0.238
	CT	22			21			22			22		
	TT	2			2			2			2		
rs6065906	TT	308	2.927	0.583	306	1.173	0.811	307	-0.018	0.138	308	0.106	0.044
	CT	67			66			67			67		
	CC	3			3			3			3		
rs181362	CC	165	1.535	0.625	165	1.257	0.664	164	0.003	0.684	165	0.005	0.878
	CT	188			185			187			188		
	TT	55			55			55			55		

Table 11. Genotype distributions (GT), adjusted p-values and effect size estimates (for minor alleles) for 34 SNPs in relation to 4 lipid traits in African Blacks

Blacks SNP	GT	TC (log)			LDL-C (sqrt)			HDL-C (sqrt)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs646776	TT	325	0.006	0.616	324	-0.044	0.601	322	0.062	0.199	328	0.004	0.857
	TC	337			339			336			341		
	CC	92			94			92			94		
rs10889353	AA	223	-0.003	0.790	225	-0.004	0.964	222	-0.029	0.546	226	-0.028	0.172
	AC	383			385			380			387		
	CC	144			143			144			146		
rs10903129	GG	459	0.002	0.897	460	0.048	0.621	456	0.014	0.800	463	0.005	0.829
	GA	242			243			241			246		
	AA	43			44			43			44		
rs1689800	AA	415	-0.017	0.170	416	-0.141	0.117	414	0.019	0.713	419	0.009	0.689
	AG	275			277			273			279		
	GG	59			59			58			60		
rs2144300	CC	684	0.039	0.172	686	0.233	0.260	680	0.100	0.401	692	-0.001	0.990
	CT	58			59			58			59		
	TT	1			1			1			1		
rs1042034	AA	544	-0.012	0.504	544	-0.102	0.432	542	0.078	0.300	551	-0.050	0.118
	AG	148			151			146			150		
	GG	10			10			10			10		

Table 11. Continued

Blacks SNP	GT	TC (log)			LDL-C (sqrt)			HDL-C (sqrt)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs12328675	TT	477	-0.016	0.260	475	-0.160	0.119	474	-0.002	0.968	481	0.022	0.383
	TC	241			244			240			244		
	CC	28			29			28			29		
rs2972146	AA	578	0.006	0.733	579	0.019	0.879	575	-0.035	0.622	584	0.027	0.361
	CA	155			156			154			157		
	CC	14			14			14			14		
rs6450176	GG	346	-0.003	0.832	345	0.004	0.967	343	-0.040	0.425	352	0.003	0.906
	GA	316			320			316			319		
	AA	77			77			76			77		
rs2814944	GG	318	0.005	0.681	320	0.055	0.527	317	-0.057	0.250	320	0.027	0.202
	AG	324			325			321			329		
	AA	87			86			87			88		
rs605066	CC	261	0.013	0.248	260	0.105	0.208	260	0.014	0.776	264	0.001	0.969
	TC	352			354			349			355		
	TT	122			123			122			125		
rs17145738	CC	627	-0.001	0.962	631	0.075	0.611	624	-0.044	0.605	635	0.014	0.697
	CT	122			121			121			123		
	TT	4			4			4			4		
rs4731702	CC	496	-0.011	0.425	501	-0.126	0.212	492	0.012	0.840	506	0.012	0.620
	TC	203			202			203			202		
	TT	38			36			38			38		

Table 11. Continued

Blacks SNP	GT	TC (log)			LDL-C (sqrt)			HDL-C (sqrt)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs9987289	GG	467	-0.038	**0.009**	467	-0.273	0.010	465	-0.065	0.276	472	0.035	0.177
	GA	216			218			214			219		
	AA	28			28			28			28		
rs2293889	GG	697	-0.012	0.680	700	0.014	0.947	693	-0.168	0.148	706	-0.002	0.969
	GT	53			53			53			53		
	TT	3			3			3			3		
rs471364	TT	468	-0.009	0.524	471	-0.060	0.551	466	0.014	0.813	475	-0.002	0.948
	TC	245			244			243			246		
	CC	32			33			32			33		
rs7395662	AA	260	-0.002	0.848	261	-0.011	0.898	259	0.025	0.608	261	-0.016	0.448
	AG	359			359			357			364		
	GG	121			123			120			124		
rs2923084	GG	212	0.006	0.578	215	0.032	0.693	212	0.019	0.688	216	0.006	0.744
	GA	354			348			352			355		
	AA	172			177			170			175		
rs7941030	TT	234	-0.011	0.315	234	-0.084	0.302	233	0.038	0.412	237	-0.040	0.043
	TC	374			374			371			377		
	CC	148			151			148			151		
rs7134375	CC	354	0.006	0.611	357	0.013	0.885	351	0.059	0.240	358	-0.002	0.910
	CA	315			313			314			318		
	AA	73			74			73			74		

Table 11. Continued

Blacks SNP	GT	TC (log)			LDL-C (sqrt)			HDL-C (sqrt)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs2338104	GG	479	-0.010	0.477	481	0.089	0.390	477	-0.187	**0.002**	487	-0.043	0.094
	GC	239			240			237			240		
	CC	26			26			26			26		
rs11613352	CC	656	-0.008	0.713	660	-0.250	0.132	652	0.198	0.035	665	0.054	0.183
	TC	88			87			88			88		
	TT	4			4			4			4		
rs2652834	CC	326	0.009	0.429	330	0.055	0.522	325	0.013	0.792	333	0.001	0.945
	TC	311			310			310			311		
	TT	94			93			92			95		
rs2271293	GG	631	0.010	0.635	634	0.126	0.387	628	0.090	0.284	640	-0.056	0.120
	GA	110			110			109			110		
	AA	7			7			7			7		
rs2925979	GG	374	-0.032	**0.008**	373	-0.203	0.022	371	-0.053	0.290	378	-0.016	0.459
	AG	288			291			287			292		
	AA	70			70			70			71		
rs11869286	GG	521	-0.022	0.128	526	-0.191	0.069	521	-0.106	0.077	530	0.034	0.182
	CG	203			202			199			203		
	CC	28			27			28			28		
rs4148008	GG	252	-0.007	0.541	258	-0.007	0.938	251	-0.008	0.874	255	0.005	0.817
	GC	375			370			372			377		
	CC	115			117			115			119		

Table 11. Continued

Blacks SNP	GT	TC (log)			LDL-C (sqrt)			HDL-C (sqrt)			TG (log)		
		Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*	Counts	beta	*P*
rs4129767	GG	354	0.027	0.026	355	0.135	0.120	353	0.064	0.205	359	0.009	0.685
	GA	315			315			312			317		
	AA	78			80			78			80		
rs12967135	GG	324	0.018	0.163	325	0.127	0.166	322	0.126	0.017	327	-0.029	0.206
	GA	358			362			356			364		
	AA	64			62			64			64		
rs2967605	CC	435	0.013	0.324	436	0.123	0.199	432	-0.003	0.959	439	-0.007	0.780
	CT	270			270			269			273		
	TT	40			42			40			42		
rs737337	CC	202	0.012	0.284	205	0.050	0.536	201	0.048	0.300	206	0.003	0.881
	CT	359			357			358			362		
	TT	183			185			181			185		
rs386000	CC	497	0.003	0.857	502	-0.025	0.810	495	0.082	0.169	505	-0.027	0.292
	GC	226			224			224			227		
	GG	26			26			26			26		
rs6065906	TT	524	-0.012	0.432	525	-0.088	0.424	521	-0.060	0.336	530	0.019	0.487
	CT	184			186			183			186		
	CC	25			24			25			25		
rs181362	CC	228	0.005	0.673	233	0.074	0.365	227	-0.017	0.710	233	0.012	0.547
	CT	373			372			372			377		
	TT	150			149			148			150		

Table 12. Summary of SNP associations with 4 lipid traits in our multi-ethnic study samples

Chr Gene(s)	SNP Traits	NHWs			Hispanics			African Blacks		
		MAF	Association		MAF	Association		MAF	Association	
1p13	**rs646776**	**C - 0.212**	**beta**	**p-value**	**C - 0.258**	**beta**	**p-value**	**C - 0.348**	**beta**	**p-value**
PSRC1	TC		-6.801	0.012		1.515	0.675		0.006	0.616
CELSR2	LDL-C		-5.754	0.023		0.712	0.830		-0.044	0.601
	HDL-C		0.003	0.711		0.012	0.163		0.062	0.199
	TG		-0.005	0.722		-0.044	0.218		0.004	0.857
1p31	**rs10889353**	**C - 0.337**	**beta**	**p-value**	**C - 0.365**	**beta**	**p-value**	**C - 0.447**	**beta**	**p-value**
DOCK7	TC		-5.183	0.031		-4.087	0.190		-0.003	0.790
	LDL-C		-2.223	0.320		-2.515	0.382		-0.004	0.964
	HDL-C		-9.2×10^{-5}	0.988		0.008	0.305		-0.029	0.546
	TG		-0.046	**4.9×10^{-5}**		-0.088	**0.004**		-0.028	0.172
1p36	**rs10903129**	**A - 0.439**	**beta**	**p-value**	**A - 0.492**	**beta**	**p-value**	**A - 0.222**	**beta**	**p-value**
TMEM57	TC		1.767	0.448		0.283	0.926		0.002	0.897
	LDL-C		1.680	0.437		-0.553	0.842		0.048	0.621
	HDL-C		-0.008	0.186		0.011	0.110		0.014	0.800
	TG		0.018	0.099		-0.013	0.669		0.005	0.829
1p34	**rs4660293**	**G - 0.212**	**beta**	**p-value**	**G - 0.206**	**beta**	**p-value**			
PABPC4	TC		0.796	0.765		1.972	0.630			
	LDL-C		0.673	0.785		3.258	0.386			
	HDL-C		3.3×10^{-4}	0.961		-0.010	0.278			
	TG		0.002	0.902		-0.004	0.917			
1q25	**rs1689800**	**G - 0.347**	**beta**	**p-value**	**G - 0.346**	**beta**	**p-value**	**G - 0.264**	**beta**	**p-value**
ZNF648	TC		-2.747	0.268		-0.820	0.798		-0.017	0.170
	LDL-C		-2.481	0.279		-0.014	0.996		-0.141	0.117
	HDL-C		-0.010	0.121		-0.007	0.378		0.019	0.713
	TG		0.013	0.261		-0.002	0.945		0.009	0.689

Table 12. Continued

Chr Gene(s)	SNP Traits	NHWs			Hispanics			African Blacks		
		MAF	Association		MAF	Association		MAF	Association	
1q42	**rs2144300**	**C - 0.384**	**beta**	**p-value**	**C - 0.425**	**beta**	**p-value**	**T - 0.040**	**beta**	**p-value**
GALNT2	TC		4.585	0.051		1.537	0.631		0.039	0.172
	LDL-C		4.498	0.040		0.636	0.830		0.233	0.260
	HDL-C		-0.011	0.060		-0.005	0.534		0.100	0.401
	TG		0.030	**0.006**		0.020	0.533		-0.001	0.990
2p24	**rs1042034**	**G - 0.215**	**beta**	**p-value**	**G - 0.298**	**beta**	**p-value**	**G - 0.119**	**beta**	**p-value**
APOB	TC		-2.519	0.361		-12.814	**2.0×10^{-4}**		-0.012	0.504
	LDL-C		-2.721	0.285		-12.508	**8.0×10^{-5}**		-0.102	0.432
	HDL-C		0.006	0.398		-0.005	0.528		0.078	0.300
	TG		-0.019	0.142		-0.015	0.669		-0.050	0.118
2q24	**rs12328675**	**C - 0.143**	**beta**	**p-value**	**C - 0.101**	**beta**	**p-value**	**C - 0.201**	**beta**	**p-value**
COBLL1	TC		-3.158	0.315		4.136	0.425		-0.016	0.260
	LDL-C		-4.021	0.169		1.913	0.686		-0.160	0.119
	HDL-C		0.002	0.814		0.007	0.559		-0.002	0.968
	TG		0.009	0.522		0.054	0.289		0.022	0.383
2q36	**rs2972146**	**C - 0.377**	**beta**	**p-value**	**C - 0.230**	**beta**	**p-value**	**C - 0.123**	**beta**	**p-value**
IRS1	TC		0.726	0.756		-1.986	0.561		0.006	0.733
	LDL-C		1.337	0.536		-0.623	0.842		0.019	0.879
	HDL-C		0.005	0.373		-0.005	0.562		-0.035	0.622
	TG		-0.004	0.710		-0.054	0.111		0.027	0.361
4q24	**rs13107325**	**T - 0.068**	**beta**	**p-value**	**T - 0.045**	**beta**	**p-value**			
SLC39A8	TC		-1.391	0.762		-3.310	0.667			
	LDL-C		-3.258	0.444		0.236	0.973			
	HDL-C		0.022	0.064		-0.032	0.066			
	TG		-0.013	0.554		0.018	0.812			
5q11	**rs6450176**	**A - 0.240**	**beta**	**p-value**	**A - 0.296**	**beta**	**p-value**	**A - 0.317**	**beta**	**p-value**
ARL15	TC		1.573	0.552		-3.270	0.337		-0.003	0.832
	LDL-C		1.193	0.627		-2.020	0.517		0.004	0.967
	HDL-C		0.003	0.668		-0.005	0.514		-0.040	0.425
	TG		-0.002	0.859		-0.015	0.662		0.003	0.906

Table 12. Continued

Chr Gene(s)	SNP Traits	NHWs			Hispanics			African Blacks		
		MAF	Association		MAF	Association		MAF	Association	
6p21	**rs2814944**	**A - 0.163**	**beta**	**p-value**	**A - 0.132**	**beta**	**p-value**	**A - 0.342**	**beta**	**p-value**
C6orf106	TC		-1.491	0.623		-6.484	0.156		0.005	0.681
	LDL-C		-0.349	0.901		-7.810	0.062		0.055	0.527
	HDL-C		-0.007	0.377		-0.008	0.428		-0.057	0.250
	TG		-0.002	0.879		0.060	0.181		0.027	0.202
6q24	**rs605066**	**C - 0.403**	**beta**	**p-value**	**C - 0.406**	**beta**	**p-value**	**T - 0.402**	**beta**	**p-value**
CITED2	TC		-4.002	0.092		1.230	0.699		0.013	0.248
LOC645434	LDL-C		-4.946	0.025		1.408	0.628		0.105	0.208
	HDL-C		0.003	0.675		-0.010	0.170		0.014	0.776
	TG		0.001	0.918		0.035	0.266		0.001	0.969
7q11	**rs17145738**	**T - 0.108**	**beta**	**p-value**	**T - 0.071**	**beta**	**p-value**	**T - 0.086**	**beta**	**p-value**
TBL2	TC		2.133	0.558		1.020	0.869		-0.001	0.962
MLXIPL	LDL-C		4.142	0.219		2.486	0.663		0.075	0.611
	HDL-C		0.011	0.219		-0.001	0.940		-0.044	0.605
	TG		-0.041	0.016		-0.046	0.448		0.014	0.697
7q32	**rs4731702**	**T - 0.494**	**beta**	**p-value**	**T - 0.436**	**beta**	**p-value**	**T - 0.184**	**beta**	**p-value**
KLF14	TC		0.120	0.960		0.638	0.845		-0.011	0.425
	LDL-C		-0.881	0.689		1.459	0.625		-0.126	0.212
	HDL-C		0.018	**0.003**		-0.009	0.222		0.012	0.840
	TG		-0.012	0.302		0.016	0.617		0.012	0.620
8p23	**rs9987289**	**A - 0.091**	**beta**	**p-value**	**A - 0.178**	**beta**	**p-value**	**A - 0.191**	**beta**	**p-value**
PPP1R3B	TC		0.971	0.808		-7.966	0.051		-0.038	**0.009**
	LDL-C		1.565	0.671		-6.099	0.106		-0.273	0.010
	HDL-C		0.002	0.867		-0.025	0.011		-0.065	0.276
	TG		0.006	0.757		0.038	0.340		0.035	0.177
8q23	**rs2293889**	**T - 0.442**	**beta**	**p-value**	**T - 0.445**	**beta**	**p-value**	**T - 0.040**	**beta**	**p-value**
TRPS1	TC		-0.410	0.861		1.852	0.551		-0.012	0.680
	LDL-C		0.715	0.743		2.085	0.467		0.014	0.947
	HDL-C		-0.005	0.399		0.005	0.506		-0.168	0.148
	TG		-0.013	0.258		0.003	0.914		-0.002	0.969

Table 12. Continued

Chr Gene(s)	SNP Traits	NHWs			Hispanics			African Blacks		
		MAF	Association		MAF	Association		MAF	Association	
9p22	**rs471364**	**C - 0.125**	**beta**	**p-value**	**C - 0.105**	**beta**	**p-value**	**C - 0.206**	**beta**	**p-value**
C9orf52	TC		-2.177	0.532		-6.466	0.233		-0.009	0.524
TTC39B	LDL-C		2.511	0.437		-3.277	0.515		-0.060	0.551
	HDL-C		-0.016	0.063		-0.015	0.253		0.014	0.813
	TG		-0.016	0.344		-0.015	0.776		-0.002	0.948
9q31	**rs1323432**	**G - 0.115**	**beta**	**p-value**	**G - 0.087**	**beta**	**p-value**			
PPP3R2	TC		11.295	**0.002**		7.703	0.148			
GRIN3A	LDL-C		8.951	**0.009**		4.455	0.364			
	HDL-C		-0.003	0.754		0.027	0.038			
	TG		0.026	0.141		-0.013	0.799			
11p11	**rs7395662**	**A - 0.373**	**beta**	**p-value**	**A - 0.323**	**beta**	**p-value**	**G - 0.407**	**beta**	**p-value**
OR4A47	TC		2.869	0.225		-8.075	0.014		-0.002	0.848
MADD-FOLH1	LDL-C		3.129	0.154		-7.407	0.014		-0.011	0.898
	HDL-C		$-2.9x10^{-6}$	1.000		0.008	0.289		0.025	0.608
	TG		0.008	0.484		-0.042	0.204		-0.016	0.448
11p11	**rs3136441**	**C - 0.137**	**beta**	**p-value**	**C - 0.268**	**beta**	**p-value**			
F2	TC		-0.523	0.874		-4.952	0.168			
LRP4	LDL-C		-0.607	0.843		-4.207	0.202			
	HDL-C		0.006	0.469		-0.006	0.444			
	TG		0.013	0.420		-0.002	0.950			
11p15	**rs2923084**	**G - 0.179**	**beta**	**p-value**	**G - 0.307**	**beta**	**p-value**	**A - 0.474**	**beta**	**p-value**
AMPD3	TC		2.969	0.307		-3.556	0.289		0.006	0.578
	LDL-C		3.145	0.243		-2.051	0.508		0.032	0.693
	HDL-C		-0.015	0.048		-0.004	0.591		0.019	0.688
	TG		0.020	0.135		-0.005	0.870		0.006	0.744
11q12	**rs174547**	**C - 0.345**	**beta**	**p-value**	**T - 0.441**	**beta**	**p-value**			
FADS1	TC		-3.992	0.112		5.627	0.072			
FADS2	LDL-C		-2.996	0.196		5.716	0.048			
FADS3	HDL-C		-0.010	0.098		0.001	0.901			
	TG		-0.011	0.371		-0.012	0.711			

Table 12. Continued

Chr Gene(s)	SNP Traits	NHWs			Hispanics			African Blacks		
		MAF	Association		MAF	Association		MAF	Association	
11q24	**rs7941030**	**C - 0.367**	**beta**	**p-value**	**C - 0.294**	**beta**	**p-value**	**C - 0.444**	**beta**	**p-value**
UBASH3B	TC		5.359	0.030		-0.860	0.797		-0.011	0.315
	LDL-C		3.490	0.131		-0.729	0.814		-0.084	0.302
	HDL-C		0.005	0.410		-0.005	0.511		0.038	0.412
	TG		0.021	0.067		0.035	0.292		-0.040	0.043
12p12	**rs7134375**	**A - 0.458**	**beta**	**p-value**	**C - 0.497**	**beta**	**p-value**	**A - 0.308**	**beta**	**p-value**
PDE3A	TC		1.439	0.525		-3.594	0.269		0.006	0.611
	LDL-C		0.531	0.799		-3.243	0.278		0.013	0.885
	HDL-C		0.011	0.049		0.003	0.695		0.059	0.240
	TG		-0.009	0.412		-0.013	0.687		-0.002	0.910
12q24	**rs2338104**	**C - 0.429**	**beta**	**p-value**	**C - 0.486**	**beta**	**p-value**	**C - 0.196**	**beta**	**p-value**
KCTD10	TC		0.357	0.877		-5.109	0.098		-0.010	0.477
MMAB-MVK	LDL-C		0.705	0.740		-5.855	0.041		0.089	0.390
	HDL-C		-0.005	0.383		0.002	0.777		-0.187	**0.002**
	TG		0.006	0.601		-0.010	0.751		-0.043	0.094
12q13	**rs11613352**	**T - 0.245**	**beta**	**p-value**	**T - 0.382**	**beta**	**p-value**	**T - 0.063**	**beta**	**p-value**
LRP1	TC		1.291	0.635		1.589	0.621		-0.008	0.713
	LDL-C		0.244	0.923		1.545	0.599		-0.250	0.132
	HDL-C		-0.002	0.722		-0.004	0.569		0.198	0.035
	TG		-0.003	0.815		0.014	0.669		0.054	0.183
15q22	**rs2652834**	**T - 0.188**	**beta**	**p-value**	**T - 0.154**	**beta**	**p-value**	**T - 0.338**	**beta**	**p-value**
LACTB	TC		-0.576	0.847		-2.073	0.638		0.009	0.429
	LDL-C		-0.532	0.848		-3.853	0.340		0.055	0.522
	HDL-C		-0.001	0.918		-0.009	0.368		0.013	0.792
	TG		0.006	0.682		0.041	0.341		0.001	0.945
16q22	**rs2271293**	**A - 0.121**	**beta**	**p-value**	**A - 0.155**	**beta**	**p-value**	**A - 0.081**	**beta**	**p-value**
NUTF2	TC		7.170	0.044		2.092	0.626		0.010	0.635
CTCF-PRMT8	LDL-C		4.285	0.192		3.055	0.440		0.126	0.387
	HDL-C		0.023	**0.009**		0.006	0.549		0.090	0.284
	TG		-0.003	0.843		-0.033	0.427		-0.056	0.120

Table 12. Continued

Chr Gene(s)	SNP Traits	NHWs			Hispanics			African Blacks		
		MAF	Association		MAF	Association		MAF	Association	
16q23	**rs2925979**	**A - 0.287**	**beta**	**p-value**	**A - 0.203**	**beta**	**p-value**	**A - 0.293**	**beta**	**p-value**
CMIP	TC		1.514	0.544		-4.750	0.224		-0.032	**0.008**
	LDL-C		3.074	0.184		-3.317	0.355		-0.203	0.022
	HDL-C		-0.007	0.273		-0.008	0.383		-0.053	0.290
	TG		-0.014	0.236		-0.016	0.687		-0.016	0.459
17q12	**rs11869286**	**G - 0.357**	**beta**	**p-value**	**G - 0.387**	**beta**	**p-value**	**C - 0.172**	**beta**	**p-value**
STARD3	TC		3.194	0.182		0.465	0.885		-0.022	0.128
	LDL-C		2.940	0.185		0.435	0.883		-0.191	0.069
	HDL-C		-0.006	0.286		-0.003	0.732		-0.106	0.077
	TG		0.009	0.431		0.006	0.845		0.034	0.182
17q24	**rs4148008**	**G - 0.332**	**beta**	**p-value**	**G - 0.272**	**beta**	**p-value**	**C - 0.409**	**beta**	**p-value**
ABCA8	TC		1.800	0.458		3.567	0.323		-0.007	0.541
	LDL-C		2.287	0.310		3.112	0.351		-0.007	0.938
	HDL-C		$3.4x10^{-4}$	0.957		-0.003	0.694		-0.008	0.874
	TG		0.008	0.488		0.022	0.539		0.005	0.817
17q25	**rs4129767**	**A - 0.498**	**beta**	**p-value**	**G - 0.461**	**beta**	**p-value**	**A - 0.313**	**beta**	**p-value**
PGS1	TC		3.112	0.183		6.496	0.038		0.027	0.026
	LDL-C		3.988	0.065		6.770	0.019		0.135	0.120
	HDL-C		-0.014	0.015		-0.007	0.385		0.064	0.205
	TG		0.027	0.013		0.034	0.269		0.009	0.685
18q21	**rs12967135**	**A - 0.235**	**beta**	**p-value**	**A - 0.175**	**beta**	**p-value**	**A - 0.324**	**beta**	**p-value**
MC4R	TC		-4.651	0.078		6.168	0.121		0.018	0.163
	LDL-C		-3.709	0.130		6.215	0.090		0.127	0.166
	HDL-C		-0.001	0.848		-0.010	0.295		0.126	0.017
	TG		-0.018	0.152		0.009	0.824		-0.029	0.206
19p13	**rs2967605**	**T - 0.169**	**beta**	**p-value**	**T - 0.214**	**beta**	**p-value**	**T - 0.237**	**beta**	**p-value**
RAB11B	TC		1.575	0.598		-1.284	0.734		0.013	0.324
ANGPTL4	LDL-C		1.256	0.649		0.781	0.823		0.123	0.199
	HDL-C		-0.003	0.678		-0.012	0.189		-0.003	0.959
	TG		0.007	0.625		-0.033	0.377		-0.007	0.780

Table 12. Continued

Chr Gene(s)	SNP Traits	NHWs			Hispanics			African Blacks		
		MAF	Association		MAF	Association		MAF	Association	
19p13	**rs737337**	**C - 0.079**	**beta**	**p-value**	**C - 0.267**	**beta**	**p-value**	**T - 0.483**	**beta**	**p-value**
LOC55908	TC		-2.436	0.555		-4.401	0.195		0.012	0.284
DOCK6	LDL-C		-4.911	0.198		-6.843	0.029		0.050	0.536
	HDL-C		0.014	0.180		-0.003	0.723		0.048	0.300
	TG		0.012	0.527		0.041	0.220		0.003	0.881
19q13	**rs386000**	**G - 0.203**	**beta**	**p-value**	**G - 0.437**	**beta**	**p-value**	**G - 0.183**	**beta**	**p-value**
LILRA3	TC		1.725	0.524		4.067	0.224		0.003	0.857
LILRB2	LDL-C		3.353	0.180		2.619	0.396		-0.025	0.810
	HDL-C		-0.003	0.629		$1.2x10^{-4}$	0.988		0.082	0.169
	TG		-0.005	0.703		0.045	0.175		-0.027	0.292
20q13	**rs1800961**	**T - 0.032**	**beta**	**p-value**	**T - 0.033**	**beta**	**p-value**			
HNF4A	TC		-0.463	0.943		-11.381	0.169			
	LDL-C		1.910	0.748		-9.706	0.211			
	HDL-C		-0.009	0.568		0.005	0.794			
	TG		-0.028	0.345		-0.096	0.238			
20q13	**rs6065906**	**C - 0.173**	**beta**	**p-value**	**C - 0.097**	**beta**	**p-value**	**C - 0.158**	**beta**	**p-value**
PLTP	TC		3.707	0.213		2.927	0.583		-0.012	0.432
PCIF1	LDL-C		2.263	0.416		1.173	0.811		-0.088	0.424
	HDL-C		-0.001	0.921		-0.018	0.138		-0.060	0.336
	TG		0.010	0.461		0.106	0.044		0.019	0.487
22q11	**rs181362**	**T - 0.201**	**beta**	**p-value**	**T - 0.366**	**beta**	**p-value**	**T - 0.445**	**beta**	**p-value**
UBE2L3	TC		0.284	0.921		1.535	0.625		0.005	0.673
	LDL-C		0.948	0.721		1.257	0.664		0.074	0.365
	HDL-C		-0.002	0.790		0.003	0.684		-0.017	0.710
	TG		-0.002	0.896		0.005	0.878		0.012	0.547

4.0 DISCUSSION

During the past few years, our group has been comprehensively investigating several HDL-C levels-associated genes (*APOA5-A4-C3-A1* cluster, *LCAT*, *CETP, LIPC, LIPG, LPL, ABCA1, SCARB1* and others) using deep-sequencing and genotyping methods to test both common and rare variant hypotheses. Several recently published GWAS prompted us to also perform this replication study to examine the GWAS signals from other genes that have not been targeted by our sequencing effort in our multiethnic sample. Although we primarily focused on the GWAS signals influencing plasma HDL-C levels (with or without effects on other lipids) for selecting the SNPs to be genotyped, we performed association analyses with all lipid levels (TC, LDL-C, HDL-C, and TG) regardless of previously reported specific associations for selected SNPs. A total of 40 SNPs from 4 recent GWAS articles (Willer et al., 2008, Aulchenko et al., 2009, Kathiresan et al., 2009, and Teslovich et al., 2010) were selected for our study and analyzed for their effects on plasma lipid levels in our multiethnic sample consisting of 3 different ethnic groups (U.S. non-Hispanic Whites - NHWs, U.S. Hispanics, and African Blacks). Of 40 SNPs, only 34 with sufficient MAFs were examined in African Blacks.

Our analysis detected a total of 22 SNPs with significant association (p-values less than 0.05) with at least one lipid trait in at least one ethnic group, several of which had also marginal p-values (0.05-0.10) for additional lipid traits. An additional 5 SNPs showed only marginal p-

values. Of these 27 SNPs, 9 were associated with at least one lipid trait in more than one ethnic group, although not always with the same trait across various ethnic groups. A total of 10 SNPs were significantly associated with more than one lipid trait in at least one ethnic group and this number was higher when taking into account the marginal p-values. The number of significant loci was 10 for TC, 12 for LDL-C, 10 for HDL-C, and 6 for TG levels. Four of our significant loci (*GALNT2*, *DOCK7, MLXIPL,* and *CELSR2*) were among those also replicated by earlier independent studies that examined selected GWAS signals in their multiethnic samples (Keebler et al., 2009, Lanktree et al., 2009).

In NHWs, 12 SNPs showed significant association (p<0.05) with at least one lipid trait and 6 were significantly associated with more than one lipid trait. These numbers were 10 and 3 in Hispanics and 7 and 2 in African Blacks. The most significant SNPs for TC levels were *GRIN3A*/rs1323432 in NHWs, *APOB*/rs1042034 in Hispanics, and *CMIP*/rs2925979 in African Blacks. The most significant SNPs for LDL-C levels were *GRIN3A*/rs1323432 in NHWs, *APOB*/rs1042034 in Hispanics, and *PPP1R3B*/rs9987289 in African Blacks. The most significant SNPs for HDL-C levels were *KLF14*/rs4731702 in NHWs, *PPP1R3B*/rs9987289 in Hispanics, and *KCTD10*/rs2338104 in African Blacks. The most significant SNPs for TG levels were *DOCK7*/rs10889353 in both NHWs and Hispanics, and *UBASH3B*/rs7941030 in African Blacks.

Table 13 summarizes our results as compared to those in original GWAS from which these SNPs were selected. Our sample sizes were relatively small as compared to those used for original GWAS (which examined several thousands subjects) and the observed associations were weaker, therefore we have also taken into account the p-values between 0.05 and 0.20 (whenever the trend was the same) when comparing our results with original GWAS signals.

Thus, **Table 13** includes all the SNPs that were significantly (p<0.05) associated with at least one lipid trait in at least one ethnic group (**bold blue trait**: decreasing effect, **bold red trait**: increasing effect) as well as the SNPs that showed trend for the same direction of association as seen for at least one genome-wide significant ($p \leq 5x10^{-8}$) lipid trait in the original GWAS (blue trait: decreasing effect with p-value between 0.05 and 0.10, red trait: increasing effect with p-value between 0.05 and 0.10, *italics blue trait*: decreasing effect with p-value between 0.10 and 0.20, *italics red trait*: increasing effect with p-value between 0.10 and 0.20). A similar color-coding (blue/red) is used to illustrate also the genome-wide significant traits and associated alleles in the original GWAS. Highlighted in gray are other alleles (and their associations) different than those observed as minor alleles in our NHW sample. Highlighted in purple are the results concordant with those in the original GWAS (for at least one genome-wide significant lipid trait) and highlighted in pink are those that are significant in our study but discordant with GWAS. Non-highlighted are the significant associations observed in our study with the lipid traits other than those reported at genome-wide level significance in the original GWAS.

For 25 of 40 SNPs analyzed (40 in NHWs and Hispanics, 34 in African Blacks), we were able to replicate the genome-wide significant associations with the same lipid trait in the same direction in at least one ethnic group that we studied; at nominal significance (p<0.05) for 14 SNPs, with marginal p-values (0.05-0.10) for 3 SNPs, and with trend for association with p-values between 0.10-0.20 for 8 SNPs. There were additional SNPs with higher p-values that showed similar trends for effects on the same lipid traits as seen in the original GWAS.

Table 13. Comparison of our association results to the genome-wide level significant findings of original GWAS

Locus	SNP	NHWs MAF	HSPs MAF	ABs MAF	NHWs Traits	HSPs Traits	ABs Traits	GWAS Allele	GWAS Traits	GWAS Cohort
1p13	rs646776				TC, LDL-C			G	TC, LDL-C	
1p31	rs10889353	C-0.337	C-0.365	C-0.447	TC, TG	TG, *TC*	*TG*	C	TC, TG	
1q25	rs1689800	G-0.347	G-0.346	G-0.264	*HDL-C*			G	HDL-C	
1q42	rs2144300	C-0.384	C-0.425	T-0.040	LDL-C HDL-C TG			T	HDL-C	
2p24	rs1042034	G-0.215	G-0.298	G-0.119	*TG*	TC, LDL-C	*TG*	C	HDL-C TG	
2q36	rs2972146	C-0.377	C-0.230	C-0.123		*TG*		G	HDL-C TG	
4q24	rs13107325	T-0.068	T-0.045			HDL-C		T	HDL-C	
6q24	rs605066	C-0.403	C-0.406	T-0.402	LDL-C	*HDL-C*		C	HDL-C	
7q11	rs17145738	T-0.108	T-0.071	T-0.086	TG			T	HDL-C TG	
7q32	rs4731702	T-0.494	T-0.436	T-0.184	HDL-C			T	HDL-C	
8p23	rs9987289	A-0.091	A-0.178	A-0.191		TC, HDL-C, *LDL-C*	TC, LDL-C	G/A	HDL-C, LDL-C, TC	EU, AA
8q23	rs2293889	T-0.442	T-0.445	T-0.040			*HDL-C*	G/T	HDL-C	EU, AA
9p22	rs471364	C-0.125	C-0.105	C-0.206	HDL-C			T/C	HDL-C	EU
9q31	rs1323432	G-0.115	G-0.087		TC, LDL-C	HDL-C		A/G	HDL-C	EU
11p11	rs7395662	A-0.373	A-0.323	G-0.407		TC, LDL-C		G/A	HDL-C	EU
11p15	rs2923084	G-0.179	G-0.307	A-0.474	HDL-C			A/G	HDL-C	EU, AA
11q12	rs174547	C-0.345	T-0.441		HDL-C	LDL-C		T/C	HDL-C, TG	EU
11q24	rs7941030	C-0.367	C-0.294	C-0.444	TC		TG	T/C	HDL-C, TC	EU, AA
12p12	rs7134375	A-0.458	C-0.497	A-0.308	HDL-C			C/A	HDL-C	EU, AA
12q24	rs2338104	C-0.429	C-0.486	C-0.196		LDL-C	HDL-C	G/C	HDL-C	EU
12q13	rs11613352	T-0.245	T-0.382	T-0.063			HDL-C	C/T	HDL-C, TG	EU, AA
16q22	rs2271293	A-0.121	A-0.155	A-0.081	TC, HDL-C			G/A	HDL-C	EU
16q23	rs2925979	A-0.287	A-0.203	A-0.293			TC, LDL-C	C/T	HDL-C	EU, AA
17q25	rs4129767	A-0.498	G-0.461	A-0.313	HDL-C, TG	TC, LDL-C	TC	A/G	HDL-C	EU, AA
18q21	rs12967135	A-0.235	A-0.175	A-0.324			HDL-C	G/A	HDL-C	EU, AA
19p13	rs2967605	T-0.169	T-0.214	T-0.237		*HDL-C*		C/T	HDL-C	EU
19p13	rs737337	C-0.079	C-0.267	T-0.483		LDL-C		T/C	HDL-C	EU, AA
19q13	rs386000	G-0.203	G-0.437	G-0.183			*HDL-C*	G/C	HDL-C	EU, AA*
20q13	rs1800961	T-0.032	T-0.033			*TC*		C/T	HDL-C, TC	EU, AA*
20q13	rs6065906	C-0.173	C-0.097	C-0.158		TG, *HDL-C*		T/C	HDL-C, TG	EU, AA

NHWs: Non-Hispanic Whites, HSPs: Hispanics, ABs: African Blacks, EU: Europeans used for primary GWAS analysis, AA: African American replication sample (*=discordant)

Of 6 SNPs showing genome-wide level significance for TC levels, we were able to replicate the associations in the same direction for 5 SNPs [at nominal significance (p<0.05) for 4 SNPs and with trend for association with p-value between 0.10-0.20 for one SNP] in at least one ethnic group studied. Of 2 SNPs showing genome-wide level significance for LDL-C levels, we were able to replicate the associations in the same direction for both SNPs at nominal significance (p<0.05) in at least one ethnic group studied. Of 36 SNPs with genome-wide level significance and one with $p=7.7x10^{-4}$ for HDL-C levels, we were able to replicate the associations in the same direction for 18 SNPs [at nominal significance (p<0.05) for 8 SNPs, with marginal p-values (0.05-0.10) for 4 SNPs and with trend for association with p-values between 0.10-0.20 for 6 SNPs] in at least one ethnic group studied. Two SNPs showed significant (p<0.05) but discordant results for association with the HDL-C levels as compared to those reported in the original GWAS. Of 7 SNPs showing genome-wide level significance for TG levels, we were able to replicate the associations in the same direction for 5 SNPs [at nominal significance (p<0.05) for 3 SNPs and with trend for association with p-values between 0.10-0.20 for 2 SNPs] in at least one ethnic group studied. For 10 SNPs, we observed significant associations with lipid traits other than those reported as genome-wide significant in the original GWAS, although we did not compare our results with the GWAS findings that did not reach genome-wide level significance. Three of these 10 SNPs did not show any association or trend for association with the traits identified as genome-wide significant in the original reports.

Overall the concordance rate was high for observed associations as compared to original GWAS findings; however, the associations were not consistently observed in all ethnic groups

that we studied. Other studies using various ethnic groups also obtained mixed results (Lanktree et al., 2009, Keebler et al., 2009, and Teslovich et al., 2010). An independent replication study (Lanktree et al. 2009) reported nominal association in the same direction for only 13 of 32 GWAS loci tested in their modest-sized multiethnic sample. Another replication study (Keebler et al., 2009) that used a larger multiethnic sample found mixed evidence by ethnic group except for five loci that appeared to be shared by all groups, most of which were established loci which were not included in this study. The largest GWAS (Teslovich et al., 2010; >100,000 European subjects) that also sought replication in non-European samples (including >8,000 African Americans) as compared to a similarly sized European replication sample (n=7,000), was able to replicate most but not all of the genome-wide significant signals identified in their primary analysis. The number of replicated SNPs was 35 of 36 for LDL-C, 44 of 47 for HDL-C, and 29 of 32 for TG in the European replication sample while the replication was more modest in African American replication sample (LDL-C: 33 of 36, HDL-C: 37 of 44 and TG: 24 of 30). Also, some of the observed effects were discordant in their replication samples.

Gender-specific effects on the genetics of lipid traits have also been suggested. We used gender as a covariate in our analyses but did not perform gender-stratified analyses due to power-related concerns. When Teslovich et al. (2010) reanalyzed their GWAS data separately in males and females, they observed gender-specific effect sizes or associations for some (but not many) loci.

In conclusion, our study showed evidence of replicated association for several but not all genome-wide significant SNP associations (or same lipid associations for each SNP) in our samples. Lack of replication of genetic associations may be related to a number of factors, such

as insufficient power, differences in study population characteristics, allele frequency or LD differences across various ethnic groups, and gene-environment interactions. Our study did not have sufficient power to detect effects of small sizes, especially for those variants with low frequencies, as we were trying to replicate the findings of the recent GWAS that examined several thousands individuals.

4.1 PUBLIC HEALTH SIGNIFICANCE

Despite medical advances that have been made in recent years, CVD is still a major public health concern and the leading cause of death for both men and women in the U.S. The relationship between dyslipidemia and CVD has long been recognized. Identification of genetic associations with plasma lipoprotein levels is relevant to public health as it may increase our understanding about lipid metabolism-related biological mechanisms and may lead to improvements in prevention and treatment of dyslipidemia. This, in turn, may have significant impact on the prevention and management of heart disease. Replication of reported genetic associations by independent groups in various populations, as exemplified by this study, is essential for establishing those associations.

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