GENETIC INFLUENCE OF SEQUENCE VARIANTS IN *SCARB1* AND *ABCA1* GENES ON MAJOR LIPID TRAITS: A CANDIDATE GENE ASSOCIATION STUDY

by

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Background: Abnormal lipid-lipoprotein levels are associated with the risk of coronary heart disease (CHD), a major public health problem worldwide. Scavenger receptor class B type 1 (SCARB1) and ATP-binding cassette transporter A1 (ABCA1) play important roles in the reverse cholesterol transport. We aimed to identify genetic variants in two lipid genes, *SCARB1* and *ABCA1*, and elucidated their contribution to major lipid traits in two populations; Non-Hispanic Whites (NHWs) and African Blacks (ABs).

Methods: We resequenced mainly the exons and exon-intron boundaries of *SCARB1* and *ABCA1* genes in 190 individuals (95 NHWs; 95 ABs) with extreme high-density lipoprotein cholesterol (HDL-C) levels, followed by genotyping of selected variants in the entire sample (623 NHWs; 788 ABs). Lipid associations were evaluated by multiple analyses.

Results: Initial sequencing identified 105 *SCARB1* (44/NHWs; 83/ABs) and 404 *ABCA1* variants, including 58 novel ones (21 *SCARB1*; 37 *ABCA1*). Among genotyped variants, 159 *SCARB1* (69/NHWs; 137/ABs) and 182 *ABCA1* variants passed quality controls and were tested for associations. Gene-based tests revealed associations (P <0.05) of

SCARB1 with HDL-C and apolipoprotein B (apoB), while *ABCA1* demonstrated association with triglycerides (TG). Eleven common *SCARB1* variants were nominally associated (P <0.05) with HDL-C or apoB in single-site analyses, and four of them (3/apoB/NHWs; 1/HDL-C/ABs) survived after multiple testing correction. The best signal of *SCARB1* was rs4765615 (apoB/P = 0.0059) in NHWs and rs11057851 (HDL-C/P = 0.0043) in ABs. Twenty-one common *ABCA1* variants were nominally associated with TG, and 16 remained significant after multiple testing correction. The best signal of *ABCA1* with TG was rs2066716 (p.Thr14217Thr; P = 0.0016) in NHWs. A group of rare *SCARB1* variants (frequency ≤1%) were associated with apoB (P = 0.0284) in NHWs, and HDL-C (P = 0.0478) in ABs. Several haplotypes and regions of *SCARB1* and *ABCA1* genes showed associations (global P <0.05) with lipid levels.

Public Health Relevance: Our findings demonstrate the genetic contribution of common and rare *SCARB1* and *ABCA1* variants to the regulation of lipoprotein-lipid levels in the general population, supporting the roles of *SCARB1* and *ABCA1* genes in lipid metabolism. Further investigations of these two genes may lead to the development of potential therapeutic interventions for CHD.

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PREFACE

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Wishing you all the best.

Thank you.

1.0. INTRODUCTION

1.1. CORONARY HEART DISEASE

1.1.1. Coronary heart disease and public health significance

Coronary heart disease (CHD) is the number one cause of deaths worldwide, causing at least 7 million deaths annually [1]. According to the World Health Organization data, the numbers of global deaths from heart attack alone increased from 6 million in 2000 (death rate: 97.6 per 100,000 population) to 7.4 million in 2012 (death rate: 104.0 per 100,000 population) [2], and approximately 9.2 million people are estimated to die from heart attack in 2030 [3]. Furthermore, as a result of urbanization, there is an increase in risk factors and disease rates for CHD in developing countries. At least 70% of total CHD deaths occur in low-income and middle-income countries [4], reflecting the burden of CHD in global scale.

In the US, the leading cause of deaths is CHD [5, 6]. Over the past four decades, the death rates for CHD in the US have been declining due to reduced CHD risk and advanced screening and treatments, but CHD is still the number one killer [5]. Recent US mortality data has shown that CHD caused 370,213 Americans deaths in 2013 (death rate: 117.1 per 100,000 population) [7]. The estimated prevalence of CHD in

adults ≥20 years of age is 6.2%, affecting 15.5 million Americans [6]. Each year, approximately 635,000 Americans experience a first episode of coronary events, and 300,000 will have a recurrent event [6]. It is estimated that every 34 seconds, one American has a coronary event, and every 1 minute and 24 seconds one will die from it [6].

Furthermore, CHD is significantly associated with disabilities, which is measured as the disability-adjusted life years loss due to health illness (DALYs; a disease burden indicator) [8]. In 2012, heart attack was reported to be the top cause of global DALYs that accounted for 5.2% of global DALYs (DALY rate: 129,820 per thousand or 1,484 per 100,000 population) [9]. In addition, of total CHD deaths in age-group of 30 to older 70 years, at least 30% occur under the age of 70 years, indicating that CHD is also the main cause of global premature deaths (<70 years of age; the global life expectancy is defined at 70 years of age) [2].

With the projection of increasing CHD prevalence, CHD has a substantial impact on economies at the household and macro levels caused by direct (medical and health care) and indirect (lost productivity due to deaths or disabilities) costs [5, 10, 11]. In the US, between 2010 and 2030, CHD prevalence is expected to increase from 8.0 % in 2010 to 9.3 % in 2030, accounting for an additional 8 million Americans with CHD [12]. By 2030, the estimated costs for CHD are projected to double, from $108.9 (direct costs, $35.7 and indirect costs, $73.2) billion dollars in 2010 to $218.7 (direct costs, $106.4 and indirect costs, $112.3) billion dollars in 2030 [12].

Emerging evidence indicates that CHD is a global pandemic, affecting a large number of people in every ethnic group, and also causing an economic burden. From a

public health point of view, it is necessary to reduce the prevalence and risk of CHD as well as to develop new interventions (therapeutic and preventive) in order to control the rising burden of CHD and improve the quality of life for all.

1.1.2. Major risk factors of coronary heart disease

CHD is a multifactorial disease determined by both genetic and environmental factors. A family history of CHD, particular premature CHD (<55 years of age in males and <65 years of age in females) [13], has been shown to be a strong risk factor for CHD [13-16]. Parental or sibling history of heart disease increases an individual's risk by 1.2-2 times CHD risk [14, 15, 17, 18]. In addition, epidemiological studies also demonstrated several independent risk factors associated with CHD events including age, sex, family history of CHD, abnormal lipoprotein-lipid levels (i.e., high levels of low-density lipoprotein cholesterol [LDL-C], low levels of high-density lipoprotein cholesterol [HDL-C], high levels of triglycerides [TG], and high levels of non-HDL-C), smoking, diabetes, and hypertension) [13, 19] (**Table 1.1**). Moreover, recent evidence indicates additional risk factors for CHD, i.e., apolipoprotein (apo) B (apoB), LDL particles, high sensitivity C-reactive protein, lipoprotein-associated phospholipase A2, lipoprotein (a), LDL or HDL subfractions, including remnant cholesterol levels [19-22].

Modification of risk factors has led to a significant reduction of the incidence and death rates for CHD [6, 23, 24], but more is required to reduce the risk factors of CHD in order to control CHD prevalence. Among major risk factors of CHD, some (i.e., age, sex, and a family history) cannot be managed, however, the majority (i.e., lipoprotein-lipid levels, smoking, diabetes, and hypertension) can be improved by lifestyle

management, including healthy dietary intake, smoking cessation, regular physical activities, weight loss, and controlling underlying conditions (dyslipidemias, diabetes and hypertension). It is estimated that lifestyle interventions alone can attribute to a 44% decrease in CHD death rates [6]. To achieve long-term and lifetime CHD risk reduction, recent guidelines for CHD management have promoted lifestyle therapies in addition to risk assessment, early detection, and medical treatments [19, 21, 22, 25].

Table 1.1. Major risk factors of coronary heart disease*.

Primary risk factors[a]	
High LDL-C levels	≥160 mg/dL
High non-HDL-C[b] levels	≥190 mg/dL
Additional risk factors	
Age	Male ≥45 years
	Female ≥55 years
Family history of CHD	<55 years in a male 1st degree relative
	<65 years in a female 1st degree relative
Low HDL-C levels	Male <40 mg/dL
	Female <45 mg/dL
High TG	≥200 mg/dL
Current smoking	
High blood pressure	≥140/ ≥90 mmHg or on anti-hypertensive drugs
Diabetes	
Chronic kidney disease	Stage ≥3B or 4[c]

1st degree relative includes parents, offspring and siblings.

[a] Primary risk factors are used for CHD risk assessment and are considered as the primary targets of treatments.

[b] Non-HDL-C = total cholesterol minus HDL-C.

[c] An estimated glomerular infiltration rate (eGFR) for chronic kidney disease (CKD): 30-44 mL/min/1.73 m^2 for CKD stage 3, and 15-29 mL/min/1.73 m^2 for CKD stage 4. No recommendation for treatment goal for CKD stage 5 (eGFR <15 mL/min/1.73 m^2) [22].

**Based on data from* Refs [13, 22].

1.2. HIGH-DENSITY LIPOPROTEIN CHOLESTEROL

1.2.1. High-density lipoprotein structure and subclasses

Lipoprotein particles are heterogeneous with varying in density, size, charges, and compositions, and categorized into five major subpopulations as shown in **Table 1.2** [26, 27].

Table 1.2. Classification and components of lipoprotein particles*.

	Chylomicrons	VLDL-C	IDL-C	LDL-C	HDL-C
Diameter (Å)	800-5000	300-800	250-350	180-280	50-120
Density (g/mL)	<0.950	0.950-1.006	1.006-1.019	1.019-1.063	1.063-1.210
Mobility in 2D-gel electrophoresis	At the origin	Pre-β	Slow β	β	α
Major molecular components (weight%)					
Cholesterol	3	10	-	26	20
Triglycerides	90	70	-	10	5
Phospholipid	5	10	-	15	25
Apolipoprotein(s)	9	10	-	25	50
Major Apolipoprotein(s)	A-I, A-II, B-I, C-I, C-II, C-III	B-I, C-I, C-II, C-III, E	B, C-III, E	B	A-I, A-II

Å, Ångstrom (length measure units, equal to 10^{-8} cm); HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; -, not determined.

Modified from* Cox, R. A., and M. R. García-Palmieri. 1990. Cholesterol, Triglycerides, and Associated Lipoproteins. *In* Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. H. K. Walker, Hall W. D., and H. J. W., editors. Butterworths, Boston [26]; and Superko, H. R. 2009. *Circulation* **119: 2383-2395 [27].

HDL-C is the smallest and densest of the lipoprotein particles, ranging in size between 7.2 and 12.9 nm in diameter with a density fraction of 1.063-1.21 g/mL [28-30]. Main components of HDL particles are composed of an inner core of cholesteryl esters (CE) and TG, surrounded by an amphipathic outer layer of free cholesterol (FC), phospholipids, and apolipoproteins (apos). The major protein composition in HDL particles includes apoA-I, apoA-II, apoA-IV, apoCs, and apoE [28-30]. ApoA-I is the most abundant HDL-C protein comprising approximately 70% of total HDL proteins, followed by apoA-II, which accounts for approximately 15-20% of total HDL proteins [29, 30]. Differences in molecular components of HDL particles result in HDL subclasses (**Table 1.3**). On the basis of density, HDL particles are classified by ultracentrifugation into two major subclasses: HDL2, the large (mean size >8-10 nm diameter), less dense (1.063-1.125 g/mL) and HDL3, the small (mean size ≤8 nm diameter), more dense (1.125-1.21 g/mL) [28-32]. Additionally, with advanced separation methods, HDL particles are categorized into further differentiated subclasses as described in **Table 1.4**.

Table 1.3. Classification and components of high-density lipoprotein (HDL) subclasses*.

	HDL2b	HDL2a	HDL3a	HDL3b	HDL3c
Size (nm)	10.4	10.3	9.9	8.0	7.3
Density (g/mL)	1.099	1.107	1.123	1.155	1.186
Molecular weight (kDa)	410	400	360	200	160
Major molecular components (mol/mol HDL)					
Cholesteryl ester	180	160	140	70	40
Triglycerides	30	20	15	10	5
Phospholipid	130	140	120	45	25
Free cholesterol	70	40	25	15	10
Apolipoprotein A-I	4	4	3-4	3	2-3
Other Apolipoprotein(s)	≤2	≤2	≤2	1	≤1

Modified from* Kontush, A., and M. J. Chapman. 2006. *Pharmacol Rev* **58: 342-374 [32].

Table 1.4. Classification of high-density lipoprotein (HDL) subclasses based on separation methods*.

	Very large HDL	Large HDL	Medium HDL	Small HDL	Very small HDL
Ultracentrifugal isolation	**HDL2**		**HDL3**		
Density (g/mL)	1.063-1.125		1.125-1.210		
Ion mobility	**HDL2b**	**HDL2a and HDL3**			
Size (nm)	10.50-14.50	7.65-10.50			
2-D gel electrophoresis	**α-1**	**α-2**	**α-3**	**α-4**	**Pre-β-1 HDL**
Size (nm)	10.8-11.2	9.0-9.4	7.5-8.5	7.0-7.5	5.0-6.0
Shape	Spherical	Spherical	Spherical	Discoidal	Discoidal
Electroimmunodiffusion	**Particles containing ApoA-I:A-II**			**Particles containing ApoA-I**	
Apolipoprotein(s)	Near absence of A-I and A-II	A-I and A-II	A-I and A-II	A-I	A-I
Gradient gel electrophoresis	**HDL2b**	**HDL2a**	**HDL3a**	**HDL3b**	**HDL3c**
Size (nm)	9.7-12.9	8.8-9.7	8.2-8.8	7.8-8.2	7.2-7.8
Density gradient ultracentrifugation	**HDL2b**	**HDL2a**	**HDL3a**	**HDL3b**	**HDL3c**
Density (g/mL)	1.063-1.087	1.088-1.110	1.110-1.129	1.129-1.154	1.154-1.170
Nuclear magnetic resonance	**Large HDL**		**Medium HDL**	**Small HDL**	
Size (nm)	9.7-13.0	8.8-9.7	8.2-8.8	7.8-8.2	7.2-7.8

Modified from* Asztalos, B. F., et al. 2011. *Curr Opin Lipidol* **22: 176-185 [28]; Rosenson, R. S., et al. 2011. *Clin Chem* **57**: 392-410 [29]; and Kontush, A., et al. 2015. *Handb Exp Pharmacol* **224**: 3-51 [30].

1.2.2. High-density lipoprotein cholesterol metabolism

The metabolism of lipoprotein particles is complex, and comprised of many complicated steps, a variety of lipoprotein particles, enzymes and protein transporters [33-40]. Basically, two main cholesterol transport processes are implicated in lipoprotein metabolism, from liver to peripheral tissues mediated by apoB-containing lipoproteins (i.e., very low-density lipoprotein [VLDL] and LDL), and from peripheral tissues back to the liver, called the reverse cholesterol transport (RCT), mediated by apoA-I-containing HDL particles [33-40].

For HDL-C metabolism, the biosynthesis of HDL is initiated with the lipid-free/lipid-poor apoA-I particles that are secreted by liver and intestine or dissociated from chylomicrons or VLDL particles in TG hydrolysis. Then, the lipid-free/lipid-poor apoA-I particles acquire free cholesterol (FC) and phospholipids (PLs) via cholesterol efflux from adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) to form pre-β, discoidal HDL (nascent HDL; see **Figure 1.1**).

The initial lipidation of pre-β, discoidal HDL particles is also generated through TG-rich lipoproteins (TRL; i.e., chylomicrons, and VLDL) hydrolyzed by lipoprotein lipase (LPL) as well as through the inter-conversion between HDL2 and HDL3 by cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP), and hepatic lipase (HL). During the remodeling of HDL maturation (spherical HDL particles), the assembly of pre-β, discoidal HDL is continually incorporated with FC and PLs from apoB-containing lipoproteins mediated by lecithin:cholesterol acyltransferase (LCAT).
The clearance of lipid and proteins of α-HDL particles occurs via the concerted actions of the selective uptake of CE into liver by scavenger receptor class B type 1 (SCARB1

or SR-BI) and the holo particle uptake by apoE or apoA receptors for degradation. The hepatic cholesterol is converted to bile acid or directly excreted into the bile. Finally, the bile is either excreted in feces or reabsorbed by the intestine. The small apolipoproteins are removed from the circulation into extravascular space, and subsequently incorporated into the biosynthesis of HDL particles. In addition, the lipid-free/lipid-poor apoA-I particles are removed from plasma through the glomerular filtration in the kidney; while, the turnover of apoA-I is mediated via cubilin in the renal proximal tubular lumen.

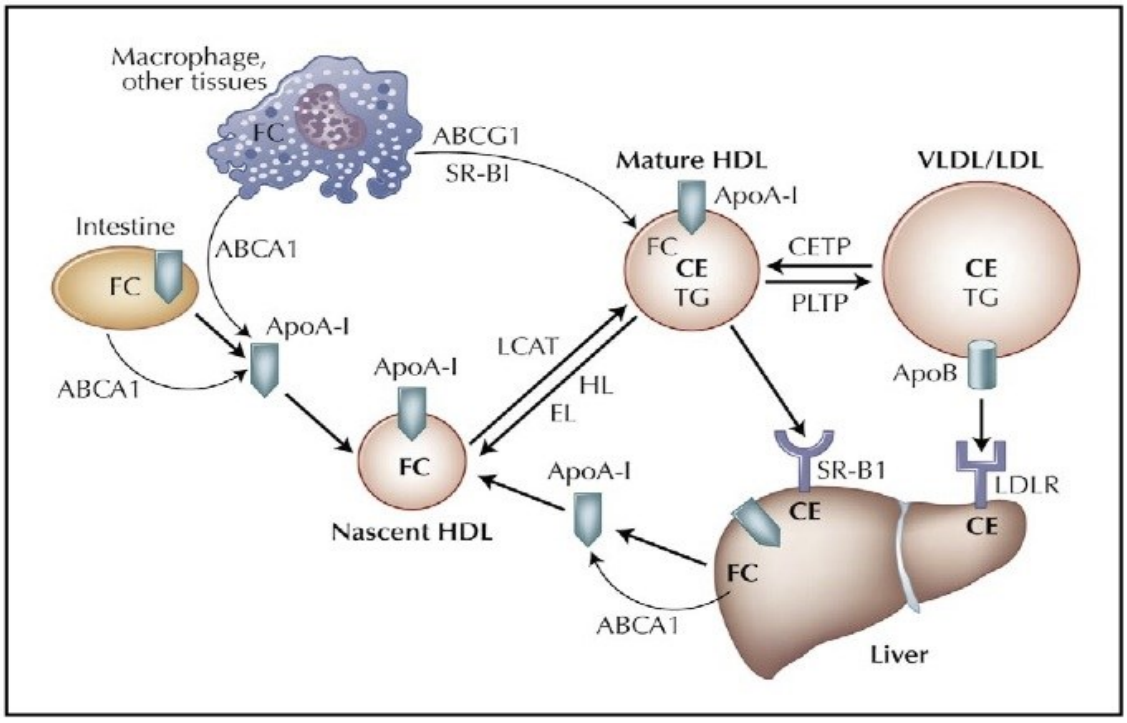

Figure 1.1. Overview of the biogenesis of high-density lipoprotein particles and the reverse cholesterol transport.

The pathways of high-density lipoprotein (HDL) metabolism and reverse cholesterol transport are depicted. Both intestine and liver secrete apolipoprotein (apo) A-I, which acquires free cholesterol (FC) via adenosine triphosphate–binding cassette transporter 1 (ABCA1) from these same tissues, forming nascent HDL. ApoA-I also acquires FC from other tissues via ABCA1. FC in nascent HDL is esterified by lecithin:cholesterol acyltransferase (LCAT) to form cholesteryl ester (CE), resulting in the formation of mature HDL. Mature HDL can also acquire FC from tissues, in this case via ABCG1 and scavenger receptor class B type I (SR-BI). CE in mature HDL can be transferred via cholesteryl ester transfer protein (CETP) to the apoB-containing lipoproteins very low- density lipoprotein (VLDL) and low-density lipoprotein (LDL) in exchange for triglycerides (TG), with subsequent hepatic uptake, or can be directly taken up by the liver via SR-BI. Mature HDL receives additional phospholipids via phospholipid transfer protein (PLTP), and can be remodeled by lipases such as hepatic lipase (HL) and endothelial lipase (EL) to smaller HDL particles. LDLR, low-density lipoprotein receptor.

Used with copyright permission from Qasim, A., and D. J. Rader. 2006. *Curr Atheroscler Rep* 8: 198-205 [37]

1.3. RELATIONSHIP BETWEEN HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AND CORONARY HEART DISEASE

1.3.1. Anti-atherogenic functions of high-density lipoprotein

HDL has a primary role in RCT that transfers cholesterol from the peripheral tissues back to the liver for catabolism [33-40] (see **Figure 1.1**). RCT also promotes the cholesterol efflux from the macrophage-derived foam cells in the arterial wall [34, 36, 39, 41, 42], which is considered to be a key mechanism for atherosclerosis regression. Thus, disruption of any steps in RCT causes abnormal lipoprotein levels, and leads to the development of atherosclerosis.

In addition, HDL also embraces a variety of anti-atherogenic functions (**Table 1.5**), including anti-inflammatory, anti-oxidative, vasoprotective (i.e., promote endothelial repair and functions), anti-thrombotic, anti-apoptosis, and anti-infectious activities [28, 31, 32, 40, 43-50]. Such heterogeneity and complexity of HDL particles also results in varying degrees of atheroprotective properties among HDL subclasses [28, 31, 40, 43, 44, 49]. Anti-oxidation activity is more potent in small, dense HDL3 than large, light HDL2 [51, 52]. Anti-inflammation, as of the inhibition capacity of endothelial cell adhesion molecular-I expression, is more effective in HDL3 than HDL2 [53]. Endothelial protection in term of anti-apoptotic activity is enhanced in HDL3, compared to HDL2 [54]. Anti-platelet aggregation capacity is higher in large, light, apoE-containing HDL2

than small, dense HDL3 [55]. Anti-coagulation activity of tissue factor pathway is more active in HDL3 than HDL2 [56].

Table 1.5. Anti-atherogenic functions of high-density lipoprotein*.

• Reverse cholesterol transport
• Anti-oxidation
• Anti-inflammation
• Anti-thrombotic activities
• Promote endothelial repair and functions
• Promote angiogenesis
• Promote hematopoietic stem cells proliferation and mobilization
• Anti-apoptosis
• Anti-diabetic effects
• Anti-infectious (anti-trypanosome)

**Based on data from* Refs [28, 31, 32, 40, 43-50].

1.3.2. Association of high-density lipoprotein cholesterol with coronary heart disease

Epidemiology studies have shown an independent association between HDL-C levels and the risk of CHD [57-59]. Low HDL-C levels (<40 mg/dL in males and <45 mg/dL in females) are considered to increase risk CHD [13]. Each 1 mg/dL decrease in HDL-C levels was associated with increased CHD risk of 2% in males and 3% in females [58]. In contrast, high HDL-C levels (≥60 mg/dL) have been considered to be protective against the risk of CHD [13]. Each 1 mg/dL increased in HDL-C level was associated

with the reduction of CHD risk by 2-3% and also mortality by 4-5% [58, 60]. In this regard, HDL-C levels also serve as a predictor for the risk of CHD including subsequent CHD events such as in patients who already had CHD as well as those who were on lipid-lowering (statin) treatment [21, 61, 62]. Moreover, HDL-C exerts several anti-atherogenic functions (see **Table 1.5**), including promoting cholesterol catabolism [33-35, 37-40] and cholesterol efflux [34, 36, 39, 41, 42], in RCT, which is suggested to be the main mechanism for atheroprotection [63, 64]. Despite LDL-C lowering drugs' success in dramatically reducing CHD risk [65-76], a residual CHD risk remains in LDL-C-treated patients whose optimal LDL-C reduction [77-80], which highlights the needs to acquire novel treatments for other prospective risk factors (i.e., HDL-C and TG). Thus, in addition to LDL-C-lowering treatments, HDL-C becomes a potential therapeutic target for the CHD risk reduction.

Consequently, several classes of HDL-C-targeted drugs have been developed [48, 50, 81-85]. However, the current HDL-C-raising therapy (i.e., niacin and fibrates) has not shown significant CHD risk reduction. In addition, clinical trials of two HDL-C-raising drugs (i.e., niacin [86, 87] and CETP inhibitors [88, 89]) failed to demonstrate the benefits of reduced CHD events, despite of elevated HDL-C levels. In parallel, the Mendelian randomization studies of HDL-C-associated variants did not provide positive relationship with CHD [90, 91]. These findings have raised the questions about the atheroprotective roles of HDL-C. Nonetheless, the inverse association between cholesterol efflux capacity, a main function of HDL in RCT, and CHD has been observed [92-94]. These results suggest that HDL-C functions seem to be more effective than HDL-C levels in relation to CHD events.

While doubts about the anti-atherogenic nature of HDL-C exist, relevant evidence has consistently demonstrated the inverse association of HDL-C with the CHD risk, reflecting the atheroprotective effect of HDL-C. However, it should be highlighted that the HDL-C concept of raising HDL-C levels related with reduced CHD risk does not infer the causal association and shows the limitation of utilizing HDL-C levels in risk assessment. In light of recent findings, the correlation of HDL-C function with CHD has established a new strategy for the HDL-C concept considering the efficacy of HDL-C functionality, rather than the elevation of HDL-C levels.

Altogether, cumulative evidence indicates that HDL-C is a potential target of HDL-C for CHD risk reduction, but the HDL-C concept has evolved to focus on HDL-C functions. Due to the complexity of HDL functions and lack of current knowledge about the relationship between the anti-atherogenic properties of HDL-C and CHD, further investigations for new aspects of HDL biology and functions are needed in order to develop alternative HDL-C-targeted drugs and reduce CHD risk.

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2.0. OVERVIEW OF GENETICS IN COMPLEX DISEASES

2.1. GENETICS AND COMPLEX DISEASES: ROLES OF COMMON AND RARE VARIANTS

Most common diseases, like cardiovascular disease, diabetes, and blood pressure, are complex (multifactorial) traits influenced by both genetic and environmental factors [1-3]. Unlike monogenic Mendelian diseases caused by a single gene, complex traits are determined by multiples genes (polygenic) as well as gene-gene (epistasis) [4] and gene-environment interactions [5, 6], resulting in a complexity of disease phenotypes [7, 8]. Therefore, the genetic determination of complex traits, including disease risk and predisposition is very challenging.

With sequencing and genotyping technologies advancements in genetics, there has been considerable success in the discovery of several disease-associated genes and variations for multiple complex diseases and phenotypes. Currently, association study designs are widely used to assess the correlation of predisposing genes or genetic variants with disease traits or phenotypes [9, 10]. The genome-wide association approach is notable for identifying disease genes and common associated disease variants with low penetrance [11-15]. Genome-wide associated studies (GWASs) have reported numerous common variants (allele frequency ≥5%) associated with complex

traits, but these GWAS-identified associated variants account for a small portion of total genetic variance (heritability) underlying complex traits [7, 16-19]. This concept of the susceptible association of common allele frequencies (allele frequency ≥5%) with complex traits is known as the common disease, common variant (CDCV) hypothesis [20].

Nonetheless, the CDCV concept does not fully explain the genetic determinant of the majority of common complex diseases [7, 16, 18, 21-23]. As noted above, in addition to genetic influence, the environment also exerts effects on trait/disease presentation [4-8]. Most susceptible or disease-associated alleles have modest magnitude of effects and mildly deleterious. If these susceptible alleles have large deleterious effects, then they should not survive under the natural selection due to weak fitness, which is not the case for late-onset common diseases [21, 22]. Moreover, the susceptible alleles with low frequency (allele frequency <5%) are shown to be associated with the same trait/disease, which represents allelic heterogeneity underlying disease [21, 22], and favors the plausible contribution of rare alleles (allele frequency <5%) to complex traits.

Because the majority of heritability for most complex traits remained unexplained, an alternative theory that part of the missing heritability is accounted by rare variants (allele frequency <5%) to complex traits. This theory is the common disease, rare variant (CDRV) hypothesis. The CDRV focuses on the potential impact of multiple rare variants with relatively large effect sizes on common diseases [14, 16, 18, 23-25].

In humans, the distribution of rare variants shows a population-specific occurrence [24-27] because these rare variants evolved relatively recently. It is also

shown that a higher proportion of functional variants have allele frequency less than 5% [27-30]. Rare functional variants tend to have stronger effects [25, 27, 31] and are more likely to alter proteins and structural properties than common variants [24, 25, 30, 32], suggesting the plausible contribution of rare variants to complex diseases.

Due to limitations of detecting rare disease-associated variants by GWASs, resequencing of candidate genes has been recommended to capture rare variants in complex traits [10, 33]. A resequencing scheme of candidate gene association can be used to identify an association between rare variants and complex quantitative traits in two extreme phenotypic groups (≤ the 5^{th}-10^{th} percentile vs. ≥ the 90^{th}-95^{th} percentile of traits) [18, 19, 34, 35]. This resequencing approach has successfully identified rare variants related to complex traits [34, 36], including lipids and lipoproteins [37-46].

In an effort to identify genetic determinants of complex diseases, GWASs and candidate gene studies have successfully identified many variants associated with common and complex diseases, which indicate the contribution of both common and rare variants to common diseases. Therefore, this suggests that the implication of the CDCV and CDRV hypotheses together can provide beneficial genetic insights in explaining the heritability of the particular complex disease. Although, there has been rapidly progress in identifying genes and associated variants for complex traits, the majority of heritability for complex traits is undetermined, and so that much work still remains to be done. With the advanced genetic technologies providing such large datasets and ever-increasing amount of information, it is not easy to integrate and translate the two into biological meanings and clinical practices [32, 35, 47-52]. For studies involving rare variants, power, analytical methods and replication are the main

concerns [27, 53-59]. In addition, the gene-environment interaction studies are required for the comprehensive assessment to determine the etiologies and risk of complex traits [5, 6, 8]. Despite these challenges, moving forward to understand complex traits is important to get insights into genetics basis of complex traits, which may help to understand the underlying mechanism of the diseases and also may lead to prediction of disease risk and development of new drugs and interventions.

2.2. GENETICS AND LIPID TRAITS

2.2.1. Genetic determination of lipid traits

Similar to coronary heart disease (CHD), lipoprotein-lipid levels are multifactorial traits influenced by genetic and environment factors. Twin and familial studies have shown significant genetic contribution to lipoprotein-lipid variation with an estimated heritability of 30-80% [60-63].

Initially, identifying candidate genes for lipid traits has mainly depended on studies of familial forms of dyslipidemias as well as animal experiments. Direct sequencing and linkage association studies have successfully revealed a number of causal genes in the Mendelian lipid disorders, for instance, familial hypercholesterolemia (MIM: 143890) caused by mutations in the *LDLR* gene, and familial high-density lipoprotein (HDL) deficiency (MIM: 604091) caused by mutations in

the *ABCA1*, and *APOA1* genes. The identification of these causative mutations has provided the physiological insights into the regulation of lipoprotein-lipid metabolism.

Since genome-wide screening was initiated in 2007, GWASs have successfully identified additional lipid genes, and to date, at least 157 GWAS loci (allele frequency ≥5%) have been identified [64, 65], attributing approximately 10-30% of total genetic variance for lipid traits. Nonetheless, the numbers of GWAS-identified variants have been increasing overtime, however, the GWAS-identified variants have small effect sizes and contribute approximately 10-30% of total genetic variance for lipid traits. Furthermore, most of the GWAS-identified variants are not truly causative, but rather in high linkage disequilibrium with functional variants, also called synthetic association [66, 67]. Despite that, GWAS findings indicate that genetic susceptibility of dyslipidemia is influenced by multiple genes, which support the CDCV hypothesis.

Because the large fraction of heritability for lipid traits is still unknown, the alternative CDRV hypothesis has gained considerable interest. With advanced sequencing technologies, the numbers of rare variants identified has been rapidly growing overtime. However, it is challenging to identify these rare variants with low allele frequencies, especially less than 1%, which can be seen in only one or a few individuals, resulting in inadequate statistical power to detect the association signals at the significance level. Despite that, a number of rare variants have been found to be associated with lipid traits in many lipid genes, including, *ABCA1*, *APOA1*, *LCAT* [68], and *PCSK9* [69]. These observed associations indicate a significant contribution of rare variants (allele frequency <5%) to lipoprotein-lipid phenotypes, and suggest a potential biological/functional role of rare variants in lipid metabolism. In fact, the discovery of

rare *PCSK9* variant associated with decreased low-density lipoprotein cholesterol (LDL-C) levels [69, 70] has led to the development of new LDL-lowering drugs targeting *PCSK9*, *PCSK9* inhibitors, which is currently in clinical trials.

2.2.2. Genetic perspective: the relationship between loci associated with high-density lipoprotein cholesterol and coronary heart disease

Several epidemiological studies have shown high HDL-C levels are associated with reduced CHD risk [71-73], strongly suggesting the protective role of HDL-C against CHD. However, two HDL-C-raising drugs (i.e., niacin [74, 75] and cholesteryl ester transfer protein (CETP) inhibitors [76, 77]) have failed in clinical trials to reduce CHD risk. In addition, the Mendelian randomization studies could not demonstrate the causal association between HDL-C and CHD events, unlike the positive associations observed with LDL-C and triglycerides (TG) [78, 79]. These findings have raised debates on the anti-atherogenic role of HDL-C in CHD. Recent evidence indicated the inverse association of HDL efflux capacity with CHD events, rather than HDL-C levels [80-82] (see **Section 1.3.2**) that still supports the atheroprotective concept of HDL-C.

Although GWASs have successfully identified several loci associated with HDL-C, these HDL-associated loci have not shown consistent associations with CHD [83, 84]. GWASs for lipids [64, 65] revealed that only small number of loci were associated with only HDL-C, while many more were associated with HDL-C along with other lipid traits. None of these loci associated with only HDL-C were found to be associated with CHD [64, 65]. Similarly, a GWAS meta-analysis from the CARDIoGRAMplusC4D

consortium found that although a small number of CHD-associated loci showed association with HDL-C, they were also associated with LDL-C and/or TG [85].

The HDL-C metabolism is a complex and dynamic process, resulting in various anti-atherogenic properties of HDL (see **Section 1.2**). Moreover, the changes in HDL-C levels also appear to affect other lipid traits such as an inverse correlation between HDL-C and TG that is commonly found in individuals suffering with heart attack or those with metabolic syndrome [86-88].

Collectively, existing evidence illustrates the undeniable fact of the relationship between HDL and CHD and also indicates that this HDL-CHD relationship may not be straightforward and the HDL metabolisms and functions are even more complicated than we expected. Genetic studies clearly indicate an important role of genetic factors in affecting lipoprotein-lipid levels, though the large portion of heritability for lipid traits remains unexplained. More importantly, there is a lack of comprehensive knowledge about the basic relationship between HDL-C and CHD. Therefore, it is essential to further explore the HDL-C relationship with CHD on the basis of genetics to find mechanistic connection between HDL-C and CHD. Additionally, gain the insights from the studies of intermediate phenotypes such as HDL-C may also help to shed further light into pathogenic mechanism underlying related disease such as CHD.

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3.0. OVERVIEW OF SCAVENGER RECEPTOR CLASS B TYPE 1 AND ATP-BINDING CASSETTE TRANSPORTER A1 IN LIPID METABOLISM

Among a variety of atheroprotective functions of HDL-C, HDL-mediated cholesterol efflux by reverse cholesterol transport (RCT) is most extensively studied in which excess cholesterol from macrophage-derived foam cells in atherosclerotic plaque is removed by this pathway [1, 2] (see **Section 1.2.2** and **Figure 1.1**). The cholesterol efflux process involves multiple factors, and among these, two membrane transporters: scavenger receptor class B type 1 (SCARB1), and adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1), play a key role in RCT.

There are two main pathways of cholesterol efflux: bidirectional, passive diffusion (via aqueous diffusion and SCARB1) and unidirectional, active transport (via ABCA1 and ATP-binding cassette transporter G1 [ABCG1]) [3-7]. In brief, aqueous diffusion is a simple passive diffusion of free cholesterol (FC) from cellular membrane into extracellular aqueous space, which is determined by the FC/phospholipids (PLs) ratio between cholesterol donors and acceptors [8]. Similar to aqueous diffusion, SCARB1 facilitates FC flux from high-density lipoprotein cholesterol (HDL-C) to cells through a passive diffusion depending on the net gradient of cholesterol. While, ABCA1 and ABCG1 mediates cholesterol efflux by active transport utilizing ATP as an energy

source for cholesterol transport. Thus, SCARB1 and ABCA1 play an important role in RCT and are relevant in coronary heart disease (CHD) development.

3.1. SCAVENGER RECEPTOR CLASS B TYPE 1 (SCARB1)

3.1.1. Structure and functions of SCARB1

SCARB1 is a multi-ligand glycoproteins membrane protein and belongs to the CD36 superfamily [9, 10]. The structure of SCARB1 is represented as a horseshoe-shape molecular feature containing two trans-membrane domains, two short cytoplasmic tails (N-, and C- terminals) [4, 11], and a large extracellular loop [9, 10] with nine glycosylation sites [12] (**Figure 3.1A**). The extracellular domain has shown to be crucial in the high-affinity binding of HDL and the selective cholesterol uptake activities of SCARB1 [13-18].

The role of SCARB1 was initially recognized as the HDL-C receptor mediating selective cholesteryl esters (CE) and FC uptake from HDL particles into cells without endocytosis and degradation of HDL particles [13, 19-21]. The multi-ligand properties allow SCARB1 interaction with a variety of acceptors besides HDL particles [10, 13, 22] and it also mediates CE and FC from low-density lipoprotein (i.e., low-density lipoprotein cholesterol [LDL-C], oxidized LDL-C and acetylated LDL particles) [10], and very low-density lipoprotein (VLDL) particles [22]. In addition, SCARB1 facilitates a bi-directional FC flux between HDL and cells including macrophages [3, 4, 6, 7, 23-27].

Furthermore, SCARB1 plays a role in other anti-atherogenic functions. The HDL-C binding to SCARB1 induces the activation of the endothelial nitric oxide synthase (eNOS) enzyme in endothelial cells, resulting in the endothelial protection [28-32]. SCARB1 has been shown to induce the HDL-C ability to promote endothelial migration and re-endothelialization [31, 33-35]. SCARB1 is also shown to be involved in protecting against apoptosis [36, 37] as well as inflammation [38, 39]. In addition, SCARB1 appears to be an HDL receptor on platelets [40, 41], and has been shown to modulate platelet functions via the SCARB1-dependent HDL binding to platelets [42-45].

3.1.2. *SCARB1* gene

The human *SCARB1* gene (Entrez gene ID: 949, http://www.ncbi.nlm.nih.gov/gene/949) is localized on chromosome 12q24.31 with a length of ~86.3 kilobases (kb). The *SCARB1* gene (NM_005505, isoform 1) comprising 13 exons encodes a 509 amino acid polypeptide (NP_005496; **Figure 3.1B**). Exon 1 contains the 5' untranslated region (UTR) and a small coding sequence, while the largest exon 13 contains all of the 3' UTR. *SCARB1* produces more than one transcript due to alternative splicing.

SCARB1 is expressed in multiple tissues with highly expression in those organs involved in cholesterol metabolism such as liver, steroidogenic tissues, and ovaries [46]. In addition, the expression of *SCARB1* is found in macrophages, [47-49], endothelial cells [50, 51] and platelets [40]. Moreover, the *SCARB1* promoter contains several important transcription factor elements for lipid/cholesterol regulation (i.e., SP1 and liver receptor homolog-1 response element binding sites) and steroid biogenesis (i.e., sterol regulatory binding protein-1 [SREBP-1] binding sites, and steroidogenic factor-1 (SF-1)

binding sites) [11, 46, 52], which support the role of *SCARB1* in cholesterol and steroid regulation.

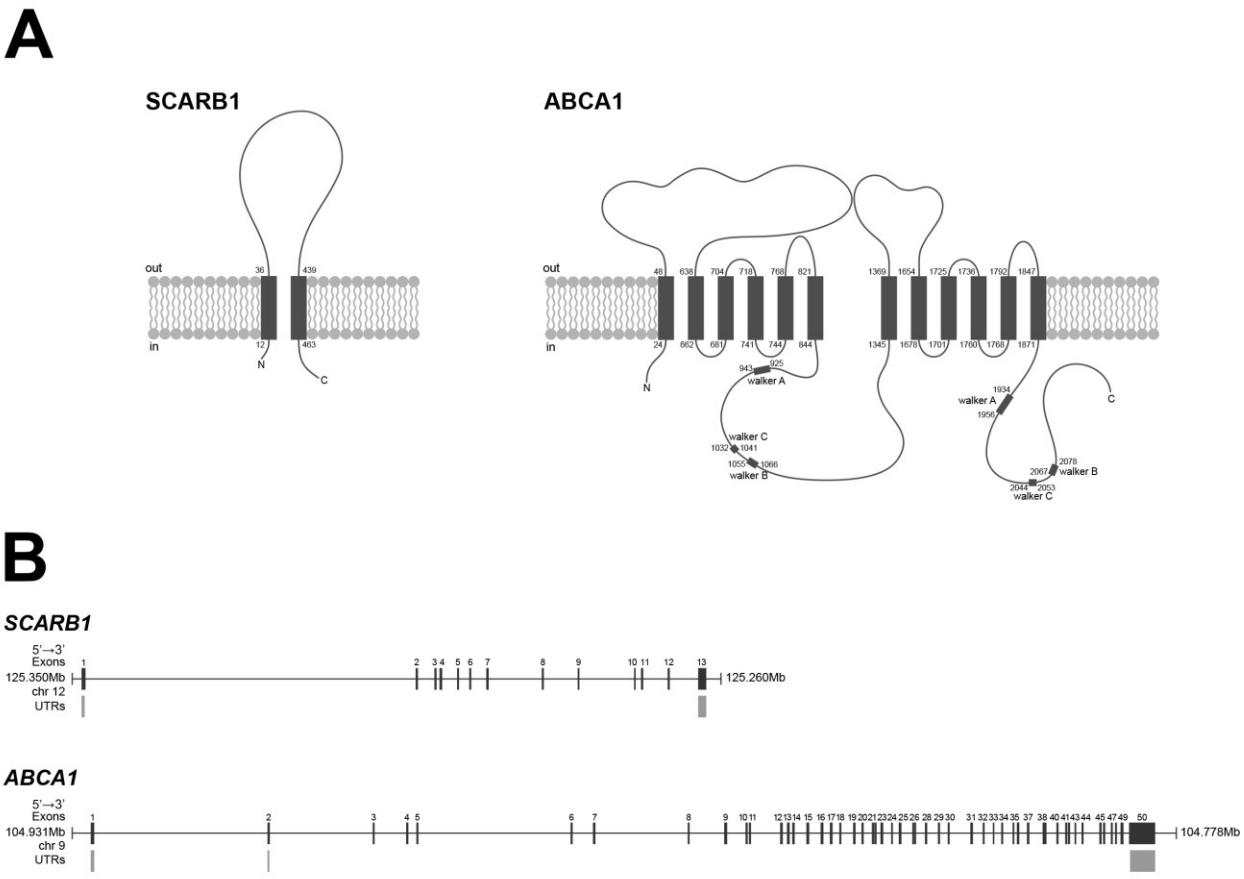

Figure 3.1. Structures of scavenger receptor class B type 1 (SCARB1)* and ATP-binding cassette transporter A1 (ABCA1) proteins (A) and *SCARB1**** and *ABCA1**** genes (B).**

The SCARB1 protein (**A**, left panel) is a horseshoe-shape molecular containing two trans-membrane domains, two short cytoplasmic tails (N-, and C- terminals) [11], and a large extracellular loop [9, 10]. The ABCA1 protein (**A**, right panel) consists of two halves with similar structural units, and each half is comprised of a trans-membrane domain containing six helices and a nucleotide binding domain containing two conserved motifs (walker A and walker B) for

ATP utilization and one signature motif (walker C) unique to ABC transporter molecules [53, 54]. The *SCARB1* gene (**B**, top panel) is located on human chromosome 12, and comprised of 13 exons, of which part of exon 1 codes for 5' untranslated region (UTR) and the whole exon 50 codes for 3' UTR. The *ABCA1* gene (**B**, bottom panel) is located on human chromosome 9, and comprised of 50 exons with an entire exon 1 and part of exon 2 codes for 5' UTRs, while part of exon 50 codes for 3' UTR.

Illustration of structure of SCARB1 protein is modified* based on Rhainds, D., and L. Brissette. 2004. *Int J Biochem Cell Biol* **36: 39-77 [11]; and Jessup, W., et al. 2006. *Curr Opin Lipidol* **17**: 247-257 [4]. The topology of SCARB1 is predicted by http://www.cbs.dtu.dk/services/TMHMM-2.0/.

***Illustration structure of ABCA1 protein is modified* based on Singaraja, R. R., et al. 2003. *Arterioscler Thromb Vasc Biol* **23**: 1322-1332 [55]; Oram, J. F., and J. W. Heinecke. 2005. *Physiol Rev* **85**: 1343-1372 [53]; Jessup, W., et al. 2006. *Curr Opin Lipidol* **17**: 247-257 [4]; and Oram, J. F., and A. M. Vaughan. 2006. *Circ Res* **99**: 1031-1043 [54].

***RefSeq *SCARB1*: hg19, NM_005505.

****RefSeq *ABCA1*: NCBI GRCh38, NC_000009.12, NM_005502.3.

3.1.3. SCARB1 and lipid metabolism

The role for SCARB1 in lipid metabolism mainly came from animal studies. An expression of *SCARB1* is shown to be inversely correlated with HDL-C levels [56-59]. Knockout *SCARB1* reduces hepatic uptake of CE and increases HDL-C levels with enlarged HDL particle size [56, 58]. The disruption of *SCARB1* also leads to an increase in non-HDL-C, apolipoprotein (apo) A-I (apoA-I) and, apolipoprotein B (apoB) levels [59]. In contrast, hepatic overexpression of *SCARB1* accelerates hepatic HDL CE uptake [60, 61] and HDL-C secretion into the bile [59, 61, 62] with subsequently enhanced HDL-C clearance rate [59], resulting in decreased levels of HDL-C including VLDL-C, LDL-C and apoB [59, 60, 62]. Additionally, attenuated expression of *SCARB1* in *LDLr*-/- mice and *APOE*-/- mice appears to promote susceptibility to atherosclerosis

[58, 63-65]. Whereas, hepatic overexpression of *SCARB1* in *LDLr*-/- mice and *APOB*-/- mice is less susceptible to atherosclerotic development [66-69].

Moreover, *SCARB1* has been implicated in the apoB-containing lipoprotein metabolism [59, 60, 62, 63, 66, 70-73]. Overexpression of *SCARB1* increases VLDL catabolism and hepatic VLDL cholesterol production [62, 71, 73] as well as it decreases apoB-containing lipoprotein (i.e., VLDL-C and LDL-C) levels [60, 66]. In contrast, the disruption of *SCARB1* is associated with a decrease in hepatic VLDL-triglycerides (VLDL-TG) and VLDL-apoB production, which leads to reduced apoB-containing lipoprotein levels [70, 72, 73].

In addition to the selective uptake of CE, SCARB1 mediates the free cholesterol flux from cells to HDL-C [6, 23-27]. The cholesterol efflux is promoted in response to an overexpression of *SCARB1* [23]. In contrast, reduced cholesterol efflux to HDL has been observed in *in vitro* trioglycolate-elicited peritoneal *SCARB1* knockout macrophages compared to peritoneal wild-type macrophages [74]. Additionally, no difference in *in vitro* macrophage cholesterol efflux was demonstrated in C57BL/6 recipient mice injected with bone marrow-derived or peritoneal macrophages from *SCARB1* knockout mice compared to those injected with macrophages from wild-type mice [75]. Nonetheless, some studies have been found negative findings of *SCARB1* in affecting cholesterol efflux [76-78]. Therefore, the function of *SCARB1* in macrophage cholesterol efflux requires further investigation.

Collectively, evolving evidence indicates that SCARB1 plays important roles in lipid metabolism as well as in cholesterol efflux.

3.1.4. Genetic association of *SCARB1* variants with lipid traits

In 1999, Acton et al. firstly reported the association of common *SCARB1* variants with LDL-C and HDL-C levels. Since then, evidence for association of common *SCARB1* variants with lipid traits, mainly with HDL-C, has been increasingly demonstrated in various populations. **Table 3.1** summarizes a number of previously reported associations between common *SCARB1* variants and lipid levels [79-90].

Most lipid-associations for *SCARB1* were observed with common coding variants. One reason is that since the size of the *SCARB1* gene is large, spanning ~86.3 kb, most studies did not completely sequence the entire *SCARB1* gene, but rather focused on the functional/coding regions. In particular, the most studies have explored the association in *SCARB1* with rs5888 (p.Ala350Ala) located in exon 8, which showed association with lipid traits in a variety of populations [79-86, 90]. Of note, the majority of common lipid-associated *SCARB1* variants were identified by candidate gene association studies, while only one common variant (rs838880) located in the 3' flanking region was identified by a large genome-wide association study (GWAS) [87]. Additionally, three rare coding *SCARB1* variants (minor allele frequency [MAF] <1%): rs397514572 (p.Ser112Phe) [88], rs187831231 (p.Thr175Ala) [88], and rs387906791 (p.Pro273Ser) [89] mutations (MIM: 601040), were found to be associated with HDL-C levels. Notably, rs387906791 (p.Pro273Ser), the first reported mutation in *SCARB1* in humans, showed its effect on adrenal hormones and platelet aggregation [89], suggesting the functional role of *SCARB1* in regulating steroid hormones and platelets in addition to HDL-C.

Overall, cumulative evidence indicates the influence of common *SCARB1* variants on lipid traits (mainly HDL-C) in humans. Despite that, there are relatively few studies that have evaluated the association of common *SCARB1* variants with lipid levels as compared to other lipid genes. Moreover, association between uncommon *SCARB1* variants (MAF <5%) and lipoprotein-lipid levels in the general population has not been explored. Therefore, more work is needed to fulfill the contribution of common and uncommon *SCARB1* variants to lipid variation. Furthermore, the inconsistency of observed lipid associations (i.e., minor allele, allele frequency, effect size, directional effect, and associated trait) of *SCARB1* among different populations implies the requirement of conclusive and complete characterization of *SCARB1* in a variety of ethnic groups in order to identify the causal variants.

In addition to HDL-C and LDL-C, *SCARB1* variants have also shown associations with TG [82, 85, 91, 92], and the risk for CHD [83, 90, 92-95], strongly indicates an important role of SCARB1 in lipid metabolism.

Table 3.1. Summary of the studies that previously reported association of *SCARB1* variants with lipid traits (*P* <0.05 for candidate gene studies; *P* <1.00 x 10^{-6} for GWASs).

Authors, year [Ref]	Type of Study	Population/Study, Region	N (F, n or %), Sample Details	SNP ID[a] (AA Change)	Chr12 Position[b]	Major/Minor Alleles	MAF	Trait(s)	Effects
Acton et al, 1999 [79]	Candidate gene, population-based	Caucasians, Spain	489 (F, n=288)	rs4238001 (G4A)	125348263	G/A	0.117	HDL-C	↑, only in males
								LDL-C	↓, only in males
								TG	↓, only in males
				rs5888 (A350A)	125284748	C/T	0.437	LDL-C	↓, only in females
Hong et al, 2002 [80]	Candidate gene, case (CAD, n=137)-control (n=124)	Korean, Korea	261 (F, n=108)	rs5888 (A350A)	125284748	C/T	0.83 in CAD; 0.67 in controls	HDL-C	↑, in CAD
Osgood et al, 2003 [81]	Candidate gene, population-based	The Framingham Offspring Study, Massachusetts, USA (mainly Caucasians)	2,650 (F, n=1,357)	rs4238001 (G4A)	125348263	G/A	0.12	HDL-C_2	↓, in carriers with type 2 diabetes
								LDL-C	↓, in carriers with type 2 diabetes
				rs5888 (A350A)	125284748	C/T	0.49	HDL-C	↑, only in males
								HDL-C particle sizes	↑
Tai et al, 2003 [82]	Candidate gene, subjects with familial hypercholesterole-mia	Spanish, Spain	77 (F, n=48)	rs4238001 (G4A)	125348263	G/A	0.19	TG	↑
				rs5888 (A350A)	125284748	C/T	0.47	TC	↑
								TG	↑
Morabia et al, 2004 [83]	Candidate gene, population-based	Swiss, Switzerland	1,756 (F, n=891)	rs5888 (A350A)	125284748	C/T	0.49	HDL-C	↑, only in males
								TC	↑, only in females
								LDL-C	↑, only in females
Boekholdt et al, 2006 [84]	Candidate gene, subjects with symptomatic coronary artery disease	The Regression Growth Evaluation Statin Study (REGRESS), a multi-center, prospective study, the Netherlands	546 (F, n=0), all males with symptomatic coronary artery disease	rs5888 (A350A)	125284748	C/T	0.49	HDL-C	↑, only in males
Roberts et al, 2007 [86]	Candidate gene, population-based	The Old Order Amish (OOA) [European Ancestry], Lancaster county, PA, USA	919 (F, n=491)	rs5888 (A350A)	125284748	C/T	0.41	HDL-C	↑, only in females <50 yrs
				rs5891 (I135V)	125299542	G/A	0.03	HDL-C	↑, only in females
Tanaka et al, 2007 [85]	Candidate gene, population-based	Spanish, Spain	59 (F, n=0), all males	rs5888 (A350A)	125284748	C/T	0.43	Postprandial small TRL-TG levels	↓, only in males
Teslovich et al, 2010 [87]	GWAS, 46 studies (population-based, family-based, and case-control)	46 multi-centered cohorts of European descent (primary study)	>100,000 individuals of European ancestry (F, range 12.80-100.00%)	rs838880	125261593	C/T	0.31	HDL-C	↑, primary meta-analysis
Brunham et al, 2011 [88]	Candidate gene, subjects with extreme HDL-C levels (≥90^{th} %tile of HDL-C distribution)	Caucasians, Canada	120 probands with extreme HDL-C levels at ≥90^{th} %tile and 80 individuals with HDL-C at the ≤10th %tile Follow-up genotyping in 17 family members of 2 probands: 5 carriers vs 12 non-carriers	rs397514572 (S112F) [MIM: 601040]	125299610	C/T	0.004, in 120 probands	HDL-C	↑, combined effects of two mutations
				rs187831231 (T175A) [MIM: 601040]	125298855	A/T	0.004, in 120 probands		
Vergeer et al, 2011 [89]	Candidate gene, subjects with extreme high HDL-C levels (>95^{th} %tile of HDL-C distribution)	Caucasians, the Netherlands	162 unrelated individuals with HDL-C levels >95^{th} %tile Follow-up genotyping in 124 family members of 1 proband: 18 carriers vs.36 non-carriers	rs387906791 (P297S) [MIM: 601040]	125292427	C/T	0.003, in 162 individuals with the >95^{th} %tile of HDL-C levels	HDL-C	↑

Table 3.1. (continued)

Wu et al, 2012 [90]	Candidate gene, population-based	Chinese, China (2 populations: Bai Ku Yao and Han)	1183 [598 Bai Ku Yao population (F, n=312) and 585 Han population (F, n=308)]	rs5888 (A350A)	125284748	C/T	0.217, in Bai Ku Yao; 0.263, in Han	HDL-C ApoA-I	↓ (TT), only in Bai Ku Yao males ↓ (TT), only in Bai Ku Yao males

AA, amino acid; ApoA-I, apolipoprotein A-I; CAD, coronary artery diseases; GWAS, genome-wide association study; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.
[a] dbSNP build 139: GRCh37.p10.
[b] RefSeq *SCARB1*: hg19, NM_005505.

3.2. ADENOSINE TRIPHOSPHATE (ATP)-BINDING CASSETTE TRANSPORTER CLASS A1 (ABCA1)

3.2.1. Structure and functions of ABCA1

ABCA1 is a member of ABC transporter family. The ABCA1 protein consists of two halves with similar structural units, and each half is comprised of a trans-membrane domain containing six helices and a nucleotide binding domain (NBD) containing two conserved motifs (walker A and walker B) for ATP utilization and one signature motif (walker C) unique to ABC transporter molecules [4, 53-55] (**Figure 3.1A**). In addition, there is a predicted a cytosolic N terminus and two large extracellular loops with high glycosylation that are linked by at least one cysteine bond [53, 54]. The two large extracellular loops of ABCA1 containing critical residues are important for an interaction with apoA-I [96].

ABCA1 transporters play a main role in mediating FC and PLs export to lipid-free/lipid-poor apoA-I to generate nascent HDL (pre-β, discoidal HDL) particles in the first step of RCT [3, 4, 6, 97-101]. Lipid-free/lipid poor apoA-I can interact with ABCA1 transporters in liver and peripheral tissue [102-104] that acquires FC and PLs to form discoidal, nascent HDL particles. The nascent HDL particles continue the acquisition of FC and PLs (lipidation) becoming spherical and mature HDL particles (see details RCT and the biosynthesis of HDL in **Section 1.2.2** and **Figure 1.1**).

In addition, ABCA1 transporter also facilitates unidirectional cellular cholesterol efflux in peripheral cells, especially cholesterol-loaded macrophages [78, 102, 103, 105-113].

3.2.2. *ABCA1* gene

The human *ABCA1* gene (Entrez gene ID: 19, http://www.ncbi.nlm.nih.gov/gene/19) is localized on chromosome 9q31.1 with a total length of ~147.2 kb. The *ABCA1* gene (NM_005502.3) with 50 exons encodes for a 2261 amino acid polypeptide (NP_005493.2; **Figure 3.1B**). Exon 1 contains the 5' UTR, and exon 2 contains the 5' UTR as well as a coding sequence. While, the largest exon 50 contains the 3' UTR and a short coding sequence.

The *ABCA1* gene is expressed in various tissues, and the most abundant expression is found in liver and macrophages [114-116]. The promoter of *ABCA1* contains several transcription factor binding motifs [117], including the transcription binding sequences for lipid regulation (i.e., SP1, AP1, and SREBPs binding sites), sterol-dependent regulation (i.e., nuclear receptor LXR [liver receptor X] binding site),

liver functions (i.e., HNF3β binding sites), as well as monocyte/macrophage differentiation (i.e., GATA and CMYB binding site) [118], indicating the function of *ABCA1* in regulating lipid metabolism and cellular cholesterol efflux.

3.2.3. ABCA1 and lipid metabolism

The defective *ABCA1* gene causes low HDL-C levels, including the hereditary forms of HDL deficiency such as Tangier disease (TD; MIM: 205400). TD was first identified in the 1960's and characterized by nearly absence of HDL-C levels, low apoA-I levels, high TC levels, a foam cell deposition in reticuloendothelial (RE) tissues, and a high risk for developing CHD [119]. An impaired ability of apoA-I lipidation for nascent HDL formation as well as a defect in intracellular cholesterol and PL efflux to HDL and apoA-I [120-122] have been demonstrated in TD, resulting in HDL-C and apoA-I deficit, and an accumulation of CE in RE tissues [119]. Moreover, kinetic studies of TD showed an enhanced catabolism of small, cholesterol-poor, TG-rich LDL particles [123, 124] together with reduced hydrolysis of VLDL by lipoprotein lipase [125], leading to a decrease in LDL levels and an increase in TG [119].

Additional evidence for the ABCA1 functions in lipid metabolism has been shown in animal studies. Knockout *ABCA1* mice have extremely low HDL-C and apoA-I [110], low LDL-C and apoB [110], and high TG [124], and are loaded with foam cells [110, 126], similar to the phenotypes of human TD. In addition, the specific disruption of hepatic *ABCA1* increases VLDL TG production and LDL clearance in conjunction with elevates TG secretion, which results in low LDL-C and high TG levels [127].

Furthermore, disruption of *ABCA1* in *APOE-/-* mice significantly promoted atherosclerotic lesions [128]. Hepatic-specific *ABCA1* knockout in *LDLr-/-* mice on atherogenic protects the progression of atherosclerosis, however, HDL-C levels is significantly reduced [129]. Whereas, hepatic-specific *ABCA1* knockout in *APOE-/-* mice on chow diet increase a susceptibility to developing atherosclerosis [128].

Mice with overexpression *ABCA1* have increased HDL-C and apoA-I levels. Silencing hepatic expression of *ABCA1* decreases large nascent HDL maturation, increases VLDL TG secretion, and attenuates phosphatidylinositol-3 (PI3) kinase activity [130].

Notably, there have been reported inconsistent findings of ABCA1 in animal model studies, of which each has distinctive characteristics (i.e., mice backgrounds, sources of the genes, types of vector, and expression sites) [131]. For instance hepatic-specific *ABCA1* overexpression in *LDLr-/-* mice displays an increase in HDL-C and apoB-containing lipoprotein levels with an atherosclerotic progression [132]. Overexpression of *ABCA1* in *APOE-/-* mice also results in an increased atherosclerotic development [133]. However, these findings appear to be contradicted by the results from other two studies that observe a protective effect against atherosclerosis in overexpression of *ABCA1* in *LDLr-/-* [128] or *APOE-/-* [134] mice. In addition, the complete disruption *ABCA1* in macrophages in *LDLr-/-* mice and *APOE-/-* mice reduces cholesterol levels, and develops foam cell accumulation in various tissues together with atherosclerosis [135]. In contrast, another study demonstrated no atherosclerosis development in macrophages-specific *ABCA1* disruption in *LDLr-/-* mice [128].

Nonetheless, all existing evidence supports the important function of ABCA1 in lipid metabolism, which is attributable to atherosclerosis susceptibility.

3.2.4. Genetic association of *ABCA1* variants with lipid traits

In 1999, the loss-of-function mutations in *ABCA1* were firstly identified in TD (MIM: 205400) [105-108], which is characterized by extremely low HDL-C levels [119]. Since then, a number of *ABCA1* variants have been identified, particularly in individuals with low HDL-C levels; at least 25 *ABCA1* variants (http://www.omim.org/entry/600046) appear to have clinical significance in TD and high-density lipoprotein deficiency (HDLD; MIM: 604091). Moreover, an increased risk for CHD development has been reported in TD [119], implying that defects in *ABCA1* lead to a susceptibility to atherogenesis due to HDL-C deficiency.

ABCA1 genetic variation is also shown to be a determinant of lipid levels in the general population. Previous studies have reported the association of *ABCA1* variants with major lipid traits, mainly with HDL-C; however, the majority of studies investigated only the potentially functional variants located in 5' UTR, promoter, and coding regions of the gene due to the large size of the *ABCA1* gene, and also focused on variants with MAF ≥5%). Some previous candidate gene studies that reported associations of the potentially functional *ABCA1* variants with lipid traits and/or heart conditions are summarized in **Table 3.2** [136-165]. The most widely studied *ABCA1* variant was rs2230806 (p.Arg219Lys) located in exon 7, followed by rs2066714/rs4149313 (p.Ile883Met) located in exon 18. These two common coding *ABCA1* variants (MAF ≥5%) have been found to be associated with lipid levels in various populations [136-

138, 143, 145, 148, 151-153, 156, 158, 160, 165], mostly with an increase in HDL-C levels. Some studies also demonstrated association of the two variants with the risk/incidence for CHD events [138, 141, 144, 147, 152, 155, 157, 164], but with inconsistent findings.

It should be noted that relatively few previous studies have successfully demonstrated the association of rare *ABCA1* variants with lipoprotein-lipid levels [137, 138, 145, 146, 149, 154] (**Table 3.2**). However, there has been more success in identifying rare variants using individuals with extreme phenotypes [137, 145, 146, 154]. Despite this, to date, there have been a small number of studies successfully reporting association of rare *ABCA1* variants (MAF <5%) with lipid traits in the general population.

Furthermore, GWASs have also shown association of *ABCA1* variants with lipid traits. **Table 3.3** summarizes GWASs that previously reported associations between common *ABCA1* variants (MAF ≥5%) and lipid traits [87, 166-177]. Consistent with the findings in candidate gene studies summarized in **Table 3.2**, the vast majority of GWASs have shown evidence for *ABCA1* variants associated with HDL-C, although most of these GWAS-identified variants are non-coding.

Overall, emerging evidence from previous studies supports the role of common and rare *ABCA1* variants in lipid metabolism in humans. However, associations of *ABCA1* variants, particularly uncommon variants (MAF <5%), remain undetermined. In addition, inconsistent associations (i.e., minor allele, allele frequency, effect size, directional effect, and associated trait) of *ABCA1* variants with lipid levels and CHD events have been noticed, most likely due to the differences in backgrounds of study populations (i.e., ethnicities, health conditions, and environmental factors) as well as the

possible effects of gene-gene, and gene-environment interactions. Furthermore, there has been a lack of knowledge for association of *ABCA1* with lipid traits in different ethnic groups, as the majority of studies have investigated in European ancestry populations. These limitations also reflect the importance of complete coverage of the *ABCA1* gene in attempt to identify the real causal/functional variants. Therefore, it is essential to further explore the genetic contribution of *ABCA1* to lipid traits as well as the association of *ABCA1* with CHD in humans.

Table 3.2. Summary of the candidate gene studies that previously reported association of potentially functional (i.e., 5' UTR promoter, and coding) *ABCA1* variants with lipid traits and/or CHD events (*P* <0.05).

Authors, year [Ref]	Type of Study	Population/Study, Region	N (F, n/%), Sample Details	Studied Phenotypes: Lipid Traits	Studied Phenotypes: CHD Events	SNP Name[a] (SNP ID[b])	Relevant Findings
Wang et al, 2000 [136]	Candidate gene, population-based	Multi-ethnic Canadians, Canada **Proband sample:** 1) 8 Canadian Tangier disease (TD) subjects from TD family; 2) 3 Aboriginal Canadians with familial hypoalphalipoproteinemia: 2 taken from the well-characterized Oji-Cree sample and 1 taken from the well-characterized Inuit sample **Genotyping sample:** 223 normo-lipidemic Canadian subjects from 6 ethnic groups: 38 Europeans, 44 Oji-Cree, 30 Inuit, 36 Africans, 37 Chinese, and 38 South Asians) were studied to determine allele and genotype frequencies for new variants **Association analysis sample For I/M823M:** 245 Canadian Inuit subjects	245 (F, 54.28%), Canadian Inuit subjects	HDL-C, TG, ApoA-I		I823M (I883M, rs2066714/ rs4149313)	In Inuit group, MM: significantly associated with higher HDL-C levels (*P*<0.05), compared to II+MI
Brousseau et al, 2001 [137]	Candidate gene, case (low HDL-C [≤40 mg/dL] and CHD, n=1014)-control (n=1014)	The Veterans Affairs Cooperative HDL Cholesterol Intervention Trial (VAHIT) and the Framingham Offspring Study (FOS), USA	2,028 (F, 0%), all males 1,014 males with low HDL-C (≤40 mg/dL) and CHD from VAHIT and 1014 males with no clinical CHD from FOS	TC, HDL-C, LDL-C, TG, TC/HDL-C ratio	CHD	A2589G (I883M, rs2066714/ rs4149313) G3456C[c] (E1172D, rs33918808)	In VAHIT, M allele: significantly associated with reduced TG (*P*<0.02) In VA, C allele: significantly associated with lower ApoB levels (*P*<0.02) In VA, C allele: significantly associated with increased risk of CHD (risk ratio [RR]=2.71 [95% CI=1.40-5.32], *P*=0.004)
Clee et al, 2001 [138]	Candidate gene, subjects with proven CAD	The Regression Growth Evaluation Statin Study (REGRESS), the Netherlands **Main sample:** 804 Dutch males with proven CAD who participated in the Regression Growth Evaluation Statin Study (REGRESS), the Netherlands **Replication sample for R216K:** 3 small cohorts 1) European descent with familial hypercholesterolemia [FH]; 2) A group of French Canadians with CAD and low HDL-C; 3) A random sample of French Canadians without clinical manifestations of CAD who were unselected for plasma lipid levels	804 (F, 0%)	HDL-C, TG	CAD	R219K (rs2230806) V399A[c] (rs9282543)	KK (REGRESS): significantly associated with decreased TG (*P*=0.001) compared to RR KK (Replication): significantly associated with increased HDL-C (*P*=0.02), and trend toward decreased TG (*P*=0.08), compared to RR RK+KK (REGRESS): significantly associated with reduced the risk of CAD (OR=0.72 [95% CI=0.54-0.95], *P*=0.02), compared to RR A allele (REGRESS): trend toward higher HDL-C *(P*=0.15) A allele (REGRESS): trend toward less progression in diffuse atherosclerosis (increased MSD: *P*=0.16)

Table 3.2. (continued)

						V771M[c] (rs2066718)	M allele (REGRESS): significantly associated with less progression in focal atherosclerosis (increased MOD; *P*=0.045)
						V825I (rs2066715)	I allele (REGRESS): increased the CAD events (*P*=0.0008)
						I883M (rs2066714/ rs4149313)	M allele (REGRESS): significantly associated with increased in CAD progression (*P*<0.001)
						R1587K (rs2230808)	K allele (REGRESS): significantly associated with decreased HDL-C (*P*=0.03)
Lututcuta et al, 2001 [139]	Candidate gene, subjects with ≥1 coronary lesion causing 30% to 75% diameter stenosis and LDL-C in the range of 115-190 mg/dL despite diet and randomized to fluvastatin (40 mg daily) or placebo	The Lipoprotein and Coronary Atherosclerosis Study (LCAS), USA	429 (F, 19%), subjects with ≥1 coronary lesion causing 30% to 75% diameter stenosis and LDL-C in the range of 115-190 mg/dL despite diet and randomized to fluvastatin (40 mg daily) or placebo	TC, HDL-C, LDL-C, TG, Lp(a), ApoA-I, ApoB	CHD by using quantitative coronary angiograms	-477C/T (rs2422493)	TT: trend toward lower levels of HDL-C (P=0.094) and ApoA-I (*P*=0.054), compared to CC+CT CT+TT: significantly associated with higher numbers of 30%-75% stenosis lesions (*P*=0.002)
Zwarts et al, 2002 [140]	Candidate gene, subjects with proven CAD	The Regression Growth Evaluation Statin Study (REGRESS), the Netherlands **<u>Main sample</u>:** 804 Dutch males with proven CAD who participated in the Regression Growth Evaluation Statin Study (REGRESS), The Netherlands **<u>Replication sample for R216K</u>:** 3 small cohorts: 1) European descent with FH; 2) A group of French Canadians with CAD and low HDL-C; 3) A random sample of French Canadians without clinical manifestations of CAD who were unselected for plasma lipid levels	804 (F, 0%), all males with CAD	HDL-C, TG	The mean segment diameter (MSD), the minimum obstruction di-ameter (MOD), and the incidence of cardiovascular events [i.e. death, myocardial infarction (MI), unscheduled coronary angio plasty or bypass surgery (PTCA, CABG), or stroke/ transient ischaemic attack (TIA)]	G-191C/Promoter C69T/5' UTR-Exon 1 C117G/ 5'UTR-Exon 1	CC (REGRESS): significantly associated with higher incidence of CAD events (*P*=0.001) with the OR of 3.96 (95% CI=1.66-9.45, *P*=0.003), compared to GG CT+TT (REGRESS): significantly associated with higher incidence of CAD events (*P*=0.03), compared to CC In placebo group, CT+TT (REGRESS): significantly associated with higher incidence of MSD (*P*=0.01), compared to CC CT+TT (Replication): significantly associated with higher incidence of CAD events (*P*=0.046) with the OR of 2.05 (95% CI=1.06-3.96, *P*=0.003), compared to CC GG (REGRESS): significantly associated with increased TG (*P*=0.003), compared to CC
Cenarro et al, 2003 [141]	Candidate gene, case (FH with premature CHD [at age <40 yrs], n=216) –control FH without premature CHD [at age <40 yrs], n=158)	The Spanish FH Register, Spain	374 (F, 44%), subjects with heterozygous FH from the Spanish FH Register, Spain	TC, HDL-C, LDL-C, TG, Lp(a)	CHD	R219K (rs2230806)	In FH with premature CHD age <40 years, K allele: significantly associated with increased risk of CHD (OR=0.51 [95% CI=0.27-0.96], *P*=0.035)
Evans and Beil, 2003 [142]	Candidate gene, outpatients at lipid clinic	Lipid outpatient clinic of the University Hospital Hamburg-Eppendorf, Germany	813 (F, 45.39%), subjects attending the lipid outpatient clinic of the University Hospital Hamburg-Eppendorf	TC, TG, ApoA-I, ApoB, Lp(a)		R219K (rs2230806)	In males at age <41 yrs, K allele: significantly associated with lower TG (*P*=0.02)
Harada et al, 2003 [143]	Candidate gene, subjects who had undergone coronary angiography	The department of cardiovascular medicine, University of Tokyo Hospital, Japan	410 (F, 16.10%), subjects who had undergone coronary angiography	TC, HDL-C, LDL-C, TG	CHD	R219K [G1051A] (rs2230806)	RK+KK: trend toward higher TG (*P*=0.055)

Table 3.2. (continued)

						I823M [A2589G] (I883M, rs2066714/ rs4149313)	MM: significantly associated with higher HDL-C than II+IM (*P*=0.003)
Bertolini et al, 2004 [144]	Candidate gene, subjects with FH	Italy	570 (F, 77.02%), FH unrelated cases (n=221), and relatives with heterozygous FH (n=349)	TC, HDL-C, LDL-C, TG, LDL-C/HDL-C ratio	CAD	R219K (rs2230806)	In FH aged >30 years, RK+KK: significantly associated with protective effect against CAH (OR=0.44 [95% CI=0.24-0.79], *P* <0.006), and more significantly in males (OR=0.37 [95% CI=0.18-0.75], *P*<0.006) than females (OR=0.48 [95% CI=0.23-1.00], *P*=0.050), compared to those carrying RR
Cohen et al, 2004 [145]	Candidate gene, Americans (Whites and Blacks) and Canadians (Whites) **Sequencing sample:** 2 groups, low HDL-C group (<5Th %tile of HDL-C distribution) and high HDL-C group (>95th %tile of HDL-C distribution)	The Dallas Heart Study (DHS), USA (for Americans) and the Ottawa Heart Institute, Canada (for Canadians)	**Sequencing sample:** 519 (F, 40.85%), comprising of: 1) 256 Americans (F, 50.00%): low HDL-C group (n=128) vs high HDL-C group (n=128); 2) 263 Canadians (F 65.62%): low HDL-C group (n=155) and high HDL-C group (n=108) **Follow-up sample:** 2873 Americans: 1043 Whites (F, 51.61%) and 1830 Blacks (F, 57.76%)	HDL-C		R85L[c] T459P[c] H551D[c] W590L[c] R965C[c] L1026P[c] W1322X[c] E1386Q[c] C1477F[c] D1706N[c] S1731C[c] N1800H[c] R1851X[c] T2073A[c]	Eleven of total 14 rare coding variants observed in the low HDL-C group: associated with cholesterol efflux rates (*P*, not shown)
						T774P[c]	In the high HDL-C group, P allele: associated with cholesterol efflux rates (*P*, not shown)
						V825I (rs2066715)	In White males (follow-up sample), I allele: associated with lower HDL-C (*P*=0.03)
						I883M (rs2066714/ rs4149313)	In Black males (follow-up sample), M allele: associated with higher HDL-C (β, not shown; *P*=0.01)
Frikke-Schmidt et al, 2004 [146]	Candidate gene, population-based	The Copenhagen City Heart Study, a prospective cardiovascular population study of individuals selected based on the Central Population Register Code, Denmark **Sequencing sample:** 190 subjects with the extreme HDL-C levels (adjusted for age and sex), the lowest 1% (n=95) and the highest 1% (n=95) **The entire general population sample:** all subjects (n=9,259) were used for genotyping 6 non-synonymous variants identified by screening	9,259 (F, 55%), the entire general sample	TC, HDL-C, TG, ApoA-I, ApoB		V771M[c] (rs2066718)	In females, M allele: significantly associated with increased HDL-C levels (*P*=0.009)
						V825I (rs2066715)	In females, I allele: significantly associated with increased HDL-C levels (*P*=0.008)
						R1587K (rs2230808)	In males, K allele: significantly associated with decreased HDL-C levels (*P*=0.007) in females and trend toward decreased HDL-C levels (*P*=0.07) in males
Tregouet et al, 2004 [147]	Candidate gene, case (MI, n=800)- control (n=776)	WHO MONICA Project, UK: Belfast, Northern Ireland; and Glasgow, Scotland	1,576 (F, ~33%)	ApoA-I	MI	C-564T	T allele: significantly associated with increased plasma ApoA-I levels (*P*=0.015)
						R219K (rs2230806)	K allele: significantly associated with decreased risk of MI (OR=0.8 [95% CI=0.68-0.94], *P*=0.007)
						R1587K (rs2230808)	K allele: significantly associated with decreased ApoA-I levels (*P*<0.0001)
Yamakawa-Kobayashi et al, 2004 [148]	Candidate gene, population-based	School-aged Japanese subjects (ages 9-15 yrs) in a school survey established by University of Tsukuba, Japan	327 (F, 49.54%)	TC, HDL-C, LDL-C, TG, ApoA-I, ApoB		K219R (rs2230806)	R allele: significantly associated with lower HDL-C levels (*P*=0.016), lower ApoA-I (*P*=0.012)
						V771M (rs2066718)	M allele: significantly associated with higher ApoA-I (*P*=0.035)

Table 3.2. (continued)

Frikke-Schmidt et al, 2005 [149]	Candidate gene, population-based	The Copenhagen City Heart Study, a prospective cardiovascular population study of individuals selected based on the Central Population Register Code, Copenhagen, Denmark	9,076 (F, 55.33%)	TC, HDL-C, TG, ApoA-I, ApoB	IHD	K776N[c] (rs13880920)	In males, N allele: marginally associated with lower HDL-C levels (*P*=0.05) and significantly associated with lower ApoA-I (*P*=0.03) N allele: significantly associated with cumulative incidence of IHD (log rank test, *P*=0.001) with the hazard ratio [HR] of 2.40 (95% CI=1.30-4.50)
Hodoğlugil et al, 2005 [150]	Candidate gene, population-based	The Turkish Heart Study database, six regions of Turkey	2,700 (F, 42.55%)	TC, HDL-C, LDL-C, TG, TC/HDL-C ratio		C-14T (rs1800977) V771M (rs2066718)	TT: significantly associated with higher HDL-C (*P*<0.02), compared to CC, in males. In males, VM: significantly associated with higher HDL-C (*P*<0.01), compared to VV
Porchay et al, 2006 [151]	Candidate gene, population-based	The Data from an Epidemiological Study on the Insulin Resistance syndrome (D.E.S.I.R.) Study, French	5,040, (F, 51%)	TC, HDL-C, LDL-C, TG, ApoA-I, ApoB		C69T (rs1800977) G378C (rs1800978) G1050A (R219K, rs2230806)	In males with normal weight (BMI<25 kg/m^2), T allele: significantly associated with higher HDL-C (*P*=0.019), and higher ApoA-I (*P*=0.023) In the entire sample, C allele: significantly associated with lower HDL-C (*P*=0.030), and lower ApoA-I (*P*=0.015) In the overweight group (BMI≥25 kg/m^2), C allele: significantly associated with lower HDL-C (*P*=0.005), and lower ApoA-I (*P*=0.005) In the normal weight group (BMI<25 kg/m^2), A allele: significantly associated with higher HDL-C (*P*=0.004) In the overweight group (BMI≥25 kg/m^2), A allele: significantly associated with lower HDL-C (*P*=0.02)
Benton et al, 2007 [152]	Candidate gene, population-based	The Multi-Ethnic Study of Atherosclerosis (MESA) from 6 communities in the USA: Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; and St. Paul, MN Multi-ethnicities: White/Caucasian (46.23%), Black/African-American (21.15%), Chinese (10.01%), and Spanish/Hispanic/Latino (22.60%)	969 (F, 57.40%)	HDL-C, LDL-C, and TG	Coronary artery calcification (CAC) and carotid IMT	565C/T (−477C/T, rs2422493) R219K [G1051A] (rs2230806)	T allele: trend towards a higher prevalence of CAC in CT (prevalence ratio [PR]=1.13, *P*=0.08) and TT (PR=1.16, *P*=0.08) AA under recessive genetic models: significantly associated with higher HDL-C (*P*=0.04), lower LDL-C (*P*=0.01) and a trend toward lower TG (*P*=0.07), compare with GG+GA AA: significantly associated with a 28% lower prevalence of CAC (*P*=0.002), compare with GG
Jensen et al, 2007 [153]	Candidate gene, population-based and case-control	The Nurses' Health Studies (NHS) I (n=745; 249 cases [CHD] vs 496 controls) and NHSII (n=465), USA	1,210 (F, 100%)	TC, HDL-C, LDL-C, TG	CHD	−565C/T (rs2422493) −191G/C (rs1800976) I833M (rs2066714/ rs4149313) R1587K (rs2230808)	T allele (co-dominant): associated with lower risk of CHD (OR=0.8 [95% CI=0.6–1.0]) C allele (co-dominant): associated with lower risk of CHD (OR=0.8 [95% CI=0.6–1.0]) In females <55 yrs, M allele: significantly associated with higher HDL-C levels (*P*<0.01) In females <55 yrs, K allele: significantly associated with lower TG (*P*=0.01)
Mantaring et al, 2007 [154]	Candidate gene, outpatients at cardiology clinic	Outpatient cardiology clinic, University of Maryland, USA	124 (F, NA), subjects that were referred to outpatient cardiology clinic, University of Maryland: very high HDL-C (n=22), high HDL-C (n=34), average HDL-C (n=36), and low HDL-C (n=32)	TC, HDL-C, LDL-C, TG, ApoA-I, ApoB		E1172D[c] (rs33918808)	ED: significantly associated with higher HDL-C (*P*<0.01), higher ApoA-I (*P*<0.05), in conjunction with lower TG (*P*<0.05), compared to EE
Nebel et al, 2007 [155]	Candidate gene, case (CHD, n=1,090)-control (n=728)	The PopGen project, Northern Schleswig-Holstein, Germany	1,818 (F, NA), subjects with CHD at age <55 yrs and had undergone coronary vascularization interventions at the University Hospital Schleswig-Holstein Campus Kiel, Kiel, Germany		CHD	R219K (rs2230806) I883M (rs2066714/ rs4149313)	K allele: marginally associated with CHD with the OR of 1.20 [95% CI=0.99-1.47], *P*=0.066) M allele: significantly association with CHD (*P*=0.034) with the OR of 1.28 [95% CI=1.02-1.61], *P*=0.034)

Table 3.2. (continued)

Genvigir et al, 2008 [156]	Candidate gene, case (hypercholesterolemic [HC], n=224)-control (normolipemic [NC], n=143)	The study for the risk of CHD at the Institute Dante Pazzanese of Cardiology and the University Hospital of the Sao Paulo University, Brazil	367 (F, 69.48%), subjects attending outpatient clinic participating the study for the risk of CHD	TC, HDL-C, LDL-C, VLDL-C, TG, TG/HDL-C ratio, ApoA-I, ApoB, ApoB/ApoA-I ratio		C-105T (rs56064613) R219K (rs2230806)	In the HC group, CT/TT: significantly associated with higher HDL-C (*P*=0.009), lower TG (*P*=0.010), lower VLDL-C (*P*=0.010), and lower TG/HDL-C ratio (*P*=0.001) In the HC group, RK+KK: significantly associated with lower TG (*P*=0.039), lower VLDL-C (*P*=0.036), lower TG/HDL-C ratio (*P*=0.030) In the NC group, RK+KK: significantly associated with higher ApoA-I (*P*=0.035)
Sandhofer et al, 2008 [157]	Candidate gene, subjects with high risk for atherosclerosis	The Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR), Austria	688 (F, 0%), all males	TC, HDL-C, LDL-C, TG, ApoA-I, ApoB, LDL size	Intima media thickness (IMT) of the carotid arteries	R219K (rs2230806) I883M (rs2066714/ rs4149313)	K allele: significantly associated with lower IMT (*P*=0.001), and reduced risk of advanced CHD (OR=0.59 [95% CI=0.39-0.88], *P*=0.009) IM+MM: significantly associated with higher LDL-C (*P*=0.048).
Porchay-Baldérelli et al, 2009 [158]	Candidate gene, Subjects with type II diabetic subjects with high urinary albumin excretion (≥20 mg/L at least 2 consecutive tests)	The DIABHYCAR (non- insulin-dependent diabetes, hypertension, microalbuminuria or proteinuria, cardiovascular events, and ramipril) study, French Follow-up: mean 4 years Clinical trials: on ramipril 1.25 mg/day, ~49%	3,129 (F, ~27%),	TC, HDL-C, LDL-C, TG	CHD history, MI history, angina history, incidence of CHD	+69C>T (rs1800977) +378G>C (rs1800978) I883M (rs2066714/ rs4149313)	T allele: significantly associated with lower risk of MI (*P*=0.002) C allele: significantly associated with lower HDL-C levels (*P*=0.04) M allele: significantly associated with higher HDL-C levels (*P*=0.03)
Acuña-Alonzo et al, 2010 [159]	Candidate gene, population-based	Multi-ethnic subjects (total n=5,268): Native Americans (n=4,405) from 12 populations: North America (USA+Mexico) (n=3,750), South American (n=655); Asians (n=623); and Europeans (n=240)	4,405 Native Americans (F, ranging from 0%-100%) The rest were not available.	TC, HDL-C, TG		R230C (rs9282541)	C allele: significantly associated with lower HDL-C levels in all Native American (US+Mexico) populations combined (*P*=1.77E-11) and in Mexican Natives combined (*P*=5.30E-08)
Delgado-Lista et al, 2010 [160]	Candidate gene, population-based	University of Cordoba, Spain	88 (F, 0%), all healthy male students	TC (total, large TRL, small TRL), HDL-C, LDL-C, TG (total, large TRL, small TRL), ApoA-I, ApoB		R219K (rs2230806) i48168 [C/T] (rs4149272) i27943 [G/A] (rs2575875)	K allele: a trend for lower fasting TG (*P*=0.056) and large TRL TG (*P*=0.07) T allele: significantly associated with lower postprandial TG (*P*<0.05), lower TRL TG (*P*<0.05), and lower ApoA-I levels (*P*<0.05) A allele: significantly associated with higher postprandial TG (*P*<0.05), higher TRL TG (*P*<0.05), lower ApoA-I levels (*P*<0.05), and higher ApoB levels (*P*<0.05).
Aguilar-Salinas et al, 2011 [161]	Candidate gene, population-based	The 2000 Mexican National Health Survey from 400 cities, Mexico	1,729 (F, 51.10%)	TC, HDL-C, LDL-C, TG, non-HDL-C		R230C (rs9282541)	RC+CC: significantly associated with lower HDL-C levels (*P*<0.001) In males, RC+CC: significantly associated with lower HDL-C levels (*P*<0.001) C allele: significantly associated with increased risk for lower HDL-C levels with the OR of 1.96 (95% CI=1.17-3.29, *P*<0.01)
Cao et al, 2011 [162]	Candidate gene, population-based	Two Chinese populations (Bai Ku Yao and Han), China	1,323 (F, 51.70%), 677 Bai Ku Tao population (F, 52.14%), and 646 Han population (F, 51.24%)	TC, HDL-C, LDL-C, TG, ApoA-I, ApoB		V825I [G/A] (rs2066715)	In Bai Ku Yao population, A allele: significantly associated with higher TC (*P*=0.022) In Han males, A allele: significantly associated with lower HDL-C (*P*=0.029) and lower ApoA-I (*P*=0.049)

Table 3.2. (continued)

Kolovou et al, 2011 [163]	Candidate gene, population-based	The University of Nursing of Technological and Educational Institution, Greek	308 (F, 100%)	TC, HDL-C, LDL-C, TG, ApoA-I		R1587K (rs2230808)	K allele: significantly associated with higher TC (*P*=0.023), higher LDL-C (*P*=0.014), and lower TG (*P*=0.047).
Akao et al, 2014 [164]	Candidate gene, subjects with pre-existing vascular disease or at least one of three major vascular risk factors	The PROSPER (PROspective Study of Pravastatin in the Elderly at Risk) study with pre-existing vascular disease (n=2,404) or at least one of three major vascular risk factors (diabetes n=575, smoking n=1433, or hypertension n=3,360), 3 centers: Cork, Ireland; Glasgow, Scotland; and Leiden, The Netherlands Follow-up: mean 3.2 years Clinical trials: on pravastatin 40 mg/day, 49.81%	5,804 (F, 51.69%)	TC, HDL-C, LDL-C, TG, ApoA-I, ApoB	CHD	R219K (rs2230806)	K allele: significantly associated with increased risk of new CHD (HR=1.22 [95% CI=1.06-1.40], *P*=0.006) In pravastatin group, K allele: significantly associated with increased risk of new CHD (HR=1.41 [95% CI=1.15-1.73], *P*=0.001)
Mokuno et al, 2015 [165]	Candidate gene, population-based	The Daiko Medical Center, as part of the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study), Japan	5,133 (F, 71.60%)	HDL-C		R219K [G1051A] (rs2230806)	K allele: significantly associated with increased HDL-C levels (trend *P*=0.033)

ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B, BMI, body mass index; CAC, coronary artery calcification; CAD, coronary artery diseases; CHD, coronary heart disease; CI, confidence interval; FH, familial hypercholesterolemia; GWAS, genome-wide association study; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; IHD, ischemic heart disease; IMT, intima media thickness; Lp(a), lipoprotein (a); LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; OR, odds ratio; PR, prevalence ratio; RR, risk ratio; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglycerides; TRL, triglyceride-rich lipoprotein; VLDL-C, very low-density lipoprotein cholesterol.

[a] Based on the original studies.

[b] dbSNP build 141.

[c] RefSeq *ABCA1*: NCBI GRCh38, NC_000009.12, NM_005502.3.

Table 3.3. Summary of the genome-wide association studies that previously reported association of *ABCA1* variants (MAF ≥5%) with lipid traits (P <1.00 x 10^{-6}).

Authors, year [Ref]	Populations and Original Studies	Primary Sample, n (F, n/%)	Replication Sample, n (F, n/%)	SNP ID[a]	Chr9 Position[b]	Major/ Minor Alleles	Allele Effect, MAF	Traits	β, unit	SE/ SEM	*P*
Kathiresan et al, 2008 [166]	Europeans from one GWAS (n=2,758) and replication in 3 cohort studies (n=18,554)	2,758 (F, 50.8%)	3 cohort studies, 18,554 (F, range: 50.2-58.6%)	rs3890182	104885374	G/A	A, 0.13	HDL-C	-0.10, mg/dL	0.02 (SEM)	*P* (primary study+3 replication samples) =3.00E-10
Wallace et al, 2008 [167]	Europeans from 3 British cohorts (BRIGHT, the GRAPHIC study, and the TwinsUK registry)	2,000 hypertensive cases (F, 60%) from the BRIGHT study **Sample for meta-analysis:** 2,931 individuals from BROAD (Saxena, et al 2007 [178], the Scandinavia, Diabetes Genetics Initiative: 1,464 cases and 1,467 controls	3,494 subjects from 2 cohorts, 2,033 individuals (F, 49.4%) from 519 families from the GRAPHIC study; and 1,461 healthy female twin individuals (F, 100%) of European descent ascertained from the TwinsUK registry, St Thomas' Hospital, London	rs3890182	104885374	G/A	A, 0.12	HDL-C	0.95, mmol/L	ND	Meta-*P* (BRIGHT+BROAD) =2.09E-06
Willer et al, 2008 [168]	European descent subjects: Finnish (FUSION, DGI), Sardinian (SardiNIA, Italy), Swedish (DGI), French (SUVIMAX), England (ISIS, BWHHS, Caerphilly), American (HAPI)	**Stage 1:** 8,816 from 3 studies: 1,874 from FUSION, 4,184 SardiNIA, and 2,758 DGI (F, range: 44.5-57%	**Stage 2:** 11,569 from 6 cohort studies: FUSION, ISIS, HAPI, SUVIMAX, BWHHS, and Caerphil (F, range: 0-100%)	rs4149268	104884939	C/T	T, 0.355	HDL-C	0.82, mg/dL	ND	*P* (stages 1+2) =1.2E-10
				rs4149274	104877133	G/A	G, 0.69	HDL-C	1.51, mg/dL	ND	*P* (stage 1) =7.40E-08
Aulchenko et al, 2009 [169]	Europeans from 16 studies (n, range: 138-4971)	>17,000 with the range from 17,798 to 22,562 (F, range: 61-63%) depending on traits	No replication	rs3905000	104894789	G/A	G, 0.865	HDL-C	0.113, mmol/L	0.016 (SEM)	Meta-*P*=8.65-13
Kathiresan et al, 2009 [170]	Europeans from 7 GWASs (n=19,840) for primary stage and from 5 cohort studies (n=20,623) for replication	**Stage 1:** 19,840 from 7 GWASs, (F, range: 18-62%)	**Stage 2:** 21,944 from 5 cohort studies (F, range: 0-59%)	rs1883025	10402020	C/T	T, 0.26	HDL-C	-0.08, mg/dL	0.02 (SEM)	*P* (stages 1+2) =1E-09
Sabatti et al, 2009 [171]	Northern Finnish, the Northern Finnish Birth Cohort 1966 (NFBC1966)	4,763 (F, 52%)	No replication	rs3847303	104886371	G/A	A, ND	HDL-C	-0.03, mmol/L	0.012 (SEM)	*P*=3.22E-03
				rs2740491	104896104	G/A	A, ND	HDL-C	-0.03, mmol/L	0.008 (SEM)	*P*=3.12E-04
Teslovich et al, 2010 [87]	Multi-ethnic subjects: European and Non-Europeans	>100,000 Europeans from 46 GWASs (F, range: 12.8-100%)	>50,000 Europeans and Non-Europeans from 9 cohort studies	rs1883025	10402020	C/T	T, 0.25	HDL-C	-0.94, mg/dL	0.09 (SEM)	Meta-*P* (primary studies)=2.00E-33
								TC	-2.24, mg/dL	0.24 (SEM)	Meta-*P* (primary studies)=3.00E-27
Waterworth et al, 2010 [172]	European descent subjects	**Stage 1:** 17,723 from 8 cohort studies (F, range: 23-58%)	**Stage 2:** 37,774 participants from 8 cohort studies (F, range: 0-59%) **Stage 3:** 9,665 Indian Asian samples from the LOLIPOP study, West London, UK (F, range: 0-18%)	rs3890182	104885374	G/A	G, 0.88	HDL-C	0.022, mmol/L	0.004 (SE)	*P* (stage 1) =4.70E-07
Lettre et al, 2011 [173]	African Americans from 12 studies	8,090 from the CARe Project, comprising of 5 studies (F, mean=60% range: 55-63%)	8,941 (7 studies; F, mean=59%, range: 44-76%)	rs3905000	104894789	G/A	A, 0.161	HDL-C	-0.043, mg/dL	0.016 (SE)	Meta-*P* (CARe) =8.65E-13

Table 3.3. (continued)

				rs13284054	104906792	T/C	C, 0.85	HDL-C	0.09, mg/dL	0.027 (SE)	Meta-*P* (CARe) =1.01E-03
Kettunen et al, 2012 [174]	Finnish from 5 cohorts	8,830 (F, mean =55.5%, range: 51-60%)	No replication	rs2575876	104903458	G/A	A, 0.19	TC/Est-C Ratio	-0.14, mmol/L	0.02 (SEM)	Meta-*P*=1.63E-11
Kristiansson et al, 2012 [175]	Finnish	11,616 non-diabetic subjects from 4 Finnish cohort studies: H2000, HBCS, YFS, NFBC1996 (F, range: 51-57%), comprising of 2,637 cases (MetS), n=2637, and 7,927 controls	1,637 from 2 studies: 906 males from the METSIM study and 731 from the FINRISK2007 study	rs1883025	10402020	C/T	T, 0.19	HDL-C	-0.10, mmol/L	ND	Meta-*P* (primary studies)=5.87E-10
Global Lipids Genetics Consortium, Willer et al, 2013 [176]	Multi-ethnic subjects: European and non-European descent	188,577 from 23 GWASs and 37 Metabochip studies (F, range: 0-69.6%)	No replication	rs1883025	10402020	C/T	T, 0.25	HDL-C	-0.07, mg/dL	ND	Meta-*P*=2.00E-65
								TC	-0.067, mg/dL	ND	Meta-*P*=6.00E-53
Weissglas-Volkov et al, 2013 [177]	Mexicans: cases (hypertriglyceridemia and low high-density lipoprotein cholesterols) and controls, from the INCMNSZ, Mexico, and University of California, Los Angeles	**Stage 1:** 2,240 Mexicans (1,122 cased with hypertriglycerides [F, 50%], and 1,118 controls [F, 50%])	**Stage 2**: 2,121 Mexicans (1,067 cased with hypertriglycerides [F, 50%], and 1,054 controls [F, 63%])	rs4149310	104826853	T/A	A, 0.36	HDL-C	0.12, mmol/L	0.02 (SE)	*P* (stages 1+2) =5.54E-08
				rs9282541 (R230C)	104858554	G/A	A, 0.11	HDL-C	-0.37, mmol/L	0.04 (SE)	*P* (stages 1+2) =6.40E-26

ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B, BMI, body mass index; CAC, coronary artery calcification; CAD, coronary artery diseases; CHD, coronary heart disease; GWAS, genome-wide association study; HDL-C, high-density lipoprotein cholesterol; IHD, ischemic heart disease; IMT, intima media thickness; Lp(a), lipoprotein (a); LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; MI, myocardial infarction; ND, not determined; SE, standard error; SEM, standard error of the mean; SNP, single nucleotide polymorphism; TC, total cholesterol; TC/Est-C, total cholesterol to esterified cholesterol; TG, triglycerides; VLDL-C, very low-density lipoprotein cholesterol.
[a] dbSNP build 141.
[b] RefSeq *ABCA1*: NCBI GRCh38, NC_000009.12, NM_005502.3.

Footnotes

Conversion units*:
For TC, HDL-C, and LDL-C: To get from SI units (mmol/L) to mg/dL multiply by 38.67. To get from mg/dL to SI (in mmol/L) multiply by 0.02586.
For TG: To get from SI units (mmol/L) to mg/dL multiply by 88.57. To get from mg/dL to SI units, multiply by 0.01129.
*Resource: Ref [179]

Abbreviations for studies:
BRIGHT, the Medical Research Council BRItish Genetics of HyperTension study
BROAD, the Diabetes Genetics Initiative from the Broad Institute of MIT and Harvard, Lund University, and Novartis Institutes; www.broad.mit.edu/diabetes/.
BWHHS, the British Women's Heart and Health Study
CARe, the Candidate gene Association Resource project
DGI, the Diabetes Genetics Initiative study
FHS, the Framingham Heart Study
FINRISK2007, the National FINRISK Study (PMID: 19959603)
FUSION, the Finland-United States Investigation of NIDDM Genetics study
GRAPHIC, the Genetic Regulation of Arterial Pressure of Humans in the Community study
H2000, the national Health 2000 survey
HBCS, the Helsinki Birth Cohort Study
HAPI, the Amish HAPI Heart study
INCMNSZ, the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán study
ISIS, the International Study of Infarct Survival study
LOLIPOP, the London Life Sciences Prospective Population Cohort study
METSIM, the Metabolic Syndrome in Men study
SardiNIA, the SardiNIA Study of Aging
SUVIMAX, the SUpplementation en VItamines et Mineraux AntioXydants study
YFS, the Cardiovascular Risk in Young Finns Study

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4.0. OBJECTIVES AND SPECIFIC AIMS

4.1. HYPOTHESIS

It is recognized that genetics plays a significant role in affecting inter-individual variation of lipoprotein-lipid levels in humans. Scavenger receptor class B type 1 (SCARB1) and ATP-binding cassette transporter A1 (ABCA1) are trans-membrane receptors that play critical roles in the regulation of lipid metabolism. In this dissertation, we investigated the association of *SCARB1* and *ABCA1* genetic variation on major lipid traits in the general population to test the common disease, common variant (CDCV) and common disease, rare variant (CDRV) hypotheses.

Specifically, we hypothesized that genetic variation of the *SCARB1* and *ABCA1* genes—both common (allele frequency ≥5%) and low-frequency (LF)/rare variants (allele frequency <5%)—contribute to lipid traits. Our study was conducted under genetic additive model in two ethnic groups: Non-Hispanic Whites (NHWs) from Colorado, USA and African Blacks (ABs) from Benin City, Nigeria.

4.2. SPECIFIC AIMS

Aim 1 **To identify genetic variation in the *SCARB1* and *ABCA1* genes by resequencing individuals with extreme high-density lipoprotein cholesterol (HDL-C) levels in order to capture both common and LF/rare variants in these two candidate genes.**

We resequenced the exons and exon-intron boundaries of the *SCARB1* and *ABCA1* genes in individuals falling in the upper (47 NHWs and 48 ABs) and lower (48 NHWs and 47 ABs) 10th percentile of the HDL-C distribution to identify both common (allele frequency ≥5%) and LF/rare (allele frequency <5%) variants.

Aim 2 **To genotype selected common tag single nucleotide polymorphisms (tagSNPs) variants and LF/rare variants in the *SCARB1* and *ABCA1* genes in the entire sample.**

Sequence variants identified in ***Aim 1*** were selected for follow-up genotyping in the entire sample of 623 NHWs and 788 ABs using Sequenom or TaqMan genotyping platforms

Sequence common variants (allele frequency ≥5%) were chosen based on SNPs correlation structure (linkage disequilibrium [LD] structure) of each ethnic group.

Additional common tagSNPs from HapMap database (Release #27) were selected for genotyping to cover the entire genes. For the *SCARB1* gene, LF/rare variants (allele frequency <5%) were selected and genotyped in the entire NHW (see details in **Chapter 6.0**) and AB samples (see details in **Chapter 7.0**). For the *ABCA1* gene, LF/rare variants were chosen based on potential functions (i.e., exons, and splice sites) and number of carriers in the NHW population (see details in **Chapter 8.0**).

Aim 3 **To evaluate the association of common tagSNPs and LF/rare variants of the *SCARB1* and *ABCA1* genes with major lipid traits.**

Genotyped variants from ***Aim 2*** that passed quality controls (QC) were analyzed using gene-based, single-site, haplotype, and rare variant analyses to test the CDCV and CDRV hypotheses.

Aim 4 **To predict the potential regulatory and transcriptional functions of identified variants in the *SCARB1* and *ABCA1* genes.**

We determined the potential regulatory and transcriptional functions of *SCARB1* and *ABCA1* variants identified in ***Aims 1 and 2*** using the RegulomeDB database (Stanford University, CA, http://regulomedb.org/) [1].

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5.0. METHODOLOGY OVERVIEW

Detailed sample descriptions and methods used for scavenger receptor class B type 1 (*SCARB1*) and ATP-binding cassette transporter A1 (*ABCA1*) in Non-Hispanic Whites (NHWs) and African Blacks (ABs) is described in **Chapter 6.0** (*SCARB1* in NHWs), **Chapter 7.0** (*SCARB1* in ABs), and **Chapter 8.0** (*ABCA1* in NHWs).

5.1. STUDY SAMPLES

The study was carried out in two population-based epidemiological samples comprising 623 NHWs from Colorado, USA and 788 ABs from Benin City, Nigeria. The NHW sample was drawn from the population-based San Luis Valley Diabetes Study (SLVDS) in southern Colorado as described previously [1]. The AB sample was part of the civil recruitment from three ministries of Edo in Benin City, Nigeria for an epidemiological study of CHD risk factors as described in details elsewhere [2].

In brief, a total of 623 NHWs, aged 20-74 years, were comprised of 295 males and 328 females. All NHW samples were non-diabetes with normoglycemic response to oral glucose tolerance test [3]. A total of 788 ABs, aged 19-70 years, were comprised of 495 males and 293 females and all were healthy. Every study sample was obtained with informed consent. The study protocols were approved by the University of Pittsburgh and University of Colorado Institutional Review Boards. The basic characteristics of both population samples are given in **Table 5.1**.

Table 5.1. Basic characteristics of the entire samples (623 Non-Hispanic Whites and 788 African Blacks).

Variables	Non-Hispanic Whites[a]	African Blacks[a]
Total N (Female, n)	623 (328)	788 (293)
Age, years	52.83 ± 11.41	40.95 ± 8.39
BMI, kg/m^2	25.51 ± 4.06	22.87 ± 4.04
Total Cholesterol, mg/dL	216.99 ± 43.55	172.01 ± 38.47
LDL-Cholesterol, mg/dL	136.99 ± 40.80	109.25 ± 34.40
HDL-Cholesterol, mg/dL	50.76 ± 14.35	47.88 ± 12.87
Triglycerides, mg/dL	142.72 ± 93.49	72.96 ± 39.32
Apolipoprotein A-I, mg/dL	149.62 ± 33.33[b]	137.03 ± 28.46
Apolipoprotein B, mg/dL	87.72 ± 24.27[b]	66.98 ± 22.19

BMI, body mass index; HDL-C or HDL-Cholesterol, high-density lipoprotein cholesterol; LDL-Cholesterol, low-density lipoprotein cholesterol.
Values are presented as unadjusted means ± standard deviation (SD), otherwise mentioned.
[a] All values included individuals with missing values or outliers (values beyond mean ± above 3.5 SD).
[b] Measured in a subset of 425 Non-Hispanic White individuals.

In the discovery resequencing stage, we selected individuals who had high-density lipoprotein cholesterol (HDL-C) levels in the upper or lower 10th percentile distribution, comprising of 95 NHWs and 95 ABs. Among the selected 95 NHWs, 47

were in the high HDL-C (high extreme) group and 48 in the low HDL-C (low extreme) group. Similarly, among the 95 selected ABs, 48 were in the high HDL-C (high extreme) group and 47 in the low HDL-C (low extreme) group. The basic characteristics including lipid profile of the extreme HDL-C groups in both population groups are given in **Table 5.2**.

Table 5.2. Basic characteristics of the resequencing samples with extreme HDL-C[a] levels (95 Non-Hispanic Whites and 95 African Blacks).

	Non-Hispanic Whites (N = 95)			African Blacks (N = 95)		
Variables	**High HDL-C Group[a]** (range[b]:58.0-106.0 mg/dL)	**Low HDL-C Group[a]** (range[b]:20.0-40.0 mg/dL)	***P*[c]**	**High HDL-C Group[a]** (range[b]:68.3-99.0 mg/dL)	**Low HDL-C Group[a]** (range[b]:10.3-35.0 mg/dL)	***P*[c]**
Total N (Female, n)	47 (23)	48 (24)	1.00	48 (24)	47 (24)	1.00
Age, years	55.45 ± 9.80	53.03 ± 10.54	0.25	41.29 ± 8.72	40.87 ± 7.12	0.80
BMI, kg/m^2	23.17 ± 3.17	27.35 ± 3.90	1.20E-07	22.06 ± 4.70	23.91 ± 5.51	0.08
TC, mg/dL	227.34 ± 51.76	208.81 ± 44.65	0.07	201.00 ± 39.68	141.68 ± 31.03	2.40E-12
LDL-C, mg/dL	126.84 ± 46.95	125.54 ± 54.97	0.90	112.55 ± 39.75	95.04 ± 28.28	0.02
HDL-C, mg/dL	77.68 ± 13.32	31.81 ± 4.37	2.20E-16	76.05 ± 7.53	25.51 ± 5.66	2.20E-16
TG, mg/dL	114.09 ± 60.88	240.21 ± 153.22	1.70E-06	61.98 ± 19.85	95.79 ± 73.21	0.004
ApoA-I, mg/dL	174.08 ± 34.78	130.20 ± 27.08	1.40E-06	166.04 ± 28.19	103.84 ± 27.23	2.20E-16
ApoB, mg/dL	87.88 ± 25.49	89.61 ± 25.18	0.80	66.00 ± 20.22	69.64 ± 21.46	0.40

ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-Cholesterol, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.
Values are presented as unadjusted means ± standard deviation (SD) unless otherwise mentioned.
[a] For each ethnic population, the distribution of HDL-C was adjusted for sex and age: HDL-C levels ≥90th %tile defined as the "High HDL-C group", and HDL-C levels ≤10th %tile defined as the "Low HDL-C group".
[b] Unadjusted HDL-C values.
[c] Unadjusted *P*-values (comparing between the high HDL-C and low HDL-C groups in each ethnic population) were calculated with t-test or χ^2 test depending on types of variables.

5.2. LIPID MEASUREMENTS

At least 8-hour fasting blood sample was collected and were centrifuged for plasma (for NHWs) or serum (for ABs). The plasma or serum samples were kept on ice and immediately stored at -80° until ready for measurement. Total cholesterol (TC) and triglycerides (TG) were measured by standardized enzymatic assays. HDL-C was measured by dextran sulfate magnesium precipitation. Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula [4] when TG levels were less than 400 mg/dl. For apolipoprotein (apo) A-I (apoA-I) and apolipoprotein B (apoB) levels were determined using the Boehringer Mannheim Turbidimetric procedure. Lipid measurement was done at the certified facilities at the Heinz Nutrition Laboratory of the University of Pittsburgh and the Clinical research laboratory of the University of Colorado Health Sciences Center [2, 5, 6].

5.3. DNA SAMPLES AND PREPARATIONS

Genomic DNA for this study was extracted from leukocytes or peripheral blood clots using standard DNA extraction protocols. Whole Genomic Amplification (WGA) was

applied for those with limited DNA resources. Selected samples for sequencing were quantified using the Quant-iT™ PicoGreen® DNA Assay Kit (Life Technologies, Waltham, MA, USA).

5.4. DNA SEQUENCING

Sequencing samples were amplified into multiple fragments with specific designed primers via polymerase chain reaction (PCR). The reference sequence (RefSeq), plus ~1 kb of 5' flanking and ~1 kb of 3' flanking regions was used for primer designs. All primers were designed in the Primer3 software program (Whitehead Institute for Biomedical Research, Steve Rozen, Andreas Untergasser, Maido Remm, Triinu Koressaar and Helen Skaletsky, http://primer3.ut.ee/) to mainly target exons and exon-intron boundaries of the gene. We obtained the RefSeq *SCARB1* (hg19: *SCARB1* [isoform 1], NM_005505; chr12:125,262,175-125,348,519) from CHIP Bioinformatics (University of Florida, FL, http://snpper.chip.org/) and the RefSeq *ABCA1* (GRCh38: NC_000009.12, NM_005502.3; chr9:104,781,002-104,928,246) from
the National Center for Biotechnology Information (NCBI, Bethesda, MD, http://www.ncbi.nlm.nih.gov/). Information of RefSeq and PCR fragments for *SCARB1* and *ABCA1* is summarized in **Table 5.3**. The distributions of exons, exon sizes, coding regions, including the assigned variant positions and names for *SCARB1* and *ABCA1* are shown in **Table 5.4** and **Table 5.5**, respectively. Primer sequences for *SCARB1* are

shown in **Appendix A Table A3**, or **Appendix B Table B2**, and for *ABCA1* are shown in **Appendix C Table C3**.

Table 5.3. Summary of genomic structures of the *SCARB1* and *ABCA1* genes and PCR fragments used for sequencing.

	***SCARB1* [*Homo sapiens*]**	***ABCA1* [*Homo sapiens*]**
Official full name	Scavenger receptor class B, member 1	ATP-binding cassette, sub-family A (ABC1), member 1
Entrez gene ID	949	19
Genomics		
Location	Chromosome 12 (12q24.31)	Chromosome 9 (9q31.1)
RefSeq assembly	hg19: *SCARB1* (isoform 1)	GRCh38: NC_000009.12
RefSeq genomic sequence	chr12:125,262,175-125,348,519	chr9:104,781,002-104,928,246
RefSeq transcript	NM_005505	NM_005502.3
RefSeq protein (aa)	NP_005496 (509 aa)	NP_005493.2 (2,261 aa)
RefSeq genomic size[a]	86.34 kb	147.24 kb
Exon count, n	13	50
PCR		
PCR fragments, n	14	96
Size of PCR fragments		
• Average (range)	910 bp (752-1,042)	1,008 bp (483-1,279)
• Sum of PCR fragments[b]	12.58 kb	80.14 kb

[a] Not included ~ 1 kb of 5' flanking and ~ 1 kb of 3' flanking regions.
[b] Total net PCR fragments after excluding overlapping regions.

Table 5.4. The distribution of exons, exon sizes, and coding regions for *SCARB1*[a].

	Exons			Coding		
SCARB1[a]	**SNP Names**[b] **(5' to 3' direction)**	**Chr12 Positions**[a] **(5' to 3' direction)**	**Size (bp)**	**SNP Names**[b] **(5' to 3' direction)**	**Chr12 Positions**[a] **(5' to 3' direction)**	**Size (bp)**
Starting	1	125349519				
5' flanking	1-1000	125349519-125348520	1,000			
5' UTR-Exon 1	1001-1253	125348519-125348367	253			
Exon 1	1001-1379	125348519-125348141	379	1254-1379	125348266-125348141	126
Exon 2	47267-47424	125302253-125302096	158	47267-47424	125302253-125302096	158
Exon 3	49860-50001	125299660-125299519	142	49860-50001	125299660-125299519	142
Exon 4	50569-50772	125298951-125298748	204	50569-50772	125298951-125298748	204
Exon 5	53009-53104	125296511-125296416	96	53009-53104	125296511-125296416	96
Exon 6	54685-54800	125294835-125294720	116	54685-54800	125294835-125294720	116
Exon 7	57047-57213	125292473-125292307	167	57047-57213	125292473-125292307	167
Exon 8	64732-64850	125284788-125284670	119	64732-64850	125284788-125284670	119
Exon 9	69706-69779	125279814-125279741	74	69706-69779	125279814-125279741	74
Exon 10	77517-77568	125272003-125271952	52	77517-77568	125272003-125271952	52
Exon 11	78471-78617	125271049-125270903	147	78471-78617	125271049-125270903	147
Exon 12	82163=82291	125267357-125267229	129	82163-82291	125267357-125267229	129
Exon 13	86388-87346	125263132-125262174	959			
3' UTR	86388-87346	125263132-125262174	959			
3' flanking	87347-88346	125262173-125261174	1,000			
Ending	88346	125261174				

[a] RefSeq *SCARB1*: hg19, NM_005505 (isoform 1).
[b] SNP Names are corresponding to positions that were assigned in our study based on RefSeq *SCARB1*. For example, in 5' flanking, p1 is corresponding to chr12:12549519, and p1000 is corresponding to chr12:125348520.

Table 5.5. The distribution of exons, exon sizes, and coding regions for *ABCA1*[a].

	Exons			Coding		
ABCA1[a]	SNP Names[b] (5' to 3' direction)	Chr12 Positions[a] (5' to 3' direction)	Size (bp)	SNP Names[b] (5' to 3' direction)	Chr12 Positions[a] (5' to 3' direction)	Size (bp)
Starting	1	104929155				
5' flanking	1-909	104929155-104928247	909			
5' UTR-Exon 1	910-1221	104928246-104927935	312			
5 UTR-Exon 2	25385-25476	104903771-104903680	92			
Exon 1	910-1221	104928246-104927935	312			
Exon 2	25385-25476	104903771-104903614	158	25477-25542	104903679-104903614	66
Exon 3	39961-40054	104889195-104889102	94	39961-40054	104889195-104889102	94
Exon 4	44588-44729	104884568-104884427	142	44588-44729	104884568-104884427	142
Exon 5	45999-46117	104883157-104883039	119	45999-46117	104883157-104883039	119
Exon 6	67356-67477	104861800-104861679	122	67356-67477	104861800-104861679	122
Exon 7	70458-70634	104858698-104858522	177	70458-70634	104858698-104858522	177
Exon 8	83587-83679	104845569-104845477	93	83587-83679	104845569-104845477	93
Exon 9	88637-88877	104840519-104840279	241	88637-88877	104840519-104840279	241
Exon 10	91589-91728	104837567-104837428	140	91589-91728	104837567-104837428	140
Exon 11	92060-92176	104837096-104836980	117	92060-92176	104837096-104836980	117
Exon 12	96385-96582	104832771-104832574	198	96385-96582	104832771-104832574	198
Exon 13	97329-97534	104831827-104831622	206	97329-97534	104831827-104831622	206
Exon 14	98055-98231	104831101-104830925	177	98055-98231	104831101-104830925	177
Exon 15	100018-100240	104829138-104828916	223	100018-100240	104829138-104828916	223
Exon 16	101987-102208	104827169-104826948	222	101987-102208	104827169-104826948	222
Exon 17	103269-103473	104825887-104825683	205	103269-103473	104825887-104825683	205
Exon 18	104578-104691	104824578-104824465	114	104578-104691	104824578-104824465	114
Exon 19	106489-106660	104822667-104822496	172	106489-106660	104822667-104822496	172
Exon 20	107650-107781	104821506-104821375	132	107650-107781	104821506-104821375	132
Exon 21	109087-109229	104820069-104819927	143	109087-109229	104820069-104819927	143
Exon 22	109433-109570	104819723-104819586	138	109433-109570	104819723-104819586	138
Exon 23	110273-110493	104818883-104818663	221	110273-110493	104818883-104818663	221
Exon 24	111752-111824	104817404-104817332	73	111752-111824	104817404-104817332	73
Exon 25	112811-113013	104816345-104816143	203	112811-113013	104816345-104816143	203
Exon 26	114681-114729	104814475-104814427	49	114681-114729	104814475-104814427	49
Exon 27	114925-115038	104814231-104814118	114	114925-115038	104814231-104814118	114
Exon 28	116434-116582	104812722-104812574	149	116434-116582	104812722-104812574	149
Exon 29	118232-118356	104810924-104810800	125	118232-118356	104810924-104810800	125
Exon 30	119592-119690	104809564-104809466	99	119592-119690	104809564-104809466	99
Exon 31	122726-122915	104806430-104806241	190	122726-122915	104806430-104806241	190
Exon 32	124436-124530	104804720-104804626	95	124436-124530	104804720-104804626	95
Exon 33	125840-125872	104803316-104803284	33	125840-125872	104803316-104803284	33
Exon 34	126997-127102	104802159-104802054	106	126997-127102	104802159-104802054	106
Exon 35	128572-128646	104800584-104800510	75	128572-128646	104800584-104800510	75
Exon 36	129168-129337	104799988-104799819	170	129168-129337	104799988-104799819	170
Exon 37	130558-130735	104798598-104798421	178	130558-130735	104798598-104798421	178

Table 5.5. (continued)

Exon 38	132732-132847	104796424-104796309	116	132732-132847	104796424-104796309	116
Exon 39	132959-133103	104796197-104796053	145	132959-133103	104796197-104796053	145
Exon 40	134646-134769	104794510-104794387	124	134646-134769	104794510-104794387	124
Exon 41	135856-135985	104793300-104793171	130	135856-135985	104793300-104793171	130
Exon 42	136250-136370	104792906-104792786	121	136250-136370	104792906-104792786	121
Exon 43	137158-137220	104791998-104791936	63	137158-137220	104791998-104791936	63
Exon 44	138128-138234	104791028-104790922	107	138128-138234	104791028-104790922	107
Exon 45	140589-140730	104788567-104788426	142	140589-140730	104788567-104788426	142
Exon 46	141102-141236	104788054-104787920	135	141102-141236	104788054-104787920	135
Exon 47	142180-142283	104786976-104786873	104	142180-142283	104786976-104786873	104
Exon 48	142766-142858	104786390-104786298	93	142766-142858	104786390-104786298	93
Exon 49	143517-143760	104785639-104785396	244	143517-143760	104785639-104785396	244
Exon 50	144701-148154	104784455-104781002	3,454	144701-144841	104784455-104784315	141
3' UTR-Exon 50	144842-148154	104784314-104781002	3,313			
3' flanking	148155-149152	104781001-104780004	998			
Ending	149152	104780004	149,152			

[a] RefSeq *ABCA1*: NCBI GRCh38, NC_000009.12, NM_005502.3.
[b] SNP Names are corresponding to positions that were assigned in our study based on RefSeq *ABCA1*. For example, in 5' flanking, p1 is corresponding to chr9:104929155, and p909 is corresponding to chr9:104928247.

PCR was performed with the GeneAMP® PCR System 9700 thermal cycler (Applied Biosystems, Waltham, MA, USA). After optimization in small-scale PCR reactions, each genomic DNA sample was subjected to PCR amplification using each primer set. Each PCR reaction was performed in a final volume of 25 µL containing 3 µL of genomic DNA (5 ng/µL), 0.4 µL of each primer (20 mM), 3.8 µL of deoxynucleoside triphosphates [dNTPs] (1.25 mM), and 0.15 µl of standard *Taq* polymerase enzyme (5 U/µL). Only the PCR amplification of exon 1 fragment of *SCARB1* required a specific enzyme, TaKaRa LA *Taq* DNA Polymerase (Clontech Laboratories, Inc., Mountain View, CA, USA) for GC-rich region instead of standard *Taq* polymerase enzyme. For PCR thermocycle setting, an initial denaturation of PCR started at 95^{o}C for 5 minutes, and then continued for 39 cycles. Each PCR cycle was consisted of denaturation at

95°C for 45 seconds, annealing at 60°C for 45 seconds and extension at 72°C for 1 minute. The final extension of PCR ended at 72°C for 10 minutes. The PCR reaction mix, including thermocycling conditions are shown in **Table 5.6** and **Table 5.7**, respectively. Following PCR amplification, all amplified products were run on 2% agarose gel electrophoresis (E-Gel® 96 Agarose Gels, 2% with SYBR® Safe [Invitrogen™, Waltham, MA, USA], or regular 2% agarose gel with ethidium bromide) to evaluate the PCR efficiency.

Sequencing reactions were performed following the manufacture's protocols and analyzed on the Applied Biosystems 3730xl DNA Analyzer (Beckman Coulter Genomics, Danvers, MA, USA). Sequencing traces of the 180 individuals with extreme HDL-C phenotypes were reviewed and analyzed using Variant Reporter (version 1.0, Applied Applied Biosystems™, Waltham, MA, USA) and Sequencher (version 4.8, Gene Codes Corporation, Ann Arbor, MI, USA) in our laboratory at the University of Pittsburgh.

Table 5.6. PCR reaction components.

PCR Reaction Components	25 µl Final Volume PCR Reaction
Genomic DNA (5 ng/µL)	3.0 µL
dH_2O	11.25-13.75 µL
10X PCR Buffer	2.5 µL
$MgCl_2$ (25mM)	1.0-3.5 µL[a]
Standard *Taq* polymerase (5 U/µL)	0.15 µL
dNTPs (1.25 mM)	3.8 µL
Forward primer (20 mM)	0.4 µL
Reverse primer (20 mM)	0.4 µL

dNTPs, deoxynucleoside triphosphates.
[a] Adjusted volume for optimal PCR reactions.

Table 5.7. PCR thermal cycling conditions.

Steps	Temperature	Time
Initial denaturation	95°C	5 min
39 cycles		
Denaturation	95°C	45 sec
Annealing	60°C	45 sec
Extension	72°C	1 min
Final extension	72°C	10 min
Hold	4°C	

5.5. VARIANT SELECTION FOR GENOTYPING

We selected sequence variants for genotyping mainly based on linkage disequilibrium (LD) pattern using Tagger analysis (a pairwise tagging method) from Haploview software (Broad Institute of MIT and Harvard, Cambridge, MA, http://www.broadinstitute.org/) [7]. Without regard to common tag single nucleotide polymorphisms (tagSNPs) that already selected, additional common tagSNPs (MAF ≥5%) from the HapMap database (Release #27, Genome build 36, dbSNP build 126) were also chosen for genotyping using Tagger analysis with an r^2 threshold of 0.90 for *SCARB1* and 0.80 for *ABCA1* to cover the entire genes, since our sequencing only focused on the coding and exon-intron boundaries. Some relevant lipid-associated variants from literature as well as sequence variants with either low sequencing success rate (%call rate, less than 40%) or deviated from Hardy-Weinberg equilibrium (HWE)

were also considered for genotyping. Specific criteria of variant selection including the lists of selected variants are presented in following chapters: **Chapter 6.0** (*SCARB1* in NHWs), **Chapter 7.0** (*SCARB1* in ABs), and **Chapter 8.0** (*ABCA1* in NHWs).

5.6. GENOTYPING AND QUALITY CONTROLS

Selected variants were genotyped in the entire study samples. Specifically, *SCARB1* variants were genotyped in 623 NWHs and 788 ABs, and *ABCA1* variants were genotyped in 623 NHWs. PCR amplification for genotyping was performed using the GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Waltham, MA, USA) or Bio-Rad PTC 200 (also called MJ Research PTC-200 thermal cyclers; GMI, Inc., Ramsey, MN).

We used either Sequenom iPLEX assays (Sequenom, Inc., San Diego, CA, USA) or TaqMan® SNP Genotyping Assay (Applied Biosystems, Waltham, MA, USA) for genotyping. Assay reactions were performed following manufacturers' instructions. Sequenom iPLEX assays were operated by the Genomics and Proteomics Core Laboratories, University of Pittsburgh (http://www.genetics.pitt.edu/). While, TaqMan assays were performed in our laboratory and used the ABI Prism® 7900HT Sequence Detection Systems (Applied Biosystems, Waltham, MA, USA) for reading plates. The TaqMan reaction mix and thermal cycling conditions are provided in **Table 5.8** and **Table 5.9**, respectively.

Table 5.8. TaqMan reaction components for 384-well reaction plate with predelivery and dry-down genomic DNA[a].

TaqMan Reaction Components	5 µl Final Volume PCR Reaction
dH_2O[b]	2.44 µL
Taqman Genotyping Master Mix	2.50 µL
Taqman Genotyping Assay Primer Mix	0.06 µL

[a] For DNA predelivery and dry-down method, each well contained 2.0-5.0 µL volume of genomic DNA (1-5 ng/µL).
[b] Molecular grade bottle water.

Table 5.9. TaqMan thermal cycling conditions.

Steps	Temperature	Time
AmpliTaq Gold, UP Enzyme Activation	95°C	10 min
50 cycles		
Denaturation	95°C	15 sec
Annealing/Extension	60°C	1 min
Hold	4°C	

For each variant, genotyping results required the genotyping quality controls (QC) checking as follows: a success rate at least 90%, not deviated from HWE (using Bonferroni correction), and a discrepancy rate among replication samples less than 1%. Subsequently, bi-allelic QC-passed genotyped variants were advanced to downstream analyses

5.7. STATISTICAL ANALYSES

An assessment of allele frequencies and HWE tests as well as LD structures was conducted with the Haploview program (Broad Institute of MIT and Harvard, Cambridge, MA, http://www.broadinstitute.org/) [7].

For each population sample, we evaluated the association between bi-allelic QC-passed genotyped variants in each gene (i.e., *SCARB1* in NHWs and ABs, and *ABCA1* in NHWs) and major lipid and apolipoprotein (apo) traits (i.e., HDL-C, LDL-C, TC, TG, apoA-I, and apoB) using additive linear regression model. See an overview of study workflow for each gene in **Figure 5.1**.

Missing phenotype or genotype data more than 2% were excluded from the study. Lipid variables were checked for normality and some required Box-Cox transformation to achieve normality (see **Table 5.10**). Phenotypic values beyond means ± 3.5 standard deviation (SD) of distribution were excluded as outliers from further analyses. The most parsimonious set of covariates for each lipid variable were identified by stepwise regression, and significant covariates were used for adjustment. A set of significant covariates was determined separately for each ethnic group and added into the model as given in **Table 5.10**.

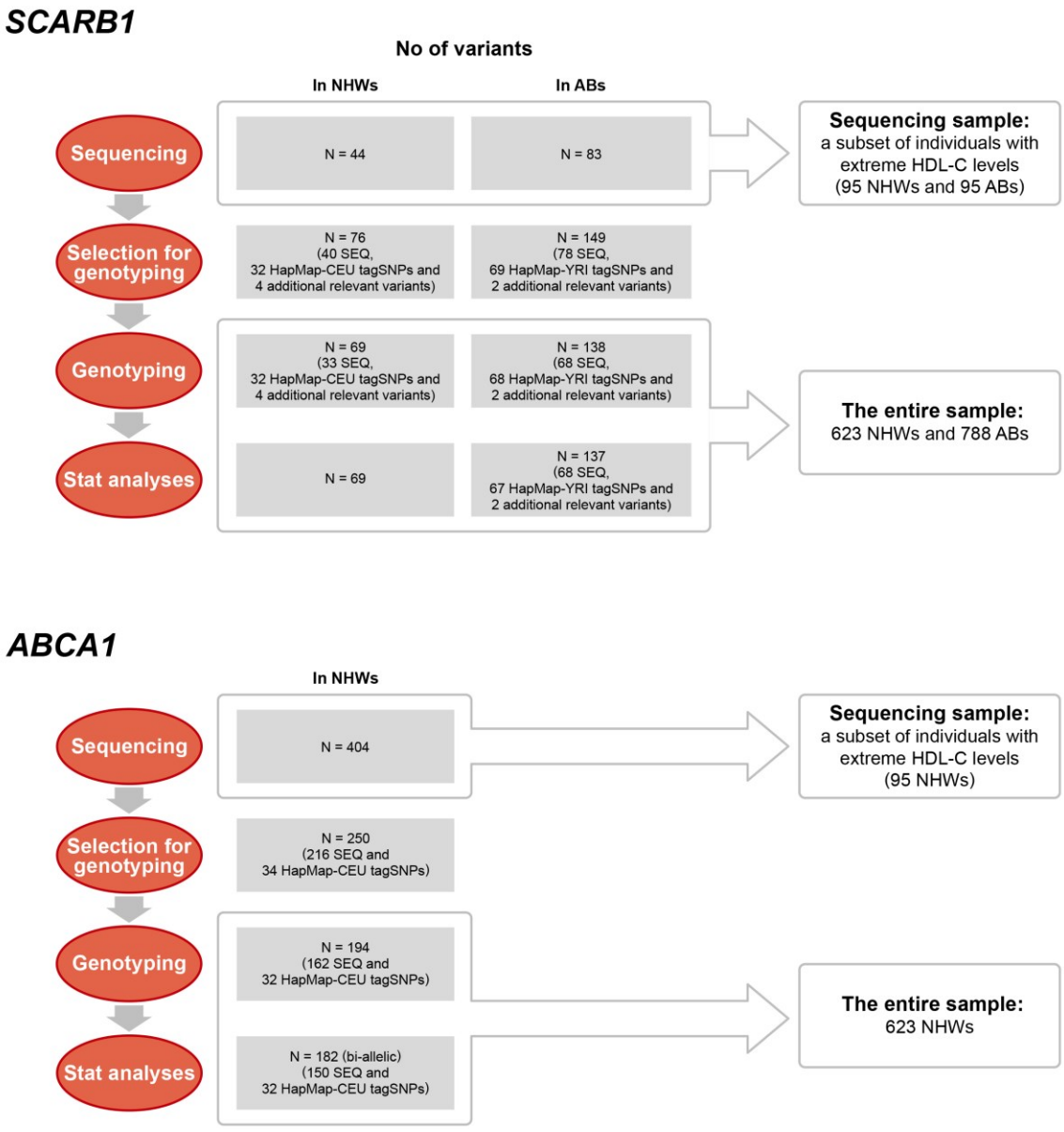

Figure 5.1. Overview of study workflows.

Summary of study workflows for *SCARB1* (**top**) in Non-Hispanic White (NHW) and African Black (AB) sample, and for *ABCA1* (**bottom**) in NHW sample. CEU, Utah residents with northern and western European ancestry; SEQ, sequencing; SNP, single nucleotide polymorphism; YRI, Yoruba people of Ibadan from Nigeria.

Table 5.10. Covariates used for lipid traits in the statistical analyses of two populations.

Covariates	Lipid Variables					
Non-Hispanic Whites	**HDL-C[a]**	**LDL-C**	**TC**	**TG[a]**	**ApoA-I**	**ApoB[a]**
Gender (M/F)	X		X	X	X	
Age, years	X	X	X	X	X	X
BMI, kg/m^2	X	X	X	X	X	
Smoking (never/current/past)	X		X	X		X
African Blacks	**HDL-C[a]**	**LDL-C[a]**	**TC[a]**	**TG[a]**	**ApoA-I[a]**	**ApoB[a]**
Gender (M/F)	X	X	X	X	X	
Age, years	X		X	X	X	
BMI, kg/m^2		X	X			X
Waist, cm	X			X		
Current smoking (yes/no)	X	X	X			
Jobmin, min	X	X	X	X		
Staff (senior/junior)		X	X			X

ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.
X, presenting the significant covariates were used for adjustment in statistical analyses.
BMI, weight (kg)/ [height (m)]^2.
Waist, the waist measurement (cm) at the narrowest point, or at the umbilicus if there was no narrowest point.
Jobmin, time (minutes) of daily walking or biking to work.
Staff, occupational status: senior (professional/administrative) or junior (non-professional).
[a] Lipid values that were required Box-Cox transformation.

5.7.1. Gene-based association analysis

We performed gene-based association tests to determine the combined effect of common and LF/rare variants within the gene on each lipid trait using the versatile gene-based association study (VEGAS) program [8]. A *P*-value threshold of 0.05 was considered a gene-based significance.

5.7.2. Single-site association analysis

We tested single-site associations of each variant with lipid traits. For each trait, we applied the Benjamini-Hochberg procedure [9] of controlling false discovery rate (FDR or *q*-value) for multiple testing adjustment. We opted to use less stringent FDR threshold of 0.20 for FDR significance, as an initial discovery. For common variants (minor allele frequency [MAF] ≥5%), the single-site significance was considered when *P*-value less than 0.05 together with FDR less than 0.20. Because our study was insufficiently powered for detecting single-site association of LF/rare variants (MAF <5%), we did not apply multiple testing correction for interpreting an individual effect of these LF/rare variants. As stated later, we performed rare variant association analyses to test the accumulative effect of LF/rare variants. For analyzing LF/rare variants between the two HDL extreme groups, we used nominal *P*-value less than 0.05, in order to look for trend of individual association results, hoping that they may provide useful information as this approach has been applied successfully in prior studies [10-14].

5.7.3. Haplotype association analysis

It has been suggested that association of haplotypes (a haplotype is a set of variants) are more informative and powerful than association of individual variant [15-19]. So, we performed haplotype analyses using the generalized linear model (GLM) with a fixed-sliding windows approach to estimate the genetic contribution of haplotypes to lipid traits. Because too many variants of each window can cause inefficient and impractical model, four variants per window with one overlapping variant were applied to maximize

the model. A global *P*-value significance was set at less than 0.05, representing an overall association of haplotypes with frequency higher than 1%.

5.7.4. Rare variant association analysis

To date, there have been several statistical methods developed for testing cumulative effects of multiple rare variants and complex traits [18, 20-23], which are generally simplified into two main approaches: burden tests or collapsing methods, and non-burden tests or variance-component tests such as the sequence kernel association test (SKAT) [24]. Basically, the burden approach assesses the effect of rare variants by collapsing rare variants into a single score based on either the proportion of rare variants carried in one individual (RVT1) or the presence or absence of rare variants carried in one individual (RVT2) [21, 22]. However, the burden test makes the assumption that all rare variants in the model are causal and influence the trait with the same effect size and direction, which is not necessary the case for all examined rare variants. With this reason, the presence of non-causal variants in the model can underpower the burden test. The sequence kernel association test (SKAT) [24], which is one of the variance-component tests, can address this concern by examining the combined effects of all rare variants by using variance-component score test (flexible weights) in the mixed model, and thus is beneficial for variants with different effects or directions. In addition, the flexible weights can be incorporated with other information such as a regulatory function to improve the statistical power and also to understand the biological mechanism. However, there is limitation of SKAT has been addressed when there is a presence of a large portion of causal rare variants in the genetic region. For

this reason, the combined test between the burden-based test and SKAT, called an optimal sequence kernel association test (SKAT-O) [25], has been proposed to improve the statistical efficiency. Because of a wide range of MAFs (from less than 1% up to ~4.99%) with variable effect sizes and directional effects of our identified LF/rare variants, SKAT-O was more appropriate for our data sets than other methods (i.e., collapsing-based test and SKAT).

We performed SKAT-O [25] using LF/rare variants that were categorized into three groups based on MAF thresholds as follows: MAF less than and equal 1%, MAF between 1 and 5%, and MAF less than 5%. A significant threshold of rare variant association was set at 0.05.

5.7.5. Statistical software

Gene-based tests were performed with VEGAS (http://gump.qimr.edu.au/VEGAS/) [8]. Other association analyses were performed in the R statistical computing software (http://www.r-project.org/). Additional R packages were implicated for specific analyses (i.e., Haplo.Stats for Haplotype analyses, and SKAT for rare variant analyses).

5.8. PREDICTED REGULATORY FUNCTIONS OF IDENTIFIED VARIANTS

As most of our identified variants in *SCARB1* and *ABCA1* were located in introns, we used the RegulomeDB database (Stanford University, CA, http://regulomedb.org/) [26] to assess their regulatory functional significance. The RegulomeDB database provides the genetic regulatory function of variants by integrating evidence from many resources, such as the ENCODE (Encyclopedia of DNA Elements), and Gene Expression Omnibus (GEO). The RegulomeDB has used the scoring scheme from 1 to 6 to present the degree of significance of evidence. Details of the RegulomeDB scores and their biological description are given in **Table 5.11**.

Table 5.11. The RegulomeDB scoring scheme and description*.

Evidence	Score	Scoring Description
Strong	*Likely to affect binding and linked to expression of a gene target*	
	1a	eQTL + TF binding + matched TF motif + matched DNase Footprint + DNase peak
	1b	eQTL + TF binding + any motif + DNase Footprint + DNase peak
	1c	eQTL + TF binding + matched TF motif + DNase peak
	1d	eQTL + TF binding + any motif + DNase peak
	1e	eQTL + TF binding + matched TF motif
	1f	eQTL + TF binding / DNase peak
Convincing	*Likely to affect binding*	
	2a	TF binding + matched TF motif + matched DNase Footprint + DNase peak
	2b	TF binding + any motif + DNase Footprint + DNase peak
	2c	TF binding + matched TF motif + DNase peak
Possible	*Less likely to affect binding*	
	3a	TF binding + any motif + DNase peak
	3b	TF binding + matched TF motif
Insufficient	*Minimal binding evidence*	
	4	TF binding + DNase peak
	5	TF binding or DNase peak
	6	Other

eQTL, expression quantitative trait loci; TF, transcription factor.

Modified from* Boyle, A. P., et al. 2012. *Genome Res* **22: 1790-1797 [26].

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6.0. GENETIC INFLUENCE OF *SCARB1* VARIANTS ON LIPID TRAITS IN US NON-HISPANIC WHITES

Impact of Genetic Variants in Human Scavenger Receptor Class B Type I (*SCARB1*) on Plasma Lipid Traits

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6.1. ABSTRACT

Background: Scavenger receptor class B type 1 (*SCARB1*) plays an important role in high-density lipoprotein cholesterol (HDL-C) metabolism in selective cholesteryl ester uptake and for free cholesterol cellular efflux.

Methods and Results: This study aims to identify common (minor allele frequency (MAF) ≥5%) and low-frequency/rare (MAF <5%) variants, using resequencing all 13 exons and exon-intron boundaries of *SCARB1* in 95 individuals with extreme HDL-C levels selected from a population-based sample of 623 US non-Hispanic whites. The sequencing step identified 44 variants, of which 11 were novel with MAF <1%. Seventy-six variants (40 sequence variants, 32 common HapMap tag single nucleotide polymorphisms, and 4 relevant variants) were selected for genotyping in the total sample of 623 subjects followed by association analyses with lipid traits. Seven variants were nominally associated with apolipoprotien B (apoB; n = 4) or HDL-C (n = 3; *P* <0.05). Three variants associated with apoB remained significant after controlling false discovery rate. The most significant association was observed between rs4765615 and apoB (*P* = 0.0059), while rs11057844 showed the strongest association with HDL-C (*P* = 0.0035). A set of 17 rare variants (MAF ≤1%) showed significant association with apoB (*P* = 0.0284). Haplotype analysis revealed 4 regions significantly associated with either apoB or HDL-C.

Conclusions: Our findings provide new information about the genetic role of *SCARB1* in affecting plasma apoB levels in addition to its established role in HDL-C metabolism.

Key words: genetic association studies; genetic variation; haplotypes; lipids; polymorphism, genetic; sequence analysis, DNA; SCARB1, protein, human

6.2. INTRODUCTION

The inverse association between high-density lipoprotein cholesterol (HDL-C) and the risk of coronary heart disease (CHD) has been widely acknowledged.[1] Raising the levels of HDL-C is among the targets for a reduced risk of CHD in addition to lowering the levels of low-density lipoprotein cholesterol (LDL-C).[2] Genetic contribution to plasma lipid and lipoprotein levels has been indicated due to the high heritability of lipid traits.[3] Genome-wide association studies (GWAS) have identified many candidate genes involved in lipid metabolism where common variants are associated with plasma lipoprotein-lipid levels.[4, 5] However, most of these reported variants are non-functional with small genetic effects on lipoprotein-lipid variation.[6] Focus on extreme phenotypic distribution has been suggested to discover disease-associated functional variants, including low-frequency (LF)/rare variants (minor allele frequency (MAF) <5%) for complex traits.[7] Sequencing candidate genes in individuals with extreme lipid levels have highlighted the important role of LF/rare variants in lipid metabolism.[8, 9]

Scavenger receptor class B type 1 (SCARB1, protein; *SCARB1*, gene) is a multi-ligand receptor involved in the regulation of HDL-C metabolism, mainly in reverse cholesterol transport.[10] SCARB1 has a high affinity for binding to HDL-C, and is abundantly expressed in liver and steroidogenic tissues.[11] SCARB1 mediates the selective cholesteryl ester (CE) uptake from HDL particles and promotes the

bidirectional cellular flux of free cholesterol (FC) between cells and HDL-C. SCARB1 is also implicated in the metabolism of apolipoprotein B (apoB)-containing lipoproteins.[12, 13]

SCARB1 is encoded by the *SCARB1* gene (human gene ID 949) located on chromosome 12q24.31, spanning 86.3-kb span. Several genetic studies in various populations have discovered multiple *SCARB1* variants and reported their relationship with lipid traits,[4, 14-17] and subclinical atherosclerosis and incidence of CHD.[18]

However, the role of *SCARB1* LF/rare variants in relation to lipoprotein-lipid levels has not been studied. In this study, we have tested the hypothesis that both common and LF/rare *SCARB1* variants have significant impact on plasma lipoprotein-lipid variation in the general population. We have resequenced all 13 exons and their exon-intron boundaries of *SCARB1* in 95 non-Hispanic White (NHW) individuals having extreme HDL-C levels in order to identify both common (MAF ≥5%) and LF/rare (MAF <5%) variants. We then genotyped selected identified variants plus common HapMap tag single nucleotide polymorphisms (SNPs) in the total sample of 623 NHWs followed by genotype-phenotype association analyses with HDL-C, LDL-C, triglycerides (TG), and apoB levels.

6.3. METHODS

6.3.1. Subjects

The study was carried out on a well-characterized epidemiological sample of 623 NHW non-diabetic subjects that were originally recruited as part of the San Luis Valley Diabetes Study in southern Colorado.[19] The subjects were between the ages of 24 and 75 years who had a normal response to a standard oral glucose test. The main characteristics for 623 NHWs used in this study are given in **Table A1**. All subjects provided written informed consent. The study protocol was approved by the University of Pittsburgh and University of Colorado Denver Institutional Review Boards.

6.3.2. Selected samples for resequencing

Ninety-five individuals with extreme HDL-C levels falling in the upper and lower 10^{th} percentile were selected from the total sample of 623 NHWs for resequencing. There were 47 individuals in the "high HDL-C" group (HDL-C ≥90^{th} %tile, range: 58-106 mg/dL) and 48 individuals in the "low HDL-C" group (HDL-C ≤10^{th} %tile, range: 20-40 mg/dL; see **Table A2**).

6.3.3. Lipid measurements

Blood samples were collected after at least 8-hour of fasting and immediately placed on ice. Plasma was separated by centrifugation at 4^{o}C and then stored at -80^{o}C before the measurement of total cholesterol (TC), HDL-C, and TG within 30 days in the General Clinical Research Laboratory of the University of Colorado Health Sciences Center, which is certified by the College of American Pathologists for determination of lipid levels.[19] TC and TG were measured by standardized enzymatic assays, and HDL-C was determined by dextran sulfate magnesium precipitation.[19] LDL-C was calculated with the Friedewald formula[20] when TG levels were less than 400 mg/dl. One of the plasma aliquots, stored at -80°C and never thawed, was used to determine apoB levels on a subset of the total sample (n = 425) using the Boehringer Mannheim Turbidimetric procedure at the University of Pittsburgh Heinz Nutrition Laboratory certified by the Clinical Laboratory Improvement Amendments.[21] The routine coefficient of variations between runs were 3.5% for TC, 4.0% for HDL-C, 3.7% for TG, and 3.3% for apoB.

6.3.4. DNA sample preparations and sequencing

Genomic DNA was extracted from leukocytes using a standard DNA extraction protocol. Sequencing samples were amplified into multiple fragments with specific designed primers via polymerase chain reaction (PCR). Primers for *SCARB1* were designed using the Primer3 software program (Whitehead Institute for Biomedical Research, Steve Rozen, and Helen Skaletsky, http://frodo.wi.mit.edu/) based on the *SCARB1* reference sequence (RefSeq) of 86.3 kb from CHIP Bioinformatics (University of

Florida, http://snpper.chip.org/; hg19, chr12:125,262,175-125,348,519, NM_005505) to PCR amplify 13 exons (isoform 1), plus 1 kb of each of 5'and 3' flanking regions. This provided 14 PCR amplicons including 2 overlapping PCR amplicons for the largest exon 13. All 14 PCR amplicons covered 2,742 bp of all 13 exons and 9,842 bp of exon-intron boundaries (see **Table A3** for primers and PCR fragment sizes). PCR reactions and cycling conditions are available upon requests.

Sequencing reactions were performed following the manufacturer's protocols and conducted on the Applied Biosystems 3730xl DNA Analyzer (Beckman Coulter Genomics, Danvers, MA). Sequencing variants were reviewed and analyzed using Variant Reporter (version 1.0, Applied Biosystems, Foster City, CA) and Sequencher (version 4.8, Gene Codes Corporation, Ann Arbor, MI) programs.

6.3.5. Variant selection for genotyping

Common tagSNPs (MAF ≥5%) from our sequencing data were selected by running Tagger analysis using Haploview (Broad Institute of MIT and Harvard, Cambridge, MA, http://www.broadinstitute.org/) with an r^2 cut-off of 0.90.[22] Since our sequencing was focused only on the coding regions and exon-intron boundaries, we selected common tagSNPs (MAF ≥5%) covering the entire *SCARB1* gene and 1 kb of each of 5' and 3' flanking regions from the HapMap data (release #27, genome build 36, dbSNP build 126) for the CEU (Utah residents with northern and western European ancestry) using Tagger analysis with an r^2 threshold of 0.80 for genotyping in our sample (**Table A4**; **Figure A1**). In addition, we also selected 4 reported *SCARB1* variants to be associated

with lipid profile for genotyping, irrespective of their linkage disequilibrium (LD) pattern with other selected variants. Altogether, we selected 76 variants for genotyping in a total of 623 NHW subjects as follows: 40 variants identified by sequencing (8 common tagSNPs and 32 LF/rare variants), 32 common HapMap-CEU tagSNPs, and 4 relevant associated variants from the literatures (**Table A5**).

6.3.6. Genotyping

Genotyping was performed using either Sequenom iPLEX Assay (Sequenom, Inc., San Diego, CA) at the Genomics and Proteomics Core Laboratories, University of Pittsburgh (http://www.genetics.pitt.edu/) or TaqMan SNP Genotyping Assays (Life Technologies Corporation, Grand Island, NY) in our laboratory. Assay reactions were performed according to manufacturers' instructions.

6.3.7. Statistical analysis

The differences in demographics and lipid profile values between the high and low extreme HDL-C groups were calculated by t-test or χ^2 test depending on types of variables.

Allele frequencies and genotype frequencies of quality control (QC) passed genotyped variants (n = 69) were calculated. The assessment of Hardy-Weinberg equilibrium (HWE) was performed using the Pearson's chi-squared test. None of the study samples had missing genotype data greater than 2%. HDL-C, TG, and apoB variables were Box-Cox transformed to achieve normality prior to analyses. The outliers

falling beyond the range of mean ± 3.5 SD (standard deviation) were excluded from further analyses. The most parsimonious set of covariates (sex, age, smoking [never, current, or past], and body mass index) specific to each trait was identified using stepwise regression, and then significant covariates for each trait were used for adjustment in all analyses.

Genetic associations between genotyped variants and 4 traits—HDL-C, LDL-C, TG, and apoB—were examined under a linear additive genetic model by coding alleles as follows: major allele homozygote = 0, minor allele heterozygote = 1, and minor allele homozygote = 2. For each variant with MAF ≥5% (n = 39), the single-site *P*-value of <0.05 was considered as suggestive evidence of association. Because of insufficient power to detect single-site association of variants with MAF <5% (n = 30), those with *P*-value <0.05 were interpreted separately. We applied Benjamini-Hochberg procedure[23] to control for false discovery rate (FDR) in single-site analysis for each trait and considered an FDR value (*q*-value) of <0.20 as statistically significant.

For LF/rare variants (MAF <5%), we used an optimal sequence kernel association test (SKAT-O)[24] for analyzing association with lipid traits. SKAT-O provided better statistical power and appropriate assumption on genetic structure in the genetic model for our data than the burden test and the sequence kernel association test. SKAT-O was applied to a total 30 LF/rare variants with MAF <5%, which were categorized into 3 subgroups on the basis of MAF: <5% (n = 30), ≤2% (n = 20), and ≤1% (n = 17). A SKAT-O *P*-value <0.05 of LF/rare variant association was considered being statistically significant.

The haplotype association of 69 variants for 4 lipid traits was estimated using the generalized linear model with a fixed-length sliding window—4 variants per window and 1 overlapping variant, as too many variants of each window can result in inefficient and impractical model. A global *P*-value <0.05 was considered significant for overall association of haplotypes with frequency >1%.

The gene-based analysis using the versatile gene-based association software (VEGAS, http://genepi.qimr.edu.au/general/softwaretools.cgi)[25] was conducted to test the combined genetic effect of all successfully genotyped 69 variants on each trait. A *P*-value of <0.05 was considered as gene-based significance. All other analyses were performed in the R statistical software (http://www.r-project.org/).

6.4. RESULTS

6.4.1. Identification of *SCARB1* sequence variants

Sequencing of 95 individuals with extreme HDL-C levels identified a total of 44 variants. Thirty-three of them (75%) were previously identified based on dbSNP build 139 and 11 were novel (submitted to dbSNP database:
http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=KAMBOH
[batch ID: SCARB1_EA]). Of 44 variants, 43 were substitutions and 1 was a deletion (**Table A5**).

Of 44 variants, 12 (27.27%) had MAF ≥5% and 32 (72.73%) had MAF <5% (MAF between 1-5%, n = 15; MAF ≤1%, n = 17). Two of the 12 variants with MAF ≥5% were coding variants (rs4238001 [Gly2Ser] in exon 1 and rs5888 [Ala350Ala] in exon 8). Among 12 common variants, 2 LD blocks were observed with r^2 ≥0.90 (**Figure A2**). Rs701104 was in perfect LD with rs838883 (r^2 = 1.0), while 3 additional variants (rs838884, rs838882, and rs838881) were in strong LD with rs838880 (r^2 ranging from 0.93 to 0.97). Of the 32 LF/rare variants with MAF <5%, 21 are known and the remaining 11 are novel. There were 4 coding variants with MAF <5% (rs73227571 [Tyr92Tyr] in exon 2, rs5891 [Val135Ile] in exon 3, rs201977189 [Gly239Arg] in exon 5, and rs10396214 [Arg484Trp] in exon 13).

Of the 32 LF/rare variants, 13 were present only in the high HDL-C group, including 2 non-synonymous variants, (rs5891 [Val135Ile] and rs201977189 [Gly239Arg]), and 9 were observed only in the low HDL-C group (**Table A5**). All 11 novel variants had MAF <1% and were non-coding; 5 and 6 were present in the high and low HDL-C groups, respectively. The proportions of individuals carrying at least 1 LF/rare variant (MAF <5%) between the high and low HDL-C groups were similar (42.55% versus 37.50%; P = 0.615).

6.4.2. Genotyping of *SCARB1* variants

Forty of the 44 sequence variants were selected for genotyping (4 were excluded because of strong LD) in the entire sample. In addition, we selected 32 common HapMap-CEU tagSNPs from the unsequenced *SCARB1* intronic regions and 4 variants with reported associations with lipid traits from the literatures. Of the 76 variants

selected for genotyping, 69 (MAF ≥5%, n = 39; MAF between 1-5%, n = 13; MAF ≤1%, n = 17) were successfully genotyped in all 623 NHWs (genotype success rate ≥90% and discrepancy rate <1% among 10% of the replication sample), and none deviated from the Hardy-Weinberg equilibrium. Characteristics and LD structure of successfully 69 genotyped variants are shown in **Table A5** and **Figure A3**.

Of 69 variants, 58 (84.05%) were located in introns, 8 (11.60%) in exons, and 3 (4.35%) in 3' flanking region (**Table A6**; **Figure A4**). The most numbers of genotyped variants (n = 25) were present in the longest intron 1. Of the 11 newly identified sequence variants, only 8 were successfully genotyped (**Table A7**). Of the 8 exonic variants, 4 were non-synonymous (rs4238001 [Gly2Ser], MAF = 0.082; rs5891 [Val135Ile], MAF = 0.0096; rs201977189 [Gly239Arg], MAF = 0.0016; rs10396214 [Arg484Trp], MAF = 0.0088), 2 were synonymous (rs73227571 [Tyr92Tyr], MAF = 0.0008; rs5888 [Ala350Ala], MAF = 0.485) and the remaining 2 (rs184715678 and p87210-chr12_126262310, MAFs of both = 0.0016) were located in the 3' UTR of exon 13.

6.4.3. Associations of *SCARB1* variants with lipid traits

Gene-based association

Initially, we performed a gene-based test including all successfully genotyped 69 variants (**Table 6.1**), and found a nominal association with apoB (*P* = 0.0425; best SNP p78334-chr12_125271186, *P* = 0.0015) and a borderline association with HDL-C (*P* = 0.132; best SNP rs11057844, *P* = 0.0035).

Association of common variants

Single-locus association analysis of 39 common variants (MAF ≥5%) revealed 7 nominal associations (P <0.05), including 3 with HDL-C (rs11057844, rs701106, and rs838880) and 4 with apoB (rs2343394, rs4765615, rs2278986, and rs11057820). Of these 7 variants with nominal association, 3 associated with apoB (rs2343394, rs4765615, and rs2278986) remained significant after multiple testing adjustment (FDR <0.20; **Tables A8** and **A9**). No significant associations were observed with either LDL-C or TG (**Tables A10** and **A11**).

Of the 3 nominally associated variants with HDL-C, the strongest signal was observed with rs11057844 (β = -0.0395; P = 0.0035; FDR = 0.227) followed by rs701106 (β = 0.0394; P = 0.0066; FDR = 0.227) and rs838880 (β = 0.0257; P = 0.025; FDR = 0.457; **Table 6.2**). The lead variant, rs11077844, was not correlated with the other 2 (r^2 = 0). Whereas rs701106 and rs838880 were in modest LD with each other (r^2 = 0.37; **Figure 6.1**). The 4 apoB-associated variants had an elevating effect on apoB levels: rs2343394 (β = 1.2544; P = 0.0082; FDR = 0.165), rs4765615 (β = 1.2493; P = 0.0059; FDR = 0.165), rs2278986 (β = 1.1926; P = 0.0122; FDR = 0.165) and rs11057820 (β = 0.87; P = 0.0436; FDR = 0.430; **Table 6.2**). Among the 3 apoB-associated variants with similar effects and FDR-significance, 2 (rs2343394 and rs2278986) were in strong LD (r^2 = 0.94) and together they were in moderate LD with rs4765615 (r^2 = 0.46-0.48; **Figure 6.1**).

Association of LF/rare variants

A total of 30 LF/rare variants with MAF <5% were tested for association using SKAT-O at three different MAF thresholds (<5%, ≤2%, and ≤1%). The group of 17 rare variants with MAF ≤1% showed significant association with apoB (*P* = 0.0284; **Table 6.3**). Next, we examined the distribution of lipid traits among variants with MAF ≤1% to determine if some of them were associated with extreme lipid phenotypes. We found 4 variants that were detected in at least 2 individuals including 2 known (rs5891 [Val135Ile] in exon 3 and rs201977189 [Gly239Arg] in exon 5) and 2 novel (p57308-chr12_125292212 in intron 7 and p78334-chr12_125271186 in intron 10) variants (**Table 6.4**). The carriers of rs5891 [Val135Ile] variant were associated with higher apoB (β = 5.8266; *P* = 0.012) and lower TG (β = -0.2536; *P* = 0.0306) levels than the wild type. Two individuals carrying the rs201977189 [Gly239Arg] variant had higher HDL-C levels than non-carriers (β = 0.2937; *P* = 0.0275). While 2 individuals carrying p57308-chr12_125292212 novel variant had lower levels of TG (β = -0.6937; *P* = 0.015), and another 2 carrying the p78334-chr12_125271186 novel variant had higher levels of apoB (β = 14.5804; *P* = 0.0015) than the respective wild types. Three additional novel rare variants were observed in 1 individual each and each carrier had extreme lipid levels above the 95th percentile of the distribution compared to non-carriers.

Association of haplotypes

Haplotype associations including all 69 variants were estimated by the fixed 4-variant-sliding window approach. A total of 23 significant haplotype windows associated with 4 lipid traits are presented in **Table A12** (see detailed haplotype windows in **Tables A14** and **A15**). The most numbers of haplotype windows that yielded significant associations

(global *P* <0.05) were observed for apoB (windows #21, #25-31, #44, #53-54; **Figure 6.2**). Of the 11 significant windows associated with apoB, the strongest signal was observed with window #28 (global *P* = 0.0005). Of note, windows #25-31 contained the 3 FDR-significant apoB-associated variants in single-site analysis (rs2343394, rs4765615, and rs2278986), indicating that functional variants exist in this region.

Eight haplotype windows showed association with HDL-C (windows #12, #14-16, #60-63; **Figure 6.2**), and the most significant window was window #62 (global *P* = 0.0034). Windows #14-16 contained rs11057844 and windows #60-63 contained rs701106; both variants (rs11057844 and rs701106) were nominally associated with HDL-C in single-site analysis. It appears that most of the effects in significant haplotype windows for apoB and HDL-C are partly derived from 6 of 7 variants that also showed significant association in single-site analysis. However, 5 significant windows for apoB (windows #21, #44, #53-54) and 1 for HDL-C (window #12) did not contain any associated variants from single-site analysis, indicating that overall haplotype approach yielded better information.

There were 3 (windows #1, #39-40) and 2 (windows #16-17) haplotype windows that showed significant association with LDL-C and TG, respectively (global *P* <0.05; **Figures A5C**, **A5D**). None of the variants included in LDL-C and TG-associated haplotype windows had evidence of association with LDL-C or TG in single-site analysis. One haplotype window (window #16) was associated with both HDL-C (global *P* = 0.0092) and TG (global *P* = 0.0315).

The consecutive haplotype windows significantly associated with HDL-C and apoB (global *P* <0.05) revealed 2 regions associated with HDL-C (region 1: windows

#14-16; region 4: windows #60-63), and 2 additional regions with apoB (region 2: windows #25-31; region 3: windows #53-54; **Figure 6.2**; **Table A13**). In region 1, the ACACGG haplotype (rs1229555, rs4765622, rs11057844, rs10846749, rs10744192, and rs10773107) showed the strongest significance associated with HDL-C. While, the GAGTCCG haplotype (rs838895, rs838893, rs797729, rs701106, rs10396214 [Arg484Trp in isoform 2], rs184715678, and p87210-chr12_125262310) was the best haplotype associated with HDL-C in region 4. Likewise, for apoB, the CCCTGGGCCG haplotype (rs11057830, rs199779577, rs73227571 [Tyr92Tyr], rs2343394, rs144194221, rs4765615, rs5891 [Val135Ile], rs2278986, p50432-chr12_125299088, and rs11057820) had the strongest signal in region 2. While, the rare haplotype (frequency <1%; rs201901986, rs34339961, rs2272310, p78334-chr12_125271186, and rs838897) yielded the most significant association with apoB in region 3.

6.4.4. Functional prediction of identified *SCARB1* variants

We determined the functionality of all 80 variants tested in this study (44 variants identified by sequencing, 32 HapMap tagSNPs, and 4 additional SNPs with reported association from the literatures) using the RegulomeDB database (version 1.0, Stanford University, http://regulome.stanford.edu/index).[26] Of the total 80 variants, 71 (88.75%) had RegulomeDB scores ranging from 1f to 6: scores 1f-2b (strong functional evidence), 6 variants; scores 3a-3b (suggestive functional evidence), 3 variants; scores 4-6 (minimal functional evidence), 62 variants (**Tables A16** and **A17**).

Seven variants that were found to be significantly associated with HDL-C or apoB showed only minimal functional evidence with RegulomeDB score of 5 (**Table 6.2**).

Although rs838880, an HDL-C associated variant (P = 0.025) was less likely to be functional (score 5), it was in strong LD (r^2 >0.90) with 3 other variants (rs838881, rs838882, and rs838884) identified by sequencing but not selected for genotyping (**Figure A2**), of which rs838884 located in 3' flanking had strong evidence of regulatory function based on RegulomeDB score of 2b. There were 6 HapMap tagged SNPs (bins #4, #12, #13) tightly linked to 3 significantly associated variants (r^2 ≥0.95; **Figure A1**), of which 5 (except rs838884) were less likely to have regulatory activities with scores 5-6 (**Table A18**).

Of the 11 rare novel variants, 9 (81.82%) were assigned RegulomeDB scores from 2b to 6 (**Table A7**). Three novel variants are likely to be functional based on RegulomeDB scores of 2b (p50432-chr12_125299088, intron 3; p87210-chr12_125262310, exon 13-3' UTR), and 3a (p64285-chr12_125285235, intron 7).

6.5. DISCUSSION

The role of SCARB1 in facilitating HDL-C clearance through a selective uptake of CE from HDL-C to the liver and a HDL-C-mediated cellular flux of FC is well established.[10, 11] In addition to HDL-C, SCARB1 also has high-affinity binding with apoB-containing lipoproteins[12, 13] and is involved in the clearance of non-HDL-C particles.[27] Hepatic overexpression of SCARB1 is associated with increased hepatic production of very low-density lipoprotein cholesterol (VLDL-C),[12, 28] leading to elevated levels of LDL-C, VLDL-

C and apoB. In contrast, SCARB1 knockout mice have significantly decreased levels of apoB-containing lipoproteins.[13] Since SCARB1 is involved in the metabolism of both HDL-C and apoB-containing lipid particles, the objective of this study was to resequence the SCARB1 gene in selected NHW individuals with extreme HDL-C levels in order to identify both common and LF/rare variants and then to examine the role of the identified variants with plasma HDL-C, LDL-C, TG and apoB levels in the entire sample of 623 NHWs.

Resequencing of all 13 *SCARB1* exons and exon-intron boundaries in selected individuals with extreme HDL-C levels identified 44 sequence variants, of which 40 were selected for genotyping. In addition, we selected 32 common HapMap tagSNPs covering the whole *SCARB1* gene, plus 4 previously significantly associated variants for genotyping in the entire sample. Finally, 69 successfully genotyped variants (MAF ≥5%, n = 39; MAF <5%, n = 30) were proceeded for association analyses in the total sample. Initial gene-based analysis including all 69 genotyped variants revealed a nominal association with apoB (P = 0.0425) and a borderline association with HDL-C (P = 0.132), reflecting the role of *SCARB1* in apoB and HDL-C metabolism. Single-locus analysis revealed three nominally significant associations with HDL-C. The most significant association was observed with rs11057844 (P = 0.0035; FDR = 0.227) followed by rs701106 (P = 0.0066; FDR = 0.227). To our knowledge, these two are novel associations observed in this study. The third association was seen with rs838880 (P = 0.025; FDR = 0.457) that has previously been reported to be genome-wide significant in a large GWAS.[4] Similar to the reported GWAS outcome, the minor allele of rs838880 was associated with increased HDL-C levels in our sample. These 3 variants

seemed to be independently associated with HDL-C based on the weak correlation among them (mean r^2 <0.40, ranging from 0 to 0.37; **Figure 6.1**). Moreover, since all 3 variants have a minimal functional role based on RegulomeDB score of 5, it is not clear if they independently affect HDL-C or their effect is mediated through a yet to be identified functional variants. Furthermore, 7 of the 8 haplotype windows (windows #14-16 spanning within intron 1, #60-63 spanning between a part of intron 11 and a part of exon 13-3' UTR) that showed significant association with HDL-C contained either rs11057844 or rs701106, implying that these 2 variants contributed to the observed haplotype effects. However, the remaining haplotype window (window #12) did not contain any HDL-C associated variants in single-locus analysis.

Three common *SCARB1* variants (rs2343394, rs4765615, and rs2278986) demonstrated novel and FDR-significant associations with apoB where the minor alleles were associated with very similar elevating effect sizes (**Table 6.2**). While rs2343394 present in intron 2 and rs2278986 present in intron 3 were in strong LD (r^2 = 0.94), together they showed moderate correlation (r^2 = 0.46–0.48) with rs4765615 present in intron 2 (**Figure 6.1**). In addition to single-locus associations, haplotype analysis also yielded association with apoB. Eleven haplotype windows were associated with apoB (windows #21, #25-31, #44, #53-54), of which 7 (windows #25-31 spanning between a part of intron 1 and a part of intron 4) contained at least 1 of the 3 FDR-significant apoB-associated variants in single-site analysis (rs2343394, rs4765615, and rs2278986). Furthermore, a rare coding variant located in exon 4, rs5891 [Val135Ile], was also found to be associated with higher apoB levels (see **Table 6.4**), but this was not in LD with the four apoB-associated variants (**Figure A6**). Our data strongly indicate that functional

variants exist in this region affecting apoB levels. Higher apoB levels are considered to be a risk factor for CHD,[29] and 1 of the 4 apoB-associated variants in our study (rs2343394) has previously been found to be associated with carotid media intima thickness and incidence of CHD.[18] Furthermore, another apoB-associated variant (rs2278986) has also been found to be associated with lower SCARB1 protein levels.[30] Taken together, our findings in conjunction with the published data suggest that *SCARB1* may have an apoB-mediated novel role in CHD.

Of the overall 23 haplotype windows that yielded significant association (global *P* <0.05), the majority were observed with HDL-C (n = 8) and apoB (n = 11; **Table A12**). Moreover, the haplotype analysis identified 4 haplotype regions harboring 5 to 10 variants (**Table A13**) that were significantly associated with HDL-C (regions: 1, 4) and apoB (regions: 2, 3; **Figure 6.2)**. Notably, 3 haplotype regions (regions: 1, 2, 4) contained 6 variants that yielded significant association in single-site analysis with HDL-C (rs11057844 and rs701106) or apoB (rs2343394, rs4765615, rs2278986, and rs11057820). Our data indicate that these 4 haplotype regions are good candidates for further deep resequencing in order to identify causal *SCARB1* variants, as our current sequencing effort was mainly focused on coding regions and we may have missed regulatory functional variants present in introns

Although no common variants showed association with either LDL-C or TG in single-locus analysis, we found 5 haplotype windows to be associated with these two traits (windows #1, #39-40 for LDL-C; windows #16-17 for TG). However, apoB is the sole apolipoprotein present on LDL particles, there was only moderate correlation between LDL-C and apoB levels in our sample (Pearson correlation coefficient: males =

0.43, females = 0.50).[21] Thus, it is not surprising that single-locus analysis yielded significant association with apoB but not with LDL-C. In addition, we found no association between 2 coding variants: rs4238001 [Gly2Ser] and rs5888 [Ala350Ala], and any of the 4 lipid traits examined in this study.

Rare variant analysis using the SKAT-O method revealed significant association of 17 variants (MAF ≤1%) with apoB (*P* = 0.0284; **Table 6.3**). As potential causal rare variants are present only in a handful of individuals, we investigated the individual contribution of 17 rare variants (MAF ≤1%) with 4 lipid traits. We found that 7 of these rare variants were associated with extremely higher or lower levels of HDL-C, TG or apoB (**Table 6.4**; **Table A7**). While 2 of them are known coding variants (rs5891 [Val135Ile] and rs201977189 [Gly239Arg]), the remaining 5 are novel intronic variants (p54866-chr12_125294654, p57308-chr12_125292212, p57618-chr12_125291902, p64285-chr12_125285235, and p78334-chr12_125271186). Four of the 7 variants (rs5891 [Val135Ile], rs201977189 [Gly239Arg], p57308-chr12_125292212, and p78334-chr12_125271186) associated with extreme lipid levels were observed in at least 2 individuals, and thus these associations seem genuine. In this regard, one of the coding variants, rs5891 [Val135Ile], has previously shown to be associated with higher HDL-C levels in Amish women from Pennsylvania.[15] The remaining 3 of 7 variants (p54866-chr12_125294654, p57618-chr12_125291902, and p64385-chr12_125285235) were observed in one individual each and they all are novel. We are not certain if the co-existence of extreme lipid phenotype and with a variant is real or an incidental finding. It would be necessary in future studies to screen these mutations in large number of NHWs to validate the observed associations. It is likely that these 3 novel rare variants

are functional, as evidence from their RegulomeDB scores of 3a (evidence of affecting transcription factor (TF) binding, any motifs and DNase peak for p64385-chr12_125285235) and 4 (evidence of affecting TF binding and DNase peak for p54866-chr12_125294654 and p57618-chr12_125291902).

There are some limitations of our study. Due to the large *SCARB1* gene size, we sequenced only the coding regions and exon-intron boundaries, and our sequencing sample was also small. It is likely that we may have missed functional less common variants present in introns and we may have not identified all functional variants present in the coding regions. Furthermore, the sample size in this study did not provide adequate statistical power for rare variant analyses, and thus, certain associations should be interpreted with caution. Despite these limitations, our haplotype analysis has identified multiple regions of potential significance, including 4 extensive haplotype regions.

In conclusion, using the resequencing approach, we were successful in identifying common and LF/rare *SCARB1* variants, including 11 novel variants. We have also identified novel single-site and haplotype associations with HDL-C and apoB levels. To our knowledge, this is the first study that has found significant association of *SCARB1* variants with apoB levels. Although additional studies are needed to replicate our findings, this study provides evidence of the genetic influence of *SCARB1*—common and LF/rare variants—on lipid and apolipoprotein levels.

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Disclosures

None.

Table 6.1. Result of gene-based association test.

Trait	No of SNPs	Test Statistics	*P*	Best SNP		
				SNP Name*-SNP ID†/Chr12 Position*	MAF	*P*
HDL-C	69	94.0639	0.1320	p28957–rs11057844	0.184	0.0035
LDL-C	69	51.4791	0.8020	p64285-chr12_125285235	0.001	0.0008
TG	69	64.9192	0.5010	p64285-chr12_125285235	0.001	0.0065
ApoB	68‡	110.7269	0.0425	p78334-chr12_125271186	0.002	0.0015

ApoB indicates apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MAF, minor allele frequency; SNP, single nucleotide polymorphism; and TG, triglycerides.
*Based on *SCARB1* RefSeq (hg19, NM_005505).
†dbSNP build 139.
‡One novel variant (p57618-chr12_125291902) was excluded due to missing phenotype data.

Table 6.2. Top seven variants associated with HDL-C (n = 3) and ApoB (n = 4) in single-site analysis (*P* <0.05).

SNP Name*	RefSNP ID†	Chr12 Position*	Location	RegulomeDB Score‡	Trait	Major/Minor Alleles	MAF	β	*P*	FDR
p28957	rs11057844	125320563	Intron 1	5	HDL-C	G/A	0.184	-0.0395	0.0035	0.227
p48969	rs2343394	125300551	Intron 2	5	ApoB	C/T	0.285	1.2544	0.0082	0.165
p49690	rs4765615	125299830	Intron 2	5	ApoB	A/G	0.450	1.2493	0.0059	0.165
p50151	rs2278986	125299369	Intron 3	5	ApoB	T/C	0.289	1.1926	0.0122	0.165
p52556	rs11057820	125296964	Intron 4	5	ApoB	A/G	0.487	0.8700	0.0436	0.430
p83884	rs701106	125265636	Intron 12	5	HDL-C	C/T	0.153	0.0394	0.0066	0.227
p87927	rs838880	125261593	3' flanking	5	HDL-C	A/G	0.324	0.0257	0.0250	0.457

ApoB indicates apolipoprotein B; FDR, false discovery rate; HDL-C, high-density lipoprotein cholesterol; MAF; minor allele frequency; and SNP, single nucleotide polymorphism.
*Based on the *SCARB1* RefSeq (hg19, NM_005505).
†dbSNP build 139.
‡Detailed RegulomeDB scoring scheme and definitions are described at Tables A16 and A17.

Table 6.3. Result of SKAT-O analysis for low-frequency/rare variants (MAF <5%).

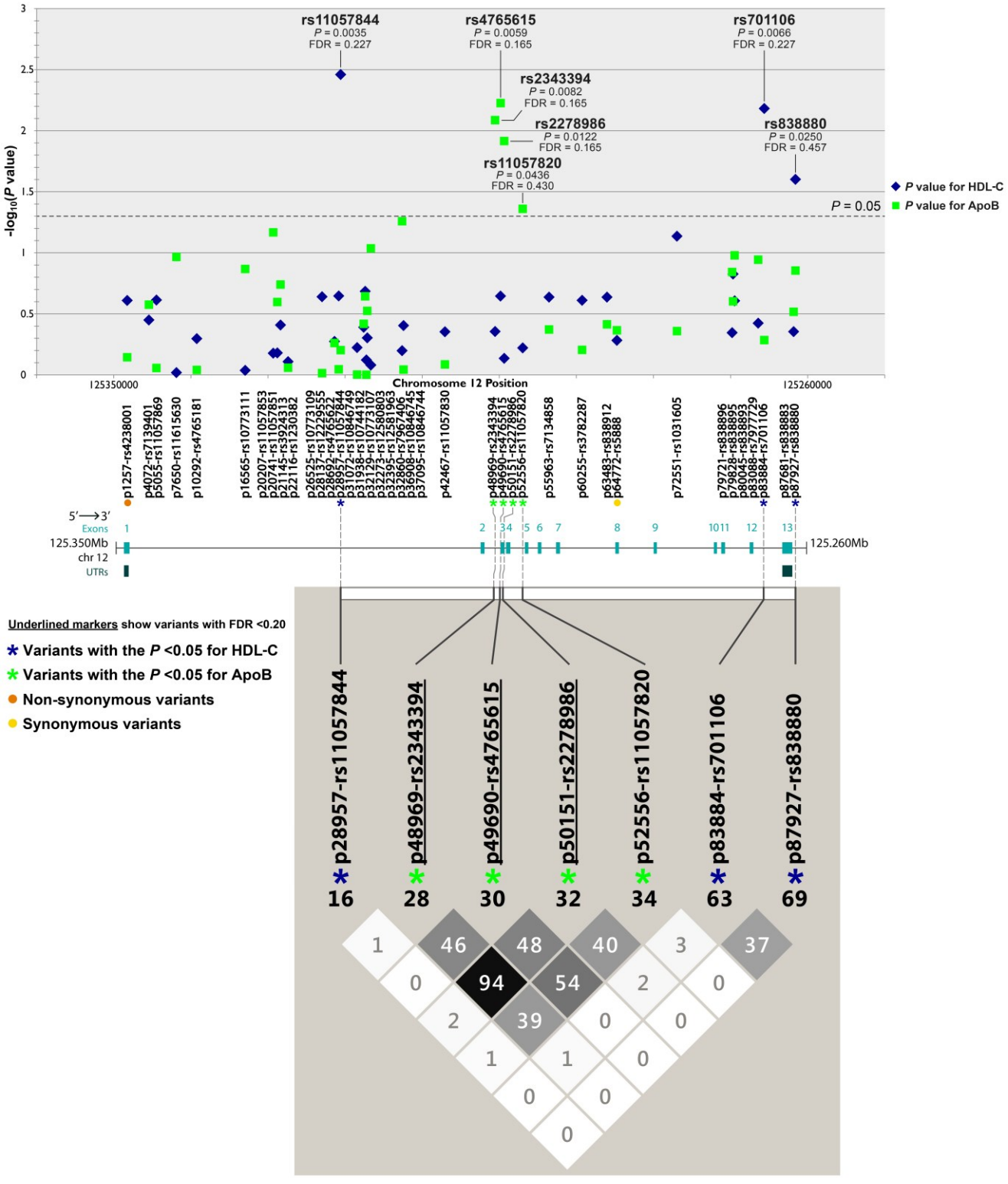
MAF	No of LF/Rare SNPs	No of Samples [with/without LF/rare SNPs]	HDL-C		LDL-C		TG		ApoB	
			Stat	*P*	Stat	*P*	Stat	*P*	Stat	*P*
≤0.01	17	52/ 571	17618.66	0.3504	26511.73	0.2990	22034.18	0.1498	41545.33	0.0284
≤0.02	20	81/ 542	20818.23	0.6619	14557.18	0.8395	35351.17	0.1737	35207.61	0.0494
<0.05	30	207/ 416	239287.65	0.3845	95602.77	0.6588	138448.60	0.3710	54901.73	0.1542

ApoB indicates apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LF, low-frequency; MAF, minor allele frequency; SKAT-O, an optimal sequence kernel association test; SNP, single nucleotide polymorphism; and TG, triglycerides.

Table 6.4. Effect of rare variants (MAF ≤1%) on HDL-C, TG, and ApoB levels.

SNP Name*	RefSNP ID†	Chr12 Position*	Location	Amino Acid Change	RegulomeDB Score‡	Minor Allele (MAF)	GT	GT Count n (carrier freq)	Mean ± SD (mg/dL)	β	SE	*P*§	FDR
HDL-C													
p53093	rs201977189	125296427	Exon 5	Gly239Arg	5	A (0.0016)	GG	613	50.51 ± 14.02	0.2937	0.1329	0.0275	0.457
							GA	2 (0.33)	71.10 ± 10.61				
p54866		125294654	Intron 6		4	A (0.0008)	GG	616	50.52 ± 13.95	0.3998	0.1873	0.0332	0.457
							GA	1 (0.16)	87.87 ± NA				
p57618		125291902	Intron 7		4	A (0.0008)	GG	615	50.54 ± 13.96	0.3727	0.1873	0.0470	0.517
							GA	1 (0.16)	86.08 ± NA				
TG													
p49978	rs5891	125299542	Exon 3	Val135Ile	5	A (0.0096)	GG	607	138.50 ± 65.91	-0.2536	0.1170	0.0306	0.704
							GA	12 (1.94)	109.21 ± 54.65				
p57308		125292212	Intron 7		4	T (0.0016)	CC	611	138.22 ± 65.81	-0.6937	0.2844	0.0150	0.517
							CT	2 (0.33)	65.73 ± 5.66				
p64285		125285235	Intron 7		3a	G (0.0008)	CC	611	137.69 ± 64.94	1.0938	0.4002	0.0065	0.446
							CG	1 (0.16)	406.12 ± NA				
ApoB													
p49978	rs5891	125299542	Exon 3	Val135Ile	5	A (0.0096)	GG	426	87.52 ± 23.82	5.8266	2.3105	0.0120	0.165
							GA	8 (1.84)	109.01 ± 20.91				
p78334		125271186	Intron 10		4	G (0.0024)	TT	432	87.66 ± 23.67	14.5804	4.5709	0.0015	0.104
							TG	2 (0.46)	144.16 ± 2.05				

ApoB indicates apolipoprotein B; FDR, false discovery rate; GT, genotype; HDL-C, high-density lipoprotein cholesterol; MAF; minor allele frequency; NA, not analyzed; SD; standard deviation; SE; standard error; SNP, single nucleotide polymorphism; and TG, triglycerides.
*Based on the *SCARB1* RefSeq (hg19, NM_005505).
†dbSNP build 139.
‡Detailed RegulomeDB scoring scheme and definitions are described at Tables A16 and A17.
§Based on single-site association analysis (Tables A8-A11).


rs11057844
P = 0.0035
FDR = 0.227
rs4765615
P = 0.0059
FDR = 0.165
rs2343394
P = 0.0082
FDR = 0.165
rs2278986
P = 0.0122
FDR = 0.165
rs11057820
P = 0.0436
FDR = 0.430
rs701106
P = 0.0066
FDR = 0.227
rs838880
P = 0.0250
FDR = 0.457
-log10(P value)
P = 0.05
P value for HDL-C
P value for ApoB
125350000
Chromosome 12 Position
125260000
p1257-rs4238001
p4072-rs7139401
p5055-rs11057869
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p10292-rs4765181
p16565-rs10773111
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p79721-rs838896
p79828-rs838895
p80045-rs838893
p83088-rs7977729
p83884-rs701106
p87681-rs838883
p87927-rs838880
5′⟶3′
Exons
125.350Mb
chr 12
UTRs
125.260Mb
Underlined markers show variants with FDR <0.20
Variants with the P <0.05 for HDL-C
Variants with the P <0.05 for ApoB
Non-synonymous variants
Synonymous variants
p28957-rs11057844
p48969-rs2343394
p49690-rs4765615
p50151-rs2278986
p52556-rs11057820
p83884-rs701106
p87927-rs838880
16
28
30
32
34
63
69
1
46
48
40
3
37
0
94
54
2
0
2
39
0
0
1
1
0
0
0
0

Figure 6.1. Single-site association *P*-values of 39 *SCARB1* variants with minor allele frequency (MAF) ≥5% for high-density lipoprotein cholesterol (HDL-C) and apolipoprotien B (ApoB; top), gene structure of *SCARB1* (middle) and linkage disequilibrium (LD) plot of 7 variants associated with HDL-C (n = 3) or ApoB (n = 4; *P* <0.05; bottom).

The $-\log_{10}$ *P*-values are in the Y-axis. A total of 39 variants with MAF ≥5% are on *SCARB1* (5'→3'; RefSeq: hg19, NM_005505) in the X-axis. Marker names are shown as "SNP name-SNP ID (dbSNP build 139)/chromosome 12 position (for novel variants)". Dash line indicates the significance threshold (*P* = 0.05). Shades and values (r^2 x 100) in each square of LD plot indicate pairwise LD: black indicating complete LD (r^2 = 1), white indicating no LD (r^2 = 0), and shade intensity indicating the degree of LD (r^2 between 0-1). FDR indicates false discovery rate; SNP, single nucleotide polymorphism.

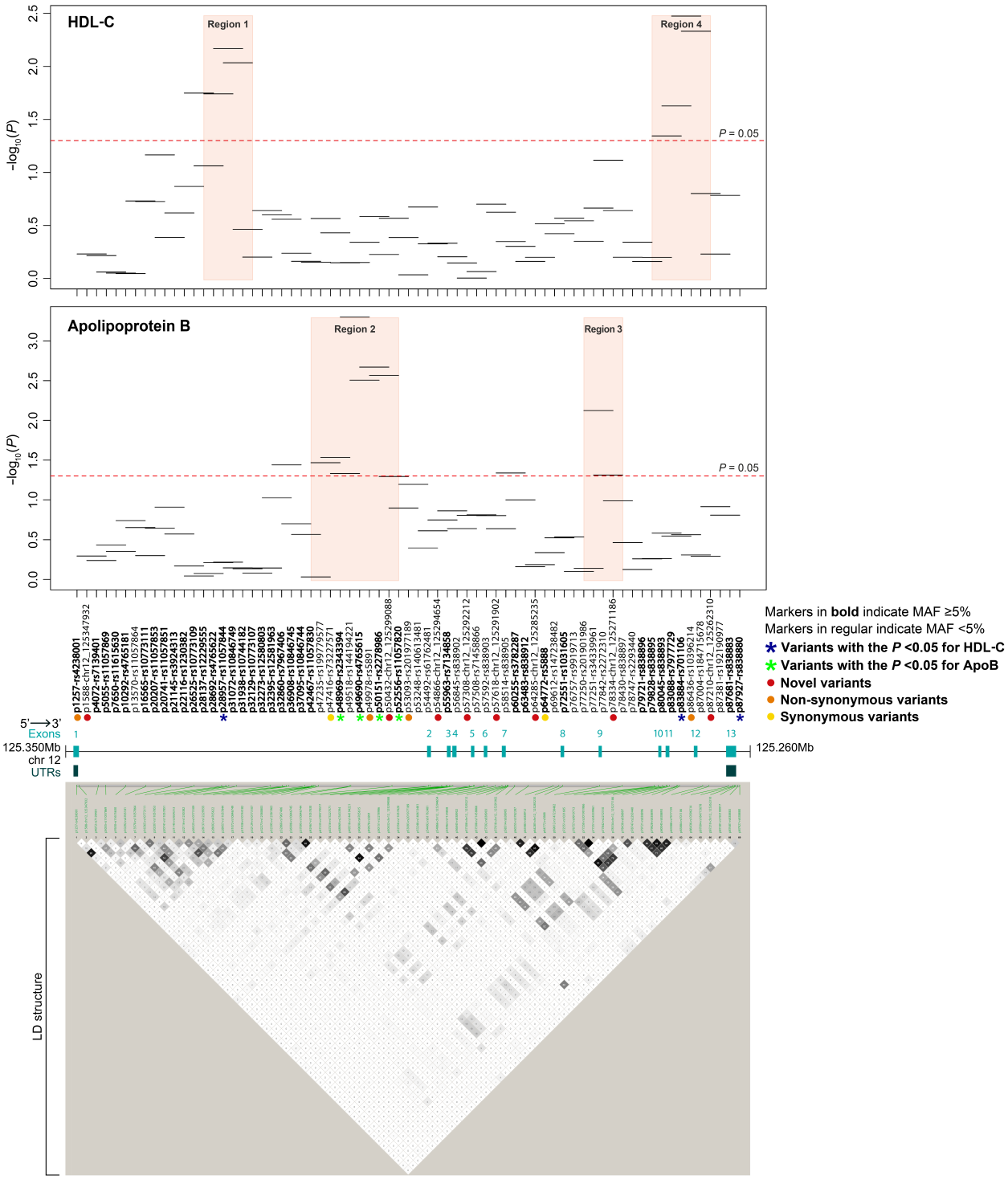
HDL-C
Region 1
Region 4
P = 0.05
−log10(P)
Apolipoprotein B
Region 2
Region 3
P = 0.05
−log10(P)
Markers in bold indicate MAF ≥5%
Markers in regular indicate MAF <5%
Variants with the P <0.05 for HDL-C
Variants with the P <0.05 for ApoB
Novel variants
Non-synonymous variants
Synonymous variants
5'⟶3'
Exons
125.350Mb
chr 12
UTRs
125.260Mb
LD structure

Figure 6.2. Haplotype association plots for high-density lipoprotein cholesterol (HDL-C) and apolipoprotien B (ApoB; top) and linkage disequilibrium (LD) structure (bottom) of 69 *SCARB1* variants.

The -$\log_{10}$ *P*-values are in the Y-axis. Total 69 variants are on *SCARB1* (5'→3'; RefSeq: hg19, NM_005505) in the X-axis. Marker names are shown as "SNP name-SNP ID (dbSNP build 139)/chromosome 12 position (for novel variants)". Dash line indicates the significance threshold (global P = 0.05). Highlighted areas in the haplotype plots represent 4 haplotype regions significantly associated with HDL-C (regions: 1, 4) and apoB (regions: 2, 3). The degree of shades and values (r^2 x 100) in each square of LD plot indicate pairwise LD: black indicating complete LD (r^2 = 1), white indicating no LD (r^2 = 0), and shade intensity indicating the degree of LD (r^2 between 0-1). MAF indicates minor allele frequency; SNP, single nucleotide polymorphism.

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7.0. GENETIC INFLUENCE OF *SCARB1* VARIANTS ON LIPID TRAITS IN AFRICAN BLACKS

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Key words: African continental ancestry group; candidate gene association study; genetic variation; haplotypes; lipids; SCARB1 protein, human; sequence analysis, DNA

7.1. ABSTRACT

Background: High-density lipoprotein cholesterol (HDL-C) exerts many anti-atherogenic properties including its role in reverse cholesterol transport (RCT). Scavenger receptor class B member 1 (SCARB1) plays a key role in RCT by selective uptake of HDL cholesteryl esters. We aimed to explore the genetic contribution of SCARB1 to affecting lipid levels in African Blacks from Nigeria.

Methods: We resequenced 13 exons and exon-intron boundaries of *SCARB1* in 95 individuals with extreme HDL-C levels using Sanger method. Then, we genotyped 147 selected variants (78 sequence variants, 69 HapMap tagSNPs, and 2 previously reported relevant variants) in the entire sample of 788 African Blacks using either the iPLEX Gold or TaqMan methods. A total of 137 successfully genotyped variants were further evaluated for association with major lipid traits.

Results: The initial gene-based analysis demonstrated evidence of association with HDL-C and apolipoprotein A-I (ApoA-I). The follow-up single-site analysis revealed nominal evidence of novel associations of 9 common variants with HDL-C and/or apoA-I (P <0.05). The strongest association was between rs11057851 and HDL-C (P = 0.0043), which remained significant after controlling for multiple testing using false discovery rate. Rare variant association testing revealed a group of 23 rare variants

(frequencies ≤1%) associated with HDL-C (P = 0.0478). Haplotype analysis identified 4 *SCARB1* regions associated with HDL-C (global P <0.05).

Conclusions: To our knowledge, this is the first report of a comprehensive association study of *SCARB1* variations with lipid traits in an African Black population. Our results showed the consistent association of *SCARB1* variants with HDL-C across various association analyses, supporting the role of *SCARB1* in lipid-lipoprotein regulatory mechanism.

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7.2. INTRODUCTION

Abnormal lipid and lipoprotein levels are a major risk factor for coronary heart disease (CHD) [1], the leading cause of death worldwide [2]. Elevated low-density lipoprotein cholesterol (LDL-C) levels and decreased high-density lipoprotein cholesterol (HDL-C) levels are correlated with the development of CHD. There is a strong genetic basis for lipoprotein-lipid levels with heritability estimates of 40-80% [3]. A large number of genes and genetic variants associated with lipid traits have been discovered in genome-wide association studies (GWASs) [4-6]. Most of the common variants (minor allele frequency [MAF] ≥5%) identified by GWASs have modest effects on lipid levels, and have overall a small contribution to total genetic variance of lipid traits (~25-30% of the heritability) [4-8]. A portion of the missing heritability of lipid traits could be explained by low frequency (LF)/rare variants (MAF <5%) as suggested by recent studies [9-11].

HDL, the smallest and densest (d = 1.063-1.21 g/mL) class of lipoprotein particles, has a variety of anti-atherogenic properties [12]. One of the HDL properties to protect against CHD is mediated by reverse cholesterol transport (RCT) from peripheral tissues back to the liver [13]. Scavenger receptor class B member 1 (SCARB1, protein; *SCARB1*, gene) serves as a HDL-C receptor in RCT that mediates selective uptake of HDL-C cholesteryl esters (CE) by the liver and free cholesterol efflux from cells to HDL-

C [14]. SCARB1 is also implicated in the metabolism of apolipoprotein B (apoB)-containing particles [15-21].

The *SCARB1* gene (Entrez Gene ID: 949) is located on human chromosome 12, and is abundantly expressed in liver and steroidogenic tissues [22, 23]. The role of *SCARB1* in HDL-C and apoB-containing lipoproteins metabolism has been established in animal studies. The disruption of *SCARB1* is associated with increased HDL-C levels and decreased CE uptake [24-26]. Whereas the overexpression of *SCARB1* reduces levels of HDL-C, apoA-I, very low-density lipoprotein cholesterol (VLDL-C), LDL-C, and apoB [15-17, 19] and promotes the hepatic uptake of CE as well as the biliary secretion of HDL-C [15, 27]. The *SCARB1* expression is also significantly associated with hepatic VLDL-triglycerides (TG) and VLDL-apoB production. Hepatic VLDL cholesterol production together with VLDL clearance is enhanced in response to *SCARB1* overexpression [21]. In contrast, reduced hepatic VLDL-TG and VLDL-apoB production is associated with *SCARB1* knockout status [18, 20, 21].

In humans, three *SCARB1* mutations (rs397514572 [p.Ser112Phe], rs187831231 [p.Thr175Ala], and rs387906791 [p.Pro297Ser]) [MIM: 601040] have been reported to be associated with significantly increased HDL-C levels [28, 29]. Moreover, several genetic studies have demonstrated the association of common *SCARB1* variation with lipoprotein-lipid levels [5, 28-38] and subclinical atherosclerosis [39].

To our knowledge, no genetic study has exclusively investigated the association between *SCARB1* and lipid traits in native African populations to date. The objective of this study was to resequence all 13 exons and exon-intron boundaries of *SCARB1* in 95 African Blacks from Nigeria with extreme HDL-C levels for variant discovery and then to

genotype selected variants in the entire sample of 788 African Blacks, followed by genotype-phenotype association analyses with five major lipid and apolipoprotein (apo) traits (HDL-C, LDL-C, TG, apoA-I and apoB). Because our initial gene-based analysis demonstrated evidence of association with HDL-C and apoA-I, our subsequent analyses focused on these two traits.

7.3. METHODS

7.3.1. Study sample

The present study was carried out on 788 African Black subjects from Benin City, Nigeria, who were recruited as part of a population-based epidemiological study on CHD risk factors. Detailed information on the study design and population description is provided elsewhere [40]. In brief, 788 recruited subjects were healthy civil servants (37.18 % females) from three government ministries of the Edo state in Benin City, Nigeria, aged between 19 and 70 years, including 464 junior staff (nonprofessional staff with salary grades 1–6), and 324 senior staff (professional and administrative staff with salary grades 7–16). The summary features, including biometric and quantitative data of the entire sample of 788 subjects are given in **Table 7.1** and **Table B1**.

For resequencing, 95 individuals with extreme HDL-C levels (within the upper and lower 10^{th} percentiles of HDL-C distribution) were chosen from the entire sample of 788 African Blacks. Resequencing sample comprised of 48 individuals with high HDL-C

levels (≥90th percentile, range: 68.3-99.0 mg/dL) and 47 individuals with low HDL-C levels (≤10th percentile, range: 10.3-35.0 mg/dL; **Table 7.1**). The University of Pittsburgh Institutional Review Board approved the study protocol. All participants gave their informed consent.

7.3.2. Lipid measurements

At least 8-hour fasting blood samples were collected from all participants. Serum specimens were separated by centrifugation of blood samples and then stored at -70^{o}C for 6-12 months until ready for testing. Lipid and apolipoprotein measurements included total cholesterol, HDL-C, TG, apoA-I, and apoB and were done with standard assays at the Heinz Nutrition Laboratory, University of Pittsburgh under the Centers for Disease Control Lipid Standardization Program [40]. LDL-C was calculated with the Friedewald equation [41] when TG levels were less than 400 mg/dL.

7.3.3. DNA sample preparations and sequencing

Genomic DNA was isolated from clotted blood using the standard DNA extraction procedure. All 13 *SCARB1* exons (isoform 1, NM_005505), exon-intron boundaries, and 1 kb of each of 5' and 3' flanking regions on chromosome 12 (hg19, chr12: 125,262,175-125,348,519) were polymerase chain reaction (PCR) amplified and sequenced. Specific primers were designed using the Primer3 software (Whitehead Institute for Biomedical Research, Steve Rozen, Andreas Untergasser, Maido Remm, Triinu Koressaar and Helen Skaletsky, http://primer3.ut.ee/) to cover 13 target regions,

resulting in 14 amplicons, including 2 overlapping amplicons for the largest last exon 13. PCR reaction and cycling conditions are available upon request. The primer sequences and amplicon sizes are given in **Table B2**.

Automated DNA sequencing of PCR products was performed in a commercial lab (Beckman Coulter Genomics, Danvers, MA, USA) using Sanger method and ABI 3730XL DNA Analyzers (Applied Biosystems, Waltham, MA, USA). Variant analysis was performed using Variant Reporter (version 1.0, Applied Biosystems, Waltham, MA, USA) and Sequencher (version 4.8, Gene Codes Corporation, Ann Arbor, MI, USA) software in our laboratory.

7.3.4. Variant selection for genotyping

Of 83 variants identified in the discovery step (see **Tables B3**, **B4**; **Figures B1**, **B2**), 78 (28 with MAF ≥5% and 50 with MAF <5%) were selected based on the pairwise linkage disequilibrium (LD) and Tagger analysis using an r^2 threshold of 0.90 (5 were excluded due to high LD) in Haploview (Broad Institute of MIT and Harvard, http://www.broadinstitute.org/) [42] for follow-up genotyping in the entire sample (n = 788). Since our sequencing was focused primarily on coding regions, in addition we selected 69 HapMap tag single nucleotide polymorphisms [SNPs] (out of total 108 HapMap tagSNPs; see **Table B5**, **Figure B3**) based on Tagger analysis (MAF ≥5% and r^2 ≥0.80) of HapMap data (Release #27) from the Yoruba people of Ibadan, Nigeria (YRI), in order to cover the entire gene for common genetic variation information. Moreover, we selected two *SCARB1* variants previously reported to be significantly associated with lipid traits in the literature (**Table B6**). Conclusively, a total of 149

variants, comprising of 78 sequence variants, 69 common HapMap-YRI tagSNPs, and 2 relevant associated variants, were selected for follow-up genotyping.

7.3.5. Genotyping

Genotyping of selected variants in the total sample of 788 individuals was performed by using either iPLEX Gold (Sequenom, Inc., San Diego, CA, USA) or TaqMan (Applied Biosystems, Waltham, MA, USA) methods and following the manufacturers' protocols.

Out of 149 selected variants, two failed assay designs and nine failed genotyping runs (see details in **Tables B3**, **B5**, and **B6**). Quality control (QC) measures for successfully genotyped variants were as follow: a genotype call rate of ≥90%, a discrepancy rate of <1% in 10% replicates, and no deviation from Hardy-Weinberg equilibrium [HWE] (P >3.62 x 10^{-4} after Bonferroni correction). Ultimately, a total of 137 QC-passed genotyped variants were included in genetic association analyses (**Tables B6**, **B7**; **Figures B4**, **B5**).

7.3.6. Statistical analysis

We used the Haploview program to determine allele frequencies, to test HWE for genotype distribution, and to evaluate the LD and pairwise correlations (r^2) between variants [42].

The values of each lipid phenotype outside the mean ± 3.5 standard deviation (SD) were excluded from downstream gene-based, single-site, and haplotype analyses. However, the extreme phenotypic values associated with rare variants (MAF ≤1%) were

maintained during rare variant analysis, as was the case for the p70201-chr12_125279319 variant (see study workflow in **Figure 7.1**). Values of the five lipid and apolipoprotein traits—HDL-C, LDL-C, TG, apoA-I, and apoB—were transformed using the Box-Cox transformation. For each trait, we used stepwise regression method to select the most parsimonious set of covariates from the following list: sex, age, waist, body mass index, current smoking (yes/no), minutes of walking or biking to work each day (jobmin), and occupational status (staff: junior [non-professional staff]/senior [professional and administrative staff]). Genetic association analyses, including gene-based, single-site, LF/rare variant, and haplotype association tests, were performed using linear regression models that included significant covariates for each variable (**Table B8**).

The gene-based association analysis was conducted under linear additive model for the combined evaluation of common and LF/rare variants (n = 136, excluding p70201-chr12_125279319; see details above in paragraph 2 of this section) for five major lipid traits using the versatile gene-based association study ([VEGAS], http://gump.qimr.edu.au/VEGAS/) software [43]. The significance threshold for the gene-based test was set at a *P*-value of 0.05.

Following gene-based analysis, which primarily implicated *SCARB1* in regulation of HDL-C and apoA-I levels, we further elucidated the association of *SCARB1* variants with these two traits using additional tests. In single-site association analysis, *P*-values for each trait were adjusted for multiple testing using Benjamini-Hochberg procedure [44] to determine the false discovery rate [FDR] (*q*-value). For common variants (MAF ≥5%), a nominal *P*-value of <0.05 was considered to be suggestive evidence of

association, and an FDR cut-off of 0.20 was used to define statistical significance. For LF/rare variants (MAF <5%), the single-site association results were interpreted separately because of inadequate power of our study to detect individual statistical significance for these variants.

We conducted an optimal sequence kernel association test (SKAT-O) [45] to evaluate the association between a total of 43 LF/rare variants (MAF <5%) and the two lipid traits (HDL-C and apoA-I) by using three different MAF thresholds: <5% (n = 43), ≤2% (n = 26), and ≤1% (n = 23). A significant SKAT-O test was set at a *P*-value of <0.05.

Haplotype association analysis was performed using the generalized linear model. We applied a fixed sliding window approach that included four variants per window and sliding for one variant at a time. For each window, a global *P*-value was used to assess the association between the haplotypes with frequency >1% and a given trait. A global *P*-value threshold of 0.05 was used to define significant haplotype association.

All analyses, except for VEGAS, were performed using the R statistical software (http://www.r-project.org/) and relevant R packages (i.e., Haplo.Stats for haplotype analysis and SKAT for SKAT-O analysis).

7.4. RESULTS

7.4.1. Identification of *SCARB1* sequence variants

Resequencing of *SCARB1* exons and exon-intron boundaries plus flanking regions in 95 African Blacks with extreme HDL-C levels identified 83 variants, of which 51 had MAF <5% (**Table B3**; **Figure B1**). The majority of 83 variants (n = 73) were previously identified (dbSNP build 139: GRCh37.p10). Most variants (n = 80) were single-nucleotide variations [SNVs] (67 transitions and 13 transversions); the rest (n = 3) were short insertion and deletion variations (indels).

Tagger analysis using an r^2 cutoff of 0.9 identified 28 bins for 32 common variants (MAF ≥5%), of which three included more than one variant (r^2 ranging from 0.95-1.0; **Figure B2**). One of these three bins contained two variants (rs204901986 and rs34339961) in complete LD (r^2 = 1.0). Of 51 LF/rare variants (MAF between 1-5%, n = 31; MAF ≤1%, n = 20), 17 were present only in the high HDL-C group (MAF ranging between 0.010-0.042) and eight were observed only in the low HDL-C group (MAF ranging between 0.011-0.033). In the high HDL-C group, 29 of 48 (~60%) individuals cumulatively carried at least one LF/rare variant, ranging from 1 to 7 variants. Similarly, in the low HDL-C group, 27 of 47 (~57%) individuals carried at least one LF/rare variant, ranging from 1 to 9 variants.

Most variants (n = 60) from our sequencing were located in intronic regions, of which two (rs113910315, MAF = 0.005 and rs10396210, MAF = 0.138) were within splice sites (defined as ± 20 bp from the start or end of an exon). The former splice site variant was observed only in the low HDL-C group.

Of the total eight coding variants observed, four were common variants (rs2070242 [p.Ser4Ser], rs10396208 [p.Cys21Cys], and rs5888 [p.Ala350Ala], and rs701103 [p.Gly499Arg]—3' untranslated region (UTR) in isoform 1 and exon 13 in isoform 2), and the remaining four were LF/rare variants (rs4238001 [p.Gly2Ser], rs5891 [p.Val135Ile], rs5892 [p.Phe301Phe], and rs141545424 [p.Gly501Gly]). Of note, two LF/rare coding variants, (rs5891 [p.Val135Ile] and rs141545424 [p.Gly501Gly]), were found only in the high HDL-C group.

Fifteen variants were located in either UTRs (n = 5) or flanking regions (n = 10). One 3' UTR variant (rs150512235, MAF = 0.006) was very close to a predicted microRNA-145 (miR-145) target site (TargetScanHuman version 6.2, http://www.targetscan.org/). One 5' flanking variant (rs181338950, MAF = 0.048) was located in the putative promoter region [46].

All 10 novel variants (9 SNVs and 1 insertion) identified in this study have been submitted to dbSNP database: http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=KAMBOH (batch ID: SCARB1_AB) and were non-coding with MAF <5% (ranging between 0.005-0.011; **Table B4**). Of these novel variants, six and four were present only in the high and low HDL-C groups, respectively.

7.4.2. Genotyping of *SCARB1* variants

Since our sequencing was focused primarily on coding regions, we selected additional HapMap tagSNPs from the HapMap-YRI data in order to cover the entire *SCARB1* gene for common genetic variation in *SCARB1*. Altogether we selected 149 variants for genotyping in our entire African Black sample as follows: 78 variants (28 common variants and 50 LF/rare variants) discovered in the sequencing step (**Table B3**; **Figures B1** and **B2**), 69 common HapMap-YRI tagSNPs (**Table B5**), and two additional variants with reported association in the literature (**Table B6**).

Of these 149 variants, eleven [10 from sequencing, including 1 promoter (rs181338950), 1 coding (rs4238001 [p.Gly2Ser]), and 1 novel (p87459-chr12_125262061); 1 from HapMap tagSNPs (rs4765180)] failed genotyping, and one (rs866793 from HapMap tagSNPs) failed QC measures. Thus, a total of 137 variants (**Table B6**; **Figure B4**) that passed QC were advanced into association analyses with five lipid-lipoprotein traits.

The majority of 137 genotyped variants (n = 120) were located in introns, 11 were in exons, and 6 were in 3' flanking region (**Table 7.2**; **Figure B5**). Ninety-four of 137 variants had MAF ≥5%, including four coding variants, one UTR variant, two deletions, and one splice site variant. The remaining 43 variants had MAF <5% (MAF between 1-5%, n = 20; MAF ≤1%, n = 23), including three coding variants, three UTR variants, one insertion, and one splice variant.

Of the 10 novel variants discovered in the sequencing step, nine (8 SNVs and 1 insertion) with MAF <1% were successfully genotyped (**Table B4**). There was one individual with plasma HDL-C levels above the mean + 3.5 SD carrying one novel

variant—p70201-chr12_125279319 (MAF = 0.0010). Although this extreme HDL-C value was excluded as outlier from the gene-based, single-site, and haplotype analyses, it was included in the SKAT-O rare variant analysis considering a possible large effect size of this variant (**Figure 7.1**).

7.4.3. Associations of S*CARB1* variants with lipid traits

Gene-based association

Gene-based tests revealed a nominally significant association (*P* = 0.0421) of *SCARB1* variants with HDL-C levels (best SNP: rs141545424 [p.Gly501Gly], exon 12, MAF = 0.0007, *P* = 0.0016; **Table 7.3**). Additionally, a trend for association (*P* = 0.1016) was also observed for apoA-I levels (best SNP: rs7134858, intron 6, MAF = 0.1560, *P* = 0.0052).

Since the gene-based tests showed evidence of associations with HDL-C and apoA-I, we primarily focused on these two traits to further examine the *SCARB1* variants in the entire sample of 788 African Blacks.

Association of common variants

Of 94 common *SCARB1* variants with MAF ≥5%, 10 showed nominal associations (*P* <0.05) with HDL-C and/or apoA-I (**Table 7.4**; see results for each trait in **Tables B9** and **B10**), of which three (rs11057851, rs4765615, and rs838895) exhibited associations with both HDL-C and apoA-I.

The most significant association was found between rs11057851 and HDL-C (β = -0.5924, *P* = 0.0043, FDR = 0.146). The second best association was between rs7134858 and apoA-I (β = 1.7537, *P* = 0.0052, FDR = 0.292), followed by the association of rs5888 [p.Ala350Ala] with apoA-I (β = 2.0962, *P* = 0.0080, FDR = 0.292).

Of 10 variants that showed nominal associations, high LD (r^2 >0.80) was observed for two pairs of variants (**Figure 7.2**), between rs8388912 and rs5888 [p.Ala350Ala] (r^2 = 0.86), and between rs838896 and rs838895 (r^2 = 0.84).

Association of low-frequency/rare variants

The LF/rare variants (n = 43) were categorized into three groups based on their frequencies for association analysis with HDL-C and apoA-I using SKAT-O: MAF <5% (n = 43), MAF ≤2% (n = 26), and MAF ≤1% (n = 23). Although no association between LF/rare variants and apoA-I was detected, the group of 23 variants with MAF ≤1% yielded nominal association with HDL-C levels (*P* = 0.0478; **Table 7.5**).

We then individually examined the association of 23 variants with MAF ≤1% with HDL-C and apoA-I. Six of these rare variants showed association with either HDL-C levels or both HDL-C and apoA-I levels (**Table 7.6**). While three of them are known variants (rs115604379, rs377124254, and rs141545424 [p.Gly501Gly]), the other three are novel (p52919-chr12_125296601, p54611-chr12_125294909, and p54856-chr12_125294664). Moreover, four of these six rare variants (rs377124254, rs141545424 [p.Gly501Gly], p54611-chr12_125294909, and p54856-chr12_125294664) were present in individuals with extreme phenotypic values (above or below the 3rd percentile). Two of these variants (rs377124254: β = 11.5518, *P* = 0.0016; rs141545424 [p.Gly501Gly]: β = 11.585, *P* = 0.0016) were found in a single

subject who had very high HDL-C level. Whereas the other two were observed in one subject who had very high HDL-C level. Whereas the other two were observed in one individual each, who had extremely low HDL-C levels (p54611-chr12_125294909: β = -9.5243, *P* = 0.0097; p54856-chr12_125294664: β = -8.4305, *P* = 0.0215) and apoA-I levels (p54611-chr12_125294909: β = -19.3821, *P* = 0.0344; p54856-chr12_125294664: β = -24.0757, *P* = 0.0082). This rare variant group also included a novel variant (p70201-chr12_125279319) that was observed in one individual with an unusually high plasma HDL-C level (above the mean + 3.5 SD).

Association of haplotypes

The 4-SNP sliding window haplotype analyses revealed associations of 32 haplotype windows with HDL-C and/or apoA-I (global *P* <0.05; **Table 7.7**; see results for each trait in **Table B11**), of which five (windows #47, #72, #111, #112, and #123) were associated with both.

Overall, a total of 21 haplotype windows showed significant associations with apoA-I, of which 10 contained seven variants associated with apoA-I in single-site analysis. Haplotype window #110 spanning introns 10-11 showed the best association signal (global *P* = 0.0012) and contained the rs838896 variant with a nominal evidence of association with apoA-I (*P* = 0.0278) in single-site analysis.

A total of 16 haplotype windows yielded significant associations with HDL-C, of which seven contained three HDL-C-associated variants detected in single-site analysis. The most significant association was found with window #111 (global *P* = 0.0040) spanning intron 11, which contained the rs838895 variant nominally associated with HDL-C (*P* = 0.0162) in single-site analysis.

We observed nine regions (5 regions for apoA-I and 4 regions for HDL-C) harboring consecutive significant haplotype windows (global *P* <0.05, ranging from 2 to 6 windows per region; **Table 7.8**; **Figure 7.3**). Seven of those regions contained at least one of the six variants that exhibited nominal associations (*P* <0.05) with HDL-C and/or apoA-I (rs4765615, rs7134858, rs838912, rs838896, rs838895, and rs701106) in single-site analysis.

7.4.4. Functional prediction of identified *SCARB1* variants

In order to examine the possible regulatory function of all 153 *SCARB1* variants (83 variants identified by our sequencing, 68 common HapMap tagSNPs [excluding rs4765180 due to genotyping failure; see **Table B5**], and two relevant variants from the literature), we used the RegulomeDB database (version 1.0, Stanford University, http://www.regulomedb.org/) [47]. Although most of 153 variants (n = 132) revealed scores ranging from 1 to 6, only 11 were supported by strong evidence for regulatory function (scores of 1f -2b): one promoter, one 5' UTR, two coding (rs2070242 [p.Ser4Ser] and rs10396208 [p.Cys21Cys]), five intronic, one 3' UTR, and one 3' flanking variants. Summary and detailed regulatory functions are provided in **Tables B12** and **B13**.

Of 10 variants associated with HDL-C and/or apoA-I, only one apoA-I associated variant (rs5888 [p.Ala350Ala] in exon 8) showed suggestive evidence of regulatory function with a score of 3a (**Table 7.4**).

Of 10 novel variants, one insertion variant (p1048insC-chr12_125348472) located in 5' UTR-exon 1 had a strong potential for regulatory function with a score of 2a (**Table B4**).

7.4.5. Comparison of *SCARB1* single-site and haplotype association analysis results between African Blacks (this study) and US Non-Hispanic Whites (previous study [48])

We compared *SCARB1* single-site and haplotype association results in African Blacks reported in this study to those in US Non-Hispanic Whites (NHWs) reported in our previously published study [48]. In the sequencing stage, the number of variants identified in African Blacks (n = 83) was greater than that in US NHWs (n = 44). Notably, most (~90%) of the 22 sequence variants that were shared between the two populations differed in minor alleles and/or MAFs. Although our major findings included the associations with HDL-C and apoA-I in African Blacks, we also sought to replicate four associations observed with apoB levels in US NHWs [48] (**Table 7.9**); the association between rs11057820 and apoB (P <0.05) that we previously reported in US NHWs [48] was also observed in African Blacks (US NHWs [G allele]: β = 0.8700, P = 0.0436; African Blacks [A allele]: β = 1.8661, P = 0.0292). In addition, we observed two variants (rs4765615 and rs701106) exhibiting nominal associations (P <0.05) in both populations, albeit with different lipid traits (US NHWs| rs4765615 [G allele]: β = 1.2493, P = 0.0059 for apoB; rs701106 [T allele]: β = 0.0394, P = 0.0066 for HDL-C; African Blacks| rs4765615 [A allele]: β = -0.4646, P = 0.013 for HDL-C and β = -0.9139, P = 0.048 for apoA-I; rs701106 [T allele]: β = 1.2967, P = 0.0156 for apoA-I). Moreover, we

noticed that two regions associated with HDL-C or apoA-I (global *P* <0.05; **Table 7.10**) in African Blacks spanning intron 2 and intron 3 overlapped with the apoB-associated region (Region I in **Figure 7.4**) previously reported in US NHWs [48]. Three haplotype regions associated with HDL-C (global *P* <0.05) spanning intron 11 and exon 13-3' UTR in African Blacks also overlapped with a large HDL-C-associated region (Region II in **Figure 7.4**) previously reported in US NHWs [48].

7.5. DISCUSSION

Our sequencing identified 83 variants, of which 78 were selected for follow-up genotyping in the total sample of 788 African Blacks. Additional 69 tagSNPs from the HapMap-YRI data along with two previously reported lipid-associated *SCARB1* variants were also genotyped in the total sample. Of 149 genotyped *SCARB1* variants, 137 that passed QC were examined for association with major lipid traits (**Table 7.2**). The initial gene-based analyses revealed a nominal association with HDL-C (*P* = 0.0421) as well as a trend for association with apoA-I (*P* = 0.1016; **Table 7.3**). Consistent with the gene-based results, single-site association analyses also revealed 10 common variants nominally associated (*P* <0.05) with HDL-C (n = 5) and/or apoA-I (n = 8; **Table 7.4**; **Figure 7.2**). The best association signal was between rs11057851 in intron 1 and HDL-C (*P* = 0.0043, FDR = 0.146) followed by two associations with apoA-I including rs7134858 in intron 6 (*P* = 0.0052, FDR = 0.292) and rs5888 [p.Ala350Ala] in exon 8 (*P*

= 0.0080, FDR = 0.292). Moreover, three variants (rs11057851, rs4765615, and rs838895) exhibited evidence of associations (P <0.05) with both HDL-C and apoA-I. These findings are supported by the fact that *SCARB1* appears to influence apoA-I in addition to HDL-C [15, 17]. In our data, there was a moderate correlation between apoA-I and HDL-C levels (r^2 = 0.61).

Except for previously reported association of rs5888 [p.Ala350Ala] with lipid traits (HDL-C or LDL-C) in non-African populations [30-38, 49], the remaining nine associations observed in this study with the lipid traits (HDL-C and/or apoA-I levels) in general population are novel and await replication in independent African or African-derived populations. Two of these nine SNPs have previously been shown to have differential effects on cholesterol levels in response to statin (rs4765615) [50] or on HDL-C and TG levels in response to estradiol in post-menopausal women (rs838895) [51]. Another variant (rs838896) was found to be associated with decreased *SCARB1* expression in liver [51]. Although the latter SNP was not associated with a low RegulomeDB score (<3), we cannot rule out the possibility that it might be affecting the *SCARB1* expression in a tissue-specific manner.

The haplotype analysis revealed evidence of significant association (global P <0.05) of 32 haplotype windows with HDL-C (n = 16) and/or apoA-I (n = 21; **Table 7.7**) and nine regions harboring consecutive overlapping haplotype windows significantly associated with either HDL-C (4 regions) or apoA-I (5 regions; **Table 7.8**; **Figure 7.3**). In addition, six variants with nominal association (P <0.05) in single-site analysis were contained in seven of these nine significantly associated regions, indicating the

presence of functional variants in these regions. Our findings demonstrate that haplotype analysis may provide more information than single-site analysis.

Our comparison of the single-site and haplotype association results between in African Blacks (this study) and US NHWs (previous study [48]) has revealed three variants (rs11057820, rs4765615 and rs701106; **Table 7.9**) and two regions (Regions I and II; **Table 7.10**; **Figure 7.4**) showing evidence of lipid-associations in both ethnic groups. However, there were differences in associated traits, and/or associated alleles or their directional effects between the two ethnic groups, which reflects the genetic heterogeneity of complex phenotypes like lipid traits among diverse populations. This phenomenon can be explained by different ancestry backgrounds associated with differences in LD structure and genetic architecture, as well as by differences in SNP-SNP, gene-gene, and gene-environment interactions. Nonetheless, the lipid associations observed across different ethnic populations provide convincing evidence that causal/functional variants are present in *SCARB1* gene that deserves comprehensive sequencing and functional studies in order to confirm and further characterize the effects of its variants on lipid metabolism.

Rare variant analysis showed significant evidence of association between a group of 23 rare variants (MAF ≤1%) and HDL-C (P = 0.0478; **Table 7.5**). Single-site analysis of these rare variants revealed six (including three novel ones) with effects on HDL-C, of which three also had effects on apoA-I (**Table 7.6**). In addition, four of these six rare variants appeared to be carried by individuals with extreme HDL-C and/or apoA-I levels (above or under the 3rd percentile). This HDL-C-associated rare variant group also included a novel variant (p70201-chr12_125279319) that was observed in

one individual with an unusually high plasma HDL-C level (above the mean + 3.5 SD). Our findings suggest that these rare variants might have functional relevance, thus screening of additional large African samples for these rare variants may help to establish their role in HDL-C and apoA-I metabolism.

To date, there has been limited information concerning possible functional effects of lipid-associated *SCARB1* variants, particularly for those located in non-coding regions. In fact, most of common and rare HDL-C/apoA-I-associated variants observed in the current study are non-coding and do not show strong evidence of regulatory function based on RegulomeDB database. Nonetheless, three of these HDL-C/ApoA-I-associated *SCARB1* variants (rs5888 [p.Ala350Ala], rs838885, and rs838886) have been previously demonstrated to influence the *SCARB1* expression [51-53]. Therefore, additional functional studies are needed and may help to determine the functional nature of the *SCARB1*-associated variants and those in LD with them.

Our study has revealed a number of novel findings, although we also acknowledge some limitations. *SCARB1* is a large gene and we sequenced only its coding regions and exon-intron junctions and also our sequencing sample size was small. Thus, we may have missed some functional LF/rare variants due to small sample size and those located in uncovered intronic regions. Moreover, consistent with generally small effect sizes of lipid-associated variants reported in the literature, most of our single-site associations reached nominal significance (P <0.05) but did not survive multiple testing corrections. Only the top variant (rs11057851) associated with HDL-C yielded an FDR cut-off of <0.20 (FDR = 0.1465). Therefore, future larger studies in

independent African or African-derived populations are necessary to validate all nominal associations observed in this study.

7.6. CONCLUSIONS

In conclusion, we report the first comprehensive association study of *SCARB1* variants with lipid traits in a native African population, which revealed a number of novel associations in single-site and haplotype analyses. In addition, resequencing allowed us to identify 10 novel rare variants, of which four were in the group of 23 rare variants that has showed association with HDL-C levels. The SCARB1 associated common and rare variants observed in our study explained ~11.09% of the variation in HDL-C levels and ~8.63% of the variation in apoA-I levels. Our findings indicate the genetic contribution of SCARB1, both common and LF/rare variants, to inter-individual lipid variation in the general African Black population, which warrants further follow-up in independent studies. Insights into the HDL-C and related lipid traits may also lead to new potential targets for CHD treatment.

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Disclosures

None.

Table 7.1. Characteristics and lipid profile of 95 individuals with extreme[a] HDL-C levels and of the entire sample of 788 African Blacks.

Variables	95 Individuals with Extreme[a] HDL-C Levels			The Entire Sample[b]
	High HDL-C Group (HDL-C range[c]: 68.30-99.00 mg/dL)	Low HDL-C Group (HDL-C range[c]: 10.30-35.00 mg/dL)	*P*[d]	
N (Females, n)	48 (24)	47 (24)	1.00	788 (293)
Age, years	41.29 ± 8.72	40.87 ± 7.12	0.80	40.95 ± 8.39
BMI, kg/m^2	22.06 ± 4.70	23.91 ± 5.51	0.08	22.87 ± 4.04
Total Cholesterol, mg/dL	201.00 ± 39.68	141.68 ± 31.03	2.40E-12	172.01 ± 38.47
LDL-Cholesterol, mg/dL	112.55 ± 39.75	95.04 ± 28.28	0.02	109.25 ± 34.40
HDL-Cholesterol, mg/dL	76.05 ± 7.53	25.51 ± 5.66	2.20E-16	47.88 ± 12.87
Triglycerides, mg/dL	61.98 ± 19.85	95.79 ± 73.21	0.004	72.96 ± 39.32
Apolipoprotein A-I, mg/dL	166.04 ± 28.19	103.84 ± 27.23	2.20E-16	137.03 ± 28.46
Apolipoprotein B, mg/dL	66.00 ± 20.22	69.64 ± 21.46	0.40	66.98 ± 22.19

BMI, body mass index; HDL-C/HDL-Cholesterol, high-density lipoprotein cholesterol; LDL-Cholesterol, low-density lipoprotein cholesterol. Values are presented as unadjusted means ± standard deviation (SD) unless otherwise mentioned.

[a] Distribution of HDL-C was adjusted for sex and age: HDL-C levels ≥90th %tile defined as the "High HDL-C group", and HDL-C levels ≤10th %tile defined as the "Low HDL-C group".

[b] All data were unadjusted and included individuals with missing values or outliers (values beyond mean ± 3.5 SD).

[c] Unadjusted range values.

[d] Unadjusted *P*-values were calculated with t-test or χ^2 test depending on types of variables.

Table 7.2. Distribution of 137 *SCARB1* genotyped variants.

	Total N (%)	MAF ≥5% n (%)	MAF between 1-5% n (%)	MAF ≤1% n (%)
Total Variants	**137 (100.00)**	**94 (68.61)**	**20 (14.60)**	**23 (16.79)**
By Known/Novel[a]				
Known	**128 (93.43)**	**94 (68.61)**	**20 (14.60)**	**14 (10.22)**
- Single-nucleotide variation	126	92	20	14
- Short indels	2	2		
Novel[a]	**9 (6.57)**			**9 (6.57)**
- Single-nucleotide variation	8			8
- Short indels	1			1
By Locations				
Exons-coding[c]	7	4[c]	1	2
Exons-UTRs	4	1	1	2
Introns	118	85	16	17
Introns-splice sites[b]	2	1		1
3' flanking	6	3	2	1
By Amino Acid Changes				
Non-synonymous[c]	2	1[c]		1
Synonymous	5	3	1	1

Indels, insertion and deletion variations; MAF, minor allele frequency; UTR, untranslated region.
[a] dbSNP build 139: GRCh37.p10; 10 novel variants were submitted to dbSNP: http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=KAMBOH (batch ID: SCARB1_AB).
[b] Splice site is defined as ± 20 bp from the start or end of an exon.
[c] Including rs701103 (p.Gly499Arg; MAF = 0.2451) that is located in exon 13-3' UTR and translated only in isoform 2.

Table 7.3. Result of gene-based association analysis.

Trait	No of Variants	Test Statistics	*P*	Best SNP		
				SNP Name[a]-RefSNP ID[b]	MAF	*P*
HDL-C	136	207.5483	**0.0421**	p82264-rs141545424	0.0007	0.0016
LDL-C	136	134.1860	0.4640	p32777-rs11057841	0.2805	0.0047
TG	136	118.1598	0.6700	p86316-rs701104	0.0487	0.0357
ApoA-I	136	183.5565	0.1016	p55963-rs7134858	0.1560	0.0052
ApoB	136	143.7284	0.3760	p22116-rs12370382	0.0645	0.0153

ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MAF, minor allele frequency; SNP, single nucleotide polymorphism; TG, triglycerides.
Significant gene-based *P*-values (*P* <0.05) are shown in **bold**.
[a] RefSeq *SCARB1*: hg19, NM_005505 (CHIP Bioinformatics).
[b] dbSNP build 139: GRCh37.p10.

Table 7.4. Significant single-site associations (*P* <0.05) of *SCARB1* common variants (MAF ≥5%).

SNP Name[a]	RefSNP ID[b]	Chr12 Position[c]	Location	Amino Acid Change	RegDB Score[d]	Major/Minor Alleles	MAF	β	SE	R^2 (%)	*P*	FDR	Secondary Trait (Effect)	Top 3 Variants
HDL-C														
p20207	rs11057853	125329313	Intron 1		5	G/A	0.4484	0.4082	0.1925	1.0650	0.0343	0.424		
p20741	rs11057851	125328779	Intron 1		5	C/T	0.3237	-0.5924	0.2067	1.3010	**0.0043**	**0.146**	ApoA-I (↓)	Top 1
p45516	rs1902569	125304004	Intron 1		5	G/A	0.1544	0.5447	0.2629	0.6390	0.0386	0.438		
p49690	rs4765615	125299830	Intron 2		5	G/A	0.4426	-0.4646	0.1866	0.9330	0.0130	0.253	ApoA-I (↓)	
p79828	rs838895	125269692	Intron 11		5	C/G	0.3171	0.4961	0.2059	0.8220	0.0162	0.276	ApoA-I (↑)	
ApoA-I														
p20741	rs11057851	125328779	Intron 1		5	C/T	0.3237	-1.2331	0.5117	0.8600	0.0162	0.319	HDL-C (↓)	
p49690	rs4765615	125299830	Intron 2		5	G/A	0.4426	-0.9139	0.4614	0.6770	0.0480	0.502	HDL-C (↓)	
p55963	rs7134858	125293557	Intron 6		6	C/T	0.1560	1.7537	0.6260	1.0710	**0.0052**	0.292		Top 2
p63483	rs838912	125286037	Intron 7		7	G/A	0.0867	1.8700	0.8230	0.6880	0.0234	0.397		
p64772	rs5888	125284748	Exon 8	Ala350Ala	3a	C/T	0.0961	2.0962	0.7888	0.9460	0.0080	0.292		Top 3
p79721	rs838896	125269799	Intron 11		5	G/C	0.3104	1.1147	0.5056	0.7270	0.0278	0.420		
p79828	rs838895	125269692	Intron 11		5	C/G	0.3171	1.2206	0.5074	0.7800	0.0164	0.319	HDL-C (↑)	
p83884	rs701106	125265636	Intron 12		5	C/T	0.2597	1.2967	0.5352	0.7770	0.0156	0.319		

ApoA-I, apolipoprotein A-I; FDR, false discovery rate; HDL-C, high-density lipoprotein cholesterol; MAF, minor allele frequency; RegDB; RegulomeDB; SE, standard error; SNP, single nucleotide polymorphism; UTR, untranslated region; R^2, a proportion of the phenotypic variance explained by the variant; ↓, decreased; ↑, increased.
All alleles on reverse strand.
The most significant *P*-value for each trait is shown in **bold**, see the single-site association ($-\log_{10} P$) plot and pairwise correlations (r^2) in Figure 7.2**.**
FDR values that passed a threshold of 0.20 are shown is shown in **bold**.
[a, c] RefSeq *SCARB1*: hg19, NM_005505 (CHIP Bioinformatics).
[b] dbSNP build 139: GRCh37.p10.
[d] Detailed RegulomeDB (version 1.0) scoring scheme and functional assignments are described in the footnote of Tables B12 (or can be seen at http://regulome.stanford.edu/help) and B13, respectively.

Table 7.5. Result of SKAT-O analysis of *SCARB1* low-frequency/rare variants (MAF <5%).

MAF	No of LF/rare Variants	No of Samples with/without LF/rare Variants	HDL-C		ApoA-I	
			Stat	*P*	Stat	*P*
≤0.01	23[a]	93/694	126653.82	**0.0478**	60151.10	0.3707
≤0.02	26	134/653	123009.08	0.1324	48439.67	0.5166
<0.05	43	442/346	135697.20	0.0737	298813.05	0.1517

ApoA-I, apolipoprotein A-I; HDL-C, high-density lipoprotein cholesterol; MAF, minor allele frequency; SKAT-O, an optimal sequence kernel association test; SNP, single nucleotide polymorphism.
Significant *P*-values (*P* <0.05) are shown in **bold**.
[a] Including p70201-chr12_125279319 that was observed in one individual with an outlier value (above the mean + 3.5 standard deviation). See details in Section 7.3.6 and Figure 7.1.

Table 7.6. Characteristics and effects of 6 *SCARB1* rare variants (MAF ≤1%) of interest.

SNP Name[a]	RefSNP ID[b]	Chr12 Position[c]	Location	Amino Acid Change	RegDB Score[d]	Major/ Minor Alleles	MAF	GT	GT Count (Carrier Freq)	Adjusted Mean ± SD (mg/dL)	β	SE	R^2 (%)	*P*	FDR	Second Assoc Trait (Effect)
HDL-C																
p52919		125296601	Intron 4		5	G/T	0.0013	GG	734	47.87 ± 12.71	-7.4063	2.5863	1.1050	0.0043	0.146	ApoA-I (↓)
								GT	2 (0.27)	24.67 ± 9.26						
p53372	rs115604379	125296148	Intron 5		5	C/T	0.0066	CC	729	47.68 ± 12.64	3.0372	1.1642	0.9140	0.0093	0.219	
								CT	10 (1.35)	58.2 ± 13.03						
p54611		125294909	Intron 5		4	T/C	0.0007	TT	742	47.86 ± 12.68	-9.5243	3.6710	0.8920	0.0097	0.219	ApoA-I (↓)
								TC	1 (0.13)	19.59 ± NA						
p54856		125294664	Intron 6		4	C/T	0.0007	CC	742	47.85 ± 12.70	-8.4305	3.6579	0.7130	0.0215	0.324	ApoA-I (↓)
								CT	1 (0.13)	21.48 ± NA						
p77620	rs377124254	125271900	Intron 10		5	G/A	0.0007	GG	735	47.77 ± 12.67	11.5518	3.6514	1.3500	0.0016	0.1104	0.110
								GA	1 (0.14)	90.2 ± NA						
p82264	rs141545424	125267256	Exon 12	Gly501Gly	5	C/A	0.0007	CC	739	47.77 ± 12.66	11.5850	3.6469	1.3530	0.0016	0.110	
								CA	1 (0.14)	90.31 ± NA						
ApoA-I																
p52919		125296601	Intron 4		5	G/T	0.0013	GG	741	136.81 ± 27.74	-13.4137	6.4689	0.5750	0.0385	0.436	HDL-C (↓)
								GT	2 (0.27)	97.42 ± 18.38						
p54611		125294909	Intron 5		4	T/C	0.0007	TT	748	136.83 ± 27.66	-19.2831	9.0970	0.5980	0.0344	0.436	HDL-C (↓)
								TC	1 (0.13)	80.62 ± NA						
p54856		125294664	Intron 6		4	C/T	0.0007	CC	748	136.87 ± 27.61	-24.0757	9.0781	0.9330	0.0082	0.292	HDL-C (↓)
								CT	1 (0.13)	67.98 ± NA						

ApoA-I, apolipoprotein A-I; FDR; false discovery rate; GT, genotype; HDL-C, high-density lipoprotein cholesterol; MAF; minor allele frequency; RegDB, RegulomeDB; SD, standard deviation; SE, standard error; SNP, single nucleotide polymorphism; R^2, a proportion of the phenotypic variance explained by the variant; ↓, decreased.
All alleles on reverse stand.
Detailed single-site association results are shown in Tables B9 and B10.
[a, c] RefSeq *SCARB1*: hg19, NM_005505 (CHIP Bioinformatics).
[b] dbSNP build 139: GRCh37.p10; 10 novel variants were submitted to dbSNP: http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=KAMBOH (batch ID: SCARB1_AB).
[d] Detailed RegulomeDB (version 1.0) scoring scheme and functional assignments are described in the footnote of Tables B12 (or can be seen at http://regulome.stanford.edu/help) and B13, respectively.

Table 7.7. Significantly associated haplotype windows (global *P* <0.05) of 136 *SCARB1* genotyped variants.

wind #	SNP 1-SNP 4 (SNP Name[a]-RefSNP ID[b] / Chr12 Position[c])	Chr12 Position[c]	Location	Amino Acid Change	Major/ Minor Alleles	MAF	β	Single -site *P*	hap #	Haplotype	Hap Freq	Coef	SE	t.stat	Hap *P*	Global *P*
HDL-C																
39	p41632-rs6488943	125307888	Intron 1		A/C	0.2954	-0.2195	0.3244	**h39.1**	CCGG	0.0315	0.4305	0.6471	0.6654	0.5060	0.0207
39	p42467-rs11057830	125307053	Intron 1		C/T	0.1523	-0.2810	0.3015	**h39.2**	CCGA	0.2508	-0.5918	0.2725	-2.1713	0.0302	
39	p45516-rs1902569	125304004	Intron 1		G/A	0.1544	0.5447	**0.0386**	**h39.3**	ATGA	0.1414	-0.6841	0.3192	-2.1433	0.0324	
39	p45627-rs12297372	125303893	Intron 1		A/G	0.0487	-0.0483	0.9156	**h39.4**	ACAA	0.1514	0.1991	0.2963	0.6720	0.5018	
									h39.5	ACGG	0.0155	-1.7144	0.9080	-1.8880	0.0594	
									h39.6 (rare)	****	0.0148	2.5239	1.0902	2.3151	0.0209	
									hap base39	ACGA	0.3946	NA	NA	NA	NA	
44	p48969-rs2343394	125300551	Intron 2		C/T	0.1898	0.3165	0.1788	**h44.1**	TCWG	0.1855	0.5292	0.2523	2.0977	0.0363	0.0271
44	p49537-rs7305310	125299983	Intron 2		C/T	0.1007	-0.3396	0.2566	**h44.2**	CCDG	0.2244	0.4676	0.2429	1.9249	0.0546	
44	p49570delC-rs145376237	125299950	Intron 2		W/D	0.2276	0.3121	0.1773	**h44.3**	CCWG	0.0446	1.0491	0.4882	2.1489	0.0320	
44	p49690-rs4765615	125299830	Intron 2		G/A	0.4426	-0.4646	**0.0130**	**h44.4**	CTWG	0.1018	-0.1197	0.3121	-0.3835	0.7015	
									h44.5 (rare)	****	0.0089	-0.9887	1.0998	-0.8990	0.3689	
									hap base44	CCWA	0.4348	NA	NA	NA	NA	
45	p49537-rs7305310	125299983	Intron 2		C/T	0.1007	-0.3396	0.2566	**h45.1**	CDGC	0.2282	0.4661	0.2393	1.9473	0.0519	0.0155
45	p49570delC-rs145376237	125299950	Intron 2		W/D	0.2276	0.3121	0.1773	**h45.2**	CWGC	0.2302	0.6926	0.2376	2.9146	0.0037	
45	p49690-rs4765615	125299830	Intron 2		G/A	0.4426	-0.4646	**0.0130**	**h45.3**	TWGC	0.1020	-0.0653	0.3085	-0.2115	0.8325	
45	p49759-rs146272788	125299761	Intron 2		C/T	0.0020	2.5988	0.2219	**h45.4 (rare)**	****	0.0030	2.0667	2.0848	0.9913	0.3219	
									hap base45	CWAC	0.4366	NA	NA	NA	NA	
46	p49570delC-rs145376237	125299950	Intron 2		W/D	0.2276	0.3121	0.1773	**h46.1**	DGCG	0.2228	0.4373	0.2413	1.8123	0.0703	0.0278
46	p49690-rs4765615	125299830	Intron 2		G/A	0.4426	-0.4646	**0.0130**	**h46.2**	WGCG	0.3311	0.4910	0.2105	2.3326	0.0199	
46	p49759-rs146272788	125299761	Intron 2		C/T	0.0020	2.5988	0.2219	**h46.3 (rare)**	****	0.0080	1.9089	1.0569	1.8061	0.0713	
46	p49978-rs5891	125299542	Exon 3	Val135Ile	G/A	0.0058	1.3374	0.2791	**hap base46**	WACG	0.4381	NA	NA	NA	NA	
47	p49690-rs4765615	125299830	Intron 2		G/A	0.4426	-0.4646	**0.0130**	**h47.1**	ACGG	0.4346	-0.4701	0.1824	-2.5777	0.0101	0.0079
47	p49759-rs146272788	125299761	Intron 2		C/T	0.0020	2.5988	0.2219	**h47.2 (rare)**	****	0.0101	1.4683	0.9441	1.5552	0.1203	
47	p49978-rs5891	125299542	Exon 3	Val135Ile	G/A	0.0058	1.3374	0.2791	**hap base47**	GCGG	0.5553	NA	NA	NA	NA	
47	p50024-rs368880622	125299496	Intron 3		G/T	0.0026	1.6506	0.4362								
63	p53359-rs112371713	125296161	Intron 5		G/A	0.1243	0.4193	0.1651	**h63.1**	ACGA	0.1237	0.3273	0.3011	1.0871	0.2773	0.0394
63	p53372-rs115604379	125296148	Intron 5		C/T	0.0066	3.0372	0.0093	**h63.2**	GCGG	0.0427	-0.1630	0.4738	-0.3441	0.7309	
63	p53790-rs4765614	125295730	Intron 5		G/A	0.2653	-0.3281	0.1218	**h63.3**	GCAA	0.2678	-0.2408	0.2194	-1.0975	0.2728	
63	p54445-rs60910935	125295075	Intron 5		A/G	0.0418	-0.1247	0.7963	**h63.4 (rare)**	****	0.0068	2.9428	1.2559	2.3432	0.0194	
									hap base63	GCGA	0.5591	NA	NA	NA	NA	

Table 7.7. (continued)

72	p55923-rs838900	125293597	Intron 6		G/A	0.3921	0.2787	0.1549	**h72.1**	ACAG	0.2725	0.4039	0.2520	1.6024	0.1095	0.0315
72	p55963-rs7134858	125293557	Intron 6		C/T	0.1560	0.4418	0.0799	**h72.2**	ACGG	0.1086	-0.1763	0.3929	-0.4486	0.6538	
72	p56845-rs838902	125292675	Intron 6		A/G	0.4249	-0.0786	0.6801	**h72.3**	GTAG	0.1284	0.3877	0.3170	1.2228	0.2218	
72	p57004-rs187562853	125292516	Intron 6		G/A	0.0098	1.6474	0.0872	**h72.4**	GTGG	0.0297	0.8722	0.6546	1.3323	0.1832	
									h72.5	GCAG	0.1716	-0.4913	0.3344	-1.4690	0.1422	
									h72.6 (rare)	****	0.0101	1.7731	0.9506	1.8653	0.0625	
									hap base72	GCGG	0.2791	NA	NA	NA	NA	
111	p78747-rs2293440	125270773	Intron 11		T/C	0.4112	-0.1684	0.3806	**h111.1**	CCCG	0.0306	0.7458	0.5599	1.3321	0.1832	0.0040
111	p78791-rs75289200	125270729	Intron 11		T/C	0.0321	0.7037	0.2078	**h111.2**	CTGC	0.1534	-0.5556	0.2830	-1.9629	0.0500	
111	p79721-rs838896	125269799	Intron 11		G/C	0.3104	0.3565	0.0817	**h111.3**	CTCG	0.2269	0.1234	0.2391	0.5162	0.6058	
111	p79828-rs838895	125269692	Intron 11		C/G	0.3171	0.4961	**0.0162**	**h111.4**	TTGG	0.0180	2.3022	0.7617	3.0225	0.0026	
									h111.5	TTCG	0.0439	0.5755	0.5317	1.0823	0.2795	
									h111.6	TTCC	0.0145	0.9606	0.8068	1.1907	0.2342	
									h111.7 (rare)	****	0.0033	0.7755	2.1917	0.3538	0.7236	
									hap base111	TTGC	0.5094	NA	NA	NA	NA	
112	p78791-rs75289200	125270729	Intron 11		T/C	0.0321	0.7037	0.2078	**h112.1**	CCGA	0.0311	0.7440	0.5559	1.3384	0.1812	0.0055
112	p79721-rs838896	125269799	Intron 11		G/C	0.3104	0.3565	0.0817	**h112.2**	TGGA	0.0171	2.3734	0.7506	3.1621	0.0016	
112	p79828-rs838895	125269692	Intron 11		C/G	0.3171	0.4961	**0.0162**	**h112.3**	TGCA	0.0112	-1.2672	0.9074	-1.3964	0.1630	
112	p80045-rs838893	125269475	Intron 11		G/A	0.3244	0.3127	0.1224	**h112.4**	TCGA	0.2704	0.2488	0.2164	1.1501	0.2505	
									h112.5	TCCG	0.0139	1.1219	0.8186	1.3704	0.1710	
									h112.6 (rare)	****	0.0068	1.6244	1.2691	1.2800	0.2009	
									hap base112	TGCG	0.6493	NA	NA	NA	NA	
113	p79721-rs838896	125269799	Intron 11		G/C	0.3104	0.3565	0.0817	**h113.1**	GGAG	0.0171	2.3949	0.7509	3.1895	0.0015	0.0048
113	p79828-rs838895	125269692	Intron 11		C/G	0.3171	0.4961	**0.0162**	**h113.2**	GCAG	0.0120	-1.1963	0.8784	-1.3619	0.1736	
113	p80045-rs838893	125269475	Intron 11		G/A	0.3244	0.3127	0.1224	**h113.3**	CGAG	0.2996	0.3071	0.2067	1.4861	0.1377	
113	p81863-rs185445624	125267657	Intron 11		G/A	0.0020	-0.9612	0.6510	**h113.4**	CCGG	0.0139	1.1509	0.8168	1.4090	0.1592	
									h113.5 (rare)	****	0.0081	1.1622	1.0896	1.0666	0.2865	
									hap base113	GCGG	0.6493	NA	NA	NA	NA	
114	p79828-rs838895	125269692	Intron 11		C/G	0.3171	0.4961	**0.0162**	**h114.1**	GAGC	0.3173	0.3755	0.2023	1.8559	0.0639	0.0447
114	p80045-rs838893	125269475	Intron 11		G/A	0.3244	0.3127	0.1224	**h114.2**	CGGT	0.0306	-0.8840	0.5344	-1.6541	0.0985	
114	p81863-rs185445624	125267657	Intron 11		G/A	0.0020	-0.9612	0.6510	**h114.3**	CAGC	0.0111	-1.2612	0.9170	-1.3754	0.1694	
114	p82019-rs838890	125267501	Intron 11		C/T	0.0320	-1.0051	0.0618	**h114.4 (rare)**	****	0.0086	0.9073	1.0936	0.8296	0.4070	
									hap base114	CGGC	0.6325	NA	NA	NA	NA	
117	p82019-rs838890	125267501	Intron 11		C/T	0.0320	-1.0051	0.0618	**h117.1**	CCAG	0.0238	-1.0596	0.6275	-1.6884	0.0917	0.0433
117	p82264-rs141545424	125267256	Exon 12	Gly501Gly	C/A	0.0007	11.5850	0.0016	**h117.2**	TCGG	0.0311	-0.9657	0.5302	-1.8215	0.0689	
117	p82340-rs77483223	125267180	Intron 12		G/A	0.0231	-1.0458	0.1012	**h117.3 (rare)**	****	0.0067	1.6191	1.2946	1.2507	0.2114	
117	p82369-rs75446635	125267151	Intron 12		G/A	0.0059	0.5896	0.6322	**hap base117**	CCGG	0.9383	NA	NA	NA	NA	

Table 7.7. (continued)

118	p82264-rs141545424	125267256	Exon 12	Gly501Gly	C/A	0.0007	11.5850	0.0016	**h118.1**	CAGT	0.0238	-1.0621	0.6274	-1.6929	0.0909	0.0375
118	p82340-rs77483223	125267180	Intron 12		G/A	0.0231	-1.0458	0.1012	**h118.2**	CGGC	0.0307	-1.0134	0.5313	-1.9073	0.0569	
118	p82369-rs75446635	125267151	Intron 12		G/A	0.0059	0.5896	0.6322	**h118.3 (rare)**	****	0.0067	1.6189	1.2762	1.2685	0.2050	
118	p82434-rs838889	125267086	Intron 12		T/C	0.0315	-1.0389	0.0526	**hap base118**	CGGT	0.9387	NA	NA	NA	NA	
123	p83884-rs701106	125265636	Intron 12		C/T	0.2597	0.2471	0.2601	**h123.1**	TCCT	0.0256	-1.2114	0.6218	-1.9483	0.0518	0.0386
123	p86245-rs188375019	125263275	Intron 12		C/T	0.0341	0.7447	0.1639	**h123.2**	TCCG	0.2327	0.5306	0.2403	2.2085	0.0275	
123	p86276-rs747155	125263244	Intron 12		C/T	0.1495	0.2793	0.2980	**h123.3**	CCTG	0.1476	0.3955	0.2811	1.4071	0.1598	
123	p86316-rs701104	125263204	Intron 12		G/T	0.0487	-0.9838	0.0286	**h123.4**	CCCT	0.0233	-0.2329	0.7038	-0.3309	0.7408	
									h123.5	CTCG	0.0330	0.8888	0.5458	1.6283	0.1039	
									h123.6 (rare)	****	0.0029	1.1191	3.2961	0.3395	0.7343	
									hap base123	CCCG	0.5348	NA	NA	NA	NA	
124	p86245-rs188375019	125263275	Intron 12		C/T	0.0341	0.7447	0.1639	**h124.1**	CTGA	0.1476	0.1530	0.2692	0.5683	0.5700	0.0368
124	p86276-rs747155	125263244	Intron 12		C/T	0.1495	0.2793	0.2980	**h124.2**	CCTG	0.0465	-1.1879	0.4699	-2.5281	0.0117	
124	p86316-rs701104	125263204	Intron 12		G/T	0.0487	-0.9838	0.0286	**h124.3**	CCGA	0.0915	0.1086	0.3376	0.3218	0.7477	
124	p86481-rs701103	125263039	Exon 13-3' UTR	Gly499Arg (isoform 2)	G/A	0.2451	0.1642	0.4492	**h124.4**	TCGG	0.0337	0.7348	0.5362	1.3702	0.1710	
									h124.5 (rare)	****	0.0045	4.0859	2.1131	1.9336	0.0535	
									hap base124	CCGG	0.6761	NA	NA	NA	NA	
125	p86276-rs747155	125263244	Intron 12		C/T	0.1495	0.2793	0.2980	**h125.1**	TGAA	0.1476	0.1543	0.2689	0.5737	0.5664	0.0307
125	p86316-rs701104	125263204	Intron 12		G/T	0.0487	-0.9838	0.0286	**h125.2**	CTGA	0.0465	-1.1980	0.4691	-2.5535	0.0109	
125	p86481-rs701103	125263039	Exon 13-3' UTR	Gly499Arg (isoform 2)	G/A	0.2451	0.1642	0.4492	**h125.3**	CGAA	0.0915	0.1139	0.3375	0.3375	0.7359	
125	p86967-rs187492239	125262553	Exon 13-3' UTR		A/G	0.0355	0.7743	0.1412	**h125.4**	CGGG	0.0352	0.7974	0.5241	1.5216	0.1285	
									h125.5 (rare)	****	0.0045	4.0989	2.1134	1.9394	0.0528	
									hap base125	CGGA	0.6747	NA	NA	NA	NA	
ApoA-I																
47	p49690-rs4765615	125299830	Intron 2		G/A	0.4426	-0.9139	**0.0480**	**h47.1**	ACGG	0.4351	-0.8907	0.4584	-1.9432	0.0524	0.0343
47	p49759-rs146272788	125299761	Intron 2		C/T	0.0020	1.5883	0.7630	**h47.2 (rare)**	****	0.0106	3.5858	2.2998	1.5592	0.1194	
47	p49978-rs5891	125299542	Exon 3	Val135Ile	G/A	0.0058	5.6762	0.0628	**hap base47**	GCGG	0.5543	NA	NA	NA	NA	
47	p50024-rs368880622	125299496	Intron 3		G/T	0.0026	1.6012	0.7255								
48	p49759-rs146272788	125299761	Intron 2		C/T	0.0020	1.5883	0.7630	**h48.1**	CGGT	0.0206	3.3555	1.6564	2.0258	0.0431	0.0293
48	p49978-rs5891	125299542	Exon 3	Val135Ile	G/A	0.0058	5.6762	0.0628	**h48.2 (rare)**	****	0.0106	4.0750	2.3644	1.7235	0.0852	
48	p50024-rs368880622	125299496	Intron 3		G/T	0.0026	1.6012	0.7255	**hap base48**	CGGC	0.9688	NA	NA	NA	NA	
48	p50118-rs58710319	125299402	Intron 3		C/T	0.0208	3.1376	0.0571								
49	p49978-rs5891	125299542	Exon 3	Val135Ile	G/A	0.0058	5.6762	0.0628	**h49.1**	GGTT	0.0213	3.3792	1.6416	2.0584	0.0399	0.0289
49	p50024-rs368880622	125299496	Intron 3		G/T	0.0026	1.6012	0.7255	**h49.2**	GGCC	0.1928	0.8864	0.5841	1.5176	0.1295	
49	p50118-rs58710319	125299402	Intron 3		C/T	0.0208	3.1376	0.0571	**h49.3 (rare)**	****	0.0086	4.7388	3.1873	1.4868	0.1375	

Table 7.7. (continued)

49	p50151-rs2278986	125299369	Intron 3	T/C	0.1933	0.8568	0.1419	**hap base49**	GGCT	0.7774	NA	NA	NA	NA	
70	p54627-chr12_125294893	125294893	Intron 5	G/C	0.0020	3.6910	0.4850	**h70.1**	GCAC	0.3873	0.8579	0.5090	1.6854	0.0923	0.0140
70	p54856-chr12_125294664	125294664	Intron 6	C/T	0.0007	-24.0757	0.0082	**h70.2**	GCGT	0.1568	2.0940	0.6700	3.1254	0.0018	
70	p55923-rs838900	125293597	Intron 6	G/A	0.3921	0.3606	0.4549	**h70.3 (rare)**	****	0.0027	-2.5567	5.2200	-0.4898	0.6244	
70	p55963-rs7134858	125293557	Intron 6	C/T	0.1560	1.7537	**0.0052**	**hap base70**	GCGC	0.4532	NA	NA	NA	NA	
71	p54856-chr12_125294664	125294664	Intron 6	C/T	0.0007	-24.0757	0.0082	**h71.1**	CACA	0.2736	0.7883	0.6210	1.2694	0.2047	0.0488
71	p55923-rs838900	125293597	Intron 6	G/A	0.3921	0.3606	0.4549	**h71.2**	CACG	0.1134	1.1284	0.9724	1.1604	0.2462	
71	p55963-rs7134858	125293557	Intron 6	C/T	0.1560	1.7537	**0.0052**	**h71.3**	CGTA	0.1296	2.1103	0.7906	2.6691	0.0078	
71	p56845-rs838902	125292675	Intron 6	A/G	0.4249	-0.3052	0.5129	**h71.4**	CGTG	0.0300	2.1358	1.6772	1.2734	0.2032	
								h71.5	CGCA	0.1706	-0.1013	0.8355	-0.1212	0.9035	
								hap base71	CGCG	0.2822	NA	NA	NA	NA	
72	p55923-rs838900	125293597	Intron 6	G/A	0.3921	0.3606	0.4549	**h72.1**	ACAG	0.2733	0.7471	0.6218	1.2016	0.2299	0.0463
72	p55963-rs7134858	125293557	Intron 6	C/T	0.1560	1.7537	**0.0052**	**h72.2**	ACGG	0.1057	0.7094	0.9850	0.7202	0.4716	
72	p56845-rs838902	125292675	Intron 6	A/G	0.4249	-0.3052	0.5129	**h72.3**	GTAG	0.1297	2.0304	0.7898	2.5707	0.0103	
72	p57004-rs187562853	125292516	Intron 6	G/A	0.0098	3.2853	0.1690	**h72.4**	GTGG	0.0299	2.1741	1.6857	1.2897	0.1975	
								h72.5	GCAG	0.1712	-0.3122	0.8263	-0.3778	0.7057	
								h72.6 (rare)	****	0.0100	3.9105	2.4373	1.6044	0.1090	
								hap base72	GCGG	0.2801	NA	NA	NA	NA	
78	p57592-rs838903	125291928	Intron 7	G/A	0.3763	-0.7661	0.1109	**h78.1**	GCAC	0.0559	1.8913	1.0469	1.8067	0.0712	0.0326
78	p58514-rs838905	125291006	Intron 7	T/C	0.4329	-0.4213	0.3646	**h78.2**	GTAC	0.0367	1.0784	1.2814	0.8415	0.4003	
78	p58664-rs865716	125290856	Intron 7	A/T	0.2708	0.5369	0.3008	**h78.3**	GTAT	0.2557	0.3365	0.6035	0.5576	0.5773	
78	p60255-rs3782287	125289265	Intron 7	C/T	0.2831	0.3715	0.4856	**h78.4**	GTTC	0.2463	0.4962	0.5864	0.8462	0.3977	
								h78.5	GTTT	0.0238	5.5715	1.6643	3.3477	0.0009	
								h78.6 (rare)	****	0.0075	0.6333	2.9303	0.2161	0.8289	
								hap base78	ACAC	0.3740	NA	NA	NA	NA	
79	p58514-rs838905	125291006	Intron 7	T/C	0.4329	-0.4213	0.3646	**h79.1**	CACT	0.1270	0.3290	0.8318	0.3955	0.6926	0.0256
79	p58664-rs865716	125290856	Intron 7	A/T	0.2708	0.5369	0.3008	**h79.2**	TACC	0.0379	0.6384	1.2921	0.4941	0.6214	
79	p60255-rs3782287	125289265	Intron 7	C/T	0.2831	0.3715	0.4856	**h79.3**	TATC	0.2563	0.1851	0.6336	0.2921	0.7703	
79	p61872-rs838909	125287648	Intron 7	C/T	0.2199	0.9232	0.1056	**h79.4**	TTCC	0.1587	-0.6020	0.7769	-0.7749	0.4386	
								h79.5	TTCT	0.0880	1.8902	0.8856	2.1342	0.0331	
								h79.6	TTTC	0.0238	5.1755	1.6851	3.0714	0.0022	
								h79.7 (rare)	****	0.0059	1.2466	3.1079	0.4011	0.6885	
								hap base79	CACC	0.3024	NA	NA	NA	NA	
80	p58664-rs865716	125290856	Intron 7	A/T	0.2708	0.5369	0.3008	**h80.1**	ACCG	0.0389	-0.3521	1.2793	-0.2753	0.7832	0.0030
80	p60255-rs3782287	125289265	Intron 7	C/T	0.2831	0.3715	0.4856	**h80.2**	ACTG	0.1274	-0.1816	0.7909	-0.2297	0.8184	
80	p61872-rs838909	125287648	Intron 7	C/T	0.2199	0.9232	0.1056	**h80.3**	ATCG	0.2611	-0.1400	0.6323	-0.2213	0.8249	
80	p62140-rs838910	125287380	Intron 7	G/T	0.3047	-0.0755	0.8821	**h80.4**	TCCG	0.1549	-1.3614	0.7489	-1.8178	0.0695	

Table 7.7. (continued)

									h80.5	TCTG	0.0901	2.0511	0.8921	2.2992	0.0218	
									h80.6	TTCG	0.0224	4.7307	1.8842	2.5107	0.0123	
									h80.7 (rare)	****	0.0083	3.1429	3.4362	0.9147	0.3607	
									hap base80	ACCT	0.2970	NA	NA	NA	NA	
81	p60255-rs3782287	125289265	Intron 7		C/T	0.2831	0.3715	0.4856	**h81.1**	CCGC	0.1740	-1.5355	0.7276	-2.1103	0.0352	0.0050
81	p61872-rs838909	125287648	Intron 7		C/T	0.2199	0.9232	0.1056	**h81.2**	CCGT	0.0215	-0.5623	1.6155	-0.3481	0.7279	
81	p62140-rs838910	125287380	Intron 7		G/T	0.3047	-0.0755	0.8821	**h81.3**	CCTC	0.0352	3.6130	1.4518	2.4886	0.0130	
81	p62409-rs838911	125287111	Intron 7		C/T	0.4211	-0.6245	0.1888	**h81.4**	CCTT	0.2683	-0.7498	0.6337	-1.1832	0.2371	
									h81.5	CTGC	0.0886	1.4787	0.9259	1.5970	0.1107	
									h81.6	CTGT	0.1287	-0.2477	0.7967	-0.3109	0.7560	
									h81.7 (rare)	****	0.0017	4.9120	8.4190	0.5834	0.5598	
									hap base81	TCGC	0.2819	NA	NA	NA	NA	
82	p61872-rs838909	125287648	Intron 7		C/T	0.2199	0.9232	0.1056	**h82.1**	CGTT	0.0214	0.3707	1.6055	0.2309	0.8175	0.0137
82	p62140-rs838910	125287380	Intron 7		G/T	0.3047	-0.0755	0.8821	**h82.2**	CTCT	0.0364	3.8641	1.3703	2.8199	0.0049	
82	p62409-rs838911	125287111	Intron 7		C/T	0.4211	-0.6245	0.1888	**h82.3**	CTTT	0.2692	-0.2007	0.5674	-0.3537	0.7237	
82	p62615-rs7138386	125286905	Intron 7		T/C	0.1137	-0.6495	0.3851	**h82.4**	TGCT	0.0869	2.1488	0.8777	2.4481	0.0146	
									h82.5	TGTT	0.0179	3.0085	1.9599	1.5351	0.1252	
									h82.6	TGTC	0.1116	-0.1961	0.7815	-0.2510	0.8019	
									h82.7 (rare)	****	0.0020	-4.7635	9.0097	-0.5287	0.5972	
									hap base82	CGCT	0.4546	NA	NA	NA	NA	
83	p62140-rs838910	125287380	Intron 7		G/T	0.3047	-0.0755	0.8821	**h83.1**	GCTA	0.0854	2.0624	0.8886	2.3211	0.0205	0.0187
83	p62409-rs838911	125287111	Intron 7		C/T	0.4211	-0.6245	0.1888	**h83.2**	GTTG	0.0389	1.3667	1.2527	1.0910	0.2756	
83	p62615-rs7138386	125286905	Intron 7		T/C	0.1137	-0.6495	0.3851	**h83.3**	GTCG	0.1129	-0.3143	0.7855	-0.4002	0.6891	
83	p63483-rs838912	125286037	Intron 7		G/A	0.0867	1.8700	**0.0234**	**h83.4**	TCTG	0.0368	3.8488	1.3757	2.7977	0.0053	
									h83.5	TTTG	0.2675	-0.1681	0.5759	-0.2918	0.7705	
									h83.6 (rare)	****	0.0031	-0.5696	5.5038	-0.1035	0.9176	
									hap base83	GCTG	0.4554	NA	NA	NA	NA	
86	p63483-rs838912	125286037	Intron 7		G/A	0.0867	1.8700	**0.0234**	**h86.1**	ATCG	0.0871	2.5431	0.8550	2.9743	0.0030	0.0290
86	p64772-rs5888	125284748	Exon 8	Ala350Ala	C/T	0.0961	2.0962	**0.0080**	**h86.2**	GCAG	0.1457	0.3613	0.6957	0.5194	0.6037	
86	p64923-rs838915	125284597	Intron 8		C/A	0.1435	-0.3684	0.5766	**h86.3**	GCCA	0.2814	1.0972	0.5782	1.8976	0.0581	
86	p65999-rs12819677	125283521	Intron 8		G/A	0.2813	0.6769	0.2052	**h86.4**	GTCG	0.0116	1.6563	2.1240	0.7798	0.4357	
									hap base86	GCCG	0.4736	NA	NA	NA	NA	
95	p71867-rs7954022	125277653	Intron 9		C/T	0.1323	0.8502	0.2241	**h95.1**	TACT	0.1311	0.8202	0.7688	1.0669	0.2864	0.0131
95	p72197-rs838861	125277323	Intron 9		A/G	0.3777	-0.1507	0.7464	**h95.2**	CACC	0.0507	0.3188	1.2809	0.2489	0.8035	
95	p72777-rs838862	125276743	Intron 9		C/T	0.0887	0.7012	0.3938	**h95.3**	CGCT	0.1846	-0.7832	0.6960	-1.1253	0.2608	
95	p75766-rs838866	125273754	Intron 9		T/C	0.2116	-0.0497	0.9306	**h95.4**	CGCC	0.1022	0.7176	0.8581	0.8362	0.4033	
									h95.5	CGTT	0.0324	4.7525	1.5071	3.1534	0.0017	

Table 7.7. (continued)

								h95.6	CGTC	0.0582	-1.3987	1.0854	-1.2887	0.1979	
								h95.7 (rare)	****	0.0009	18.2723	NA	NA	NA	
								hap base95	CACT	0.4399	NA	NA	NA	NA	
96	p72197-rs838861	125277323	Intron 9	A/G	0.3777	-0.1507	0.7464	**h96.1**	ACCT	0.0443	1.0796	1.2832	0.8413	0.4004	0.0484
96	p72777-rs838862	125276743	Intron 9	C/T	0.0887	0.7012	0.3938	**h96.2**	GCTC	0.1849	-0.7979	0.6554	-1.2176	0.2238	
96	p75766-rs838866	125273754	Intron 9	T/C	0.2116	-0.0497	0.9306	**h96.3**	GCCT	0.0727	-0.3866	0.9478	-0.4079	0.6835	
96	p75778-rs7301120	125273742	Intron 9	C/T	0.1135	0.3767	0.6174	**h96.4**	GCCC	0.0282	1.9372	1.6107	1.2027	0.2295	
								h96.5	GTTC	0.0319	4.2363	1.4400	2.9419	0.0034	
								h96.6	GTCC	0.0595	-1.3421	1.0101	-1.3286	0.1844	
								h96.7 (rare)	****	0.0058	-3.2342	3.8265	-0.8452	0.3983	
								hap base96	ACTC	0.5728	NA	NA	NA	NA	
97	p72777-rs838862	125276743	Intron 9	C/T	0.0887	0.7012	0.3938	**h97.1**	CTCT	0.1997	-1.0781	0.6237	-1.7287	0.0843	0.0098
97	p75766-rs838866	125273754	Intron 9	T/C	0.2116	-0.0497	0.9306	**h97.2**	CCTT	0.1141	0.2005	0.7597	0.2639	0.7919	
97	p75778-rs7301120	125273742	Intron 9	C/T	0.1135	0.3767	0.6174	**h97.3**	CCCT	0.0336	0.7963	1.3894	0.5731	0.5667	
97	p76757-rs9919713	125272763	Intron 9	A/T	0.4390	-0.1860	0.6921	**h97.4**	TTCT	0.0301	4.3773	1.4494	3.0201	0.0026	
								h97.5	TCCT	0.0588	-1.4125	1.0117	-1.3961	0.1631	
								h97.6 (rare)	****	0.0050	-6.5869	3.6167	-1.8213	0.0690	
								hap base97	CTCA	0.5587	NA	NA	NA	NA	
109	p78402-rs838898	125271118	Intron 10	G/A	0.0714	-0.9806	0.2889	**h109.1**	AGCT	0.0288	-1.4134	1.6436	-0.8600	0.3901	0.0195
109	p78430-rs838897	125271090	Intron 10	C/G	0.3830	-0.1887	0.6887	**h109.2**	AGTT	0.0451	-1.5093	1.2496	-1.2078	0.2275	
109	p78747-rs2293440	125270773	Intron 11	T/C	0.4112	-0.2984	0.5352	**h109.3**	GGCC	0.0317	3.0784	1.3763	2.2366	0.0256	
109	p78791-rs75289200	125270729	Intron 11	T/C	0.0321	3.6568	0.0086	**h109.4**	GGCT	0.1633	-0.4126	0.6911	-0.5971	0.5506	
								h109.5	GGTT	0.1088	-1.6537	0.8639	-1.9142	0.0560	
								h109.6	GCCT	0.1851	-1.8104	0.7168	-2.5256	0.0118	
								hap base109	GCTT	0.4363	NA	NA	NA	NA	
110	p78430-rs838897	125271090	Intron 10	C/G	0.3830	-0.1887	0.6887	**h110.1**	GCCC	0.0305	3.0357	1.4224	2.1342	0.0331	0.0012
110	p78747-rs2293440	125270773	Intron 11	T/C	0.4112	-0.2984	0.5352	**h110.2**	GCTG	0.0189	-3.0973	2.2833	-1.3565	0.1753	
110	p78791-rs75289200	125270729	Intron 11	T/C	0.0321	3.6568	0.0086	**h110.3**	GCTC	0.1696	-0.0290	0.6830	-0.0424	0.9662	
110	p79721-rs838896	125269799	Intron 11	G/C	0.3104	1.1147	**0.0278**	**h110.4**	GTTG	0.1400	-2.3158	0.7741	-2.9914	0.0029	
								h110.5	GTTC	0.0189	1.3536	2.3385	0.5788	0.5629	
								h110.6	CCTG	0.1379	-2.4014	0.7888	-3.0443	0.0024	
								h110.7	CCTC	0.0514	-0.8677	1.2628	-0.6871	0.4922	
								h110.8	CTTC	0.0398	-0.1892	1.4963	-0.1264	0.8994	
								h110.9 (rare)	****	0.0012	7.8235	8.0313	0.9741	0.3303	
								hap base110	CTTG	0.3918	NA	NA	NA	NA	
111	p78747-rs2293440	125270773	Intron 11	T/C	0.4112	-0.2984	0.5352	**h111.1**	CCCG	0.0305	3.5704	1.4077	2.5364	0.0114	0.0038
111	p78791-rs75289200	125270729	Intron 11	T/C	0.0321	3.6568	0.0086	**h111.2**	CTGC	0.1514	-2.1697	0.7058	-3.0742	0.0022	

Table 7.7. (continued)

111	p79721-rs838896	125269799	Intron 11	G/C	0.3104	1.1147	**0.0278**	**h111.3**	CTCG	0.2233	0.3086	0.5985	0.5157	0.6062	
111	p79828-rs838895	125269692	Intron 11	C/G	0.3171	1.2206	**0.0164**	**h111.4**	TTGG	0.0173	1.0502	1.9388	0.5417	0.5882	
								h111.5	TTGC	0.0431	0.3464	1.3140	0.2637	0.7921	
								h111.6	TTCC	0.0150	0.6429	1.9745	0.3256	0.7448	
								h111.7 (rare)	****	0.0047	3.8853	4.0634	0.9562	0.3393	
								hap base111	TTGC	0.5147	NA	NA	NA	NA	
112	p78791-rs75289200	125270729	Intron 11	T/C	0.0321	3.6568	0.0086	**h112.1**	CCGA	0.0309	3.7315	1.3947	2.6755	0.0076	0.0412
112	p79721-rs838896	125269799	Intron 11	G/C	0.3104	1.1147	**0.0278**	**h112.2**	TGGA	0.0179	1.8646	1.8467	1.0097	0.3130	
112	p79828-rs838895	125269692	Intron 11	C/G	0.3171	1.2206	**0.0164**	**h112.3**	TGCA	0.0109	-3.3720	2.3180	-1.4547	0.1462	
112	p80045-rs838893	125269475	Intron 11	G/A	0.3244	0.8859	0.0774	**h112.4**	TCGA	0.2661	0.7087	0.5428	1.3056	0.1921	
								h112.5	TCCG	0.0144	1.0316	2.0147	0.5120	0.6088	
								h112.6 (rare)	****	0.0068	2.8715	3.2105	0.8944	0.3714	
								hap base112	TGCG	0.6530	NA	NA	NA	NA	
123	p83884-rs701106	125265636	Intron 12	C/T	0.2597	1.2967	**0.0156**	**h123.1**	TCCT	0.0235	-1.7638	1.7393	-1.0141	0.3109	0.0468
123	p86245-rs188375019	125263275	Intron 12	C/T	0.0341	1.8399	0.1674	**h123.2**	TCCG	0.2351	1.8726	0.6006	3.1179	0.0019	
123	p86276-rs747155	125263244	Intron 12	C/T	0.1495	-0.2164	0.7433	**h123.3**	CCTG	0.1485	0.3912	0.6981	0.5604	0.5754	
123	p86316-rs701104	125263204	Intron 12	G/T	0.0487	-0.6627	0.5579	**h123.4**	CCCT	0.0238	1.6476	1.7546	0.9390	0.3480	
								h123.5	CTCG	0.0328	2.3144	1.3655	1.6949	0.0905	
								h123.6 (rare)	****	0.0024	1.2704	8.8153	0.1441	0.8855	
								hap base123	CCCG	0.5340	NA	NA	NA	NA	

ApoA-I, apolipoprotein A-I; Coef, coefficient; del/D, deletion; HDL-C, high-density lipoprotein cholesterol; MAF, minor allele frequency; NA, not analyzed; SE, standard error; SNP, single nucleotide polymorphism; UTR, untranslated region; W, wild type allele on RefSeq for deletion.
All alleles on reverse strand. HDL-C and ApoA-I values were Box-Cox transformed.
SNP 1-SNP 4 for each window are shown as "SNP name-SNP ID/Chr12 Position (for novel variants)", and corresponding to 5' to 3' direction.
For SNPs with MAF ≥5%, nominally significant single-site *P*-values (*P* <0.05) are shown in **bold**.
Detailed haplotype association results of all haplotype windows for each trait are shown in Table B11; see haplotype association plots in Figure 7.3.
[a, c] RefSeq *SCARB1*: hg19, NM_005505 (CHIP Bioinformatics).
[b] dbSNP build 139: GRCh37.p10; 10 novel variants were submitted to dbSNP database: http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=KAMBOH (batch ID: SCARB1_AB).

Table 7.8. Significantly associated haplotype regions (global *P* <0.05) of 136 *SCARB1* genotyped variants.

Region #	Trait	Consecutive Significantly Associated Haplotype Windows (global *P* <0.05)							
		Haplotype Windows #	Chr12 Position[a] (Location)		The Composited Variants from 5' to 3' Direction		Most Relevant Haplotype		
			Start (5')	End (3')	SNP Name[b]-RefSNP ID[c]/Chr12 Position[a]	Major/Minor Alleles	hap #	Sequence	β (range: min-max)
1	HDL-C	44	125300551	125299542	p48969-rs2343394	C/T	h44.3	CCWGCGG	0.4910-1.0491
		45	(intron 2)	(exon 3)	p49537-rs7305310	C/T	h45.2		
		46			p49570delC-rs145376237	W/D	h46.2		
		47			**p49690-rs4765615**	G/A	hap base47		
					p49759-rs146272788	C/T	hap base44	CCWACGG	-0.4701
					p49978-rs5891 (p.Val135Ile)	G/A	hap base45		
					p50024-rs368880622	G/T	hap base46		
							h47.1		
2	ApoA-I	47	125299830	125299369	**p49690-rs4765615**	G/A	h47.1	ACGGTT	(-0.8907)-3.3792
		48	(intron 2)	(intron 3)	p49759-rs146272788	C/T	h48.1		
		49			p49978-rs5891 (p.Val135Ile)	G/A	h49.1		
					p50024-rs368880622	G/T			
					p50118-rs58710319	C/T			
					p50151-rs2278986	T/C			
3	ApoA-I	**70**	125294893	125292516	p54627-chr12_125294893	G/C	h70.2	GCGTAG	2.0304-2.1103
		71	(intron 5)	(intron 6)	p54856-chr12_125294664[d]	C/T	h71.3		
		72			p55923-rs838900	G/A	h72.3		
					p55963-rs7134858	C/T			
					p56845-rs838902	A/G			
					p57004-rs187562853	G/A			
4	ApoA-I	78	125291928	125286037	p57592-rs838903	G/A	h78.5	GTTTCGCTG	4.7307-5.5715
		79	(intron 7)	(intron 7)	p58514-rs838905	T/C	h79.6		
		80			p58664-rs865716	A/T	h80.6		
		81			p60255-rs3782287	C/T	hap base81		
		82			p61872-rs838909	C/T	hap base82		
		83			p62140-rs838910	G/T	hap base83		
					p62409-rs838911	C/T	h78.2	GTACCTCTG	0.6384-3.8641
					p62615-rs7138386	T/C	h79.2		
					p63483-rs838912	G/A	hap base80		
							h81.3		
							h82.2		
							h83.4		
5	ApoA-I	95	125277653	125272763	p71867-rs7954022	C/T	h95.5	CGTTCT	4.2363-4.7525
		96	(intron 9)	(intron 9)	p72197-rs838861	A/G	h96.5		
		97			p72777-rs838862	C/T	h97.4		
					p75766-rs838866	T/C			
					p75778-rs7301120	C/T			
					p76757-rs9919713	A/T			

Table 7.8. (continued)

6*	ApoA-I	109	125271118	125269475	p78402-rs838898	G/A	h109.6	GCCTGCA	(-3.3720)-(-1.8104)
		110	(intron 10)	(intron 11)	p78430-rs838897	C/G	h110.6		
		111			p78747-rs2293440	T/C	h111.2		
		112			p78791-rs75289200	T/C	h112.3		
					p79721-rs838896	G/C			
					p79828-rs838895	C/G			
					p80045-rs838893	G/A			
7*	HDL-C	**111**	125270773	125267501	p78747-rs2293440	T/C	h111.4	TTGGAGC	0.3755-2.3949
		112	(intron 11)	(intron 11)	p78791-rs75289200	T/C	h112.2		
		113			p79721-rs838896	G/C	h113.1		
		114			**p79828-rs838895**	C/G	h114.1		
					p80045-rs838893	G/A			
					p81863-rs185445624	G/A			
					p82019-rs838890	C/T			
8	HDL-C	117	125267501	125267086	p82019-rs838890	C/T	h117.2	TCGGC	(-1.0134)-(-0.9657)
		118	(intron 11)	(intron 12)	p82264-rs141545424 (p.Gly501Gly)[d]	C/A	h118.2		
					p82340-rs77483223	G/A			
					p82369-rs75446635	G/A			
					p82434-rs838889	T/C			
9	HDL-C	123	125265636	125262553	***p83884-rs701106***	C/T	h123.4	CCCTGA	(-1.180)-(-0.2329)
		124	(intron 12)	(exon 13-3' UTR)	p86245-rs188375019	C/T	h124.2		
		125			p86276-rs747155	C/T	h125.2		
					p86316-rs701104	G/T			
					p86481-rs701103 (p.Gly499Arg, isoform 2)	G/A			
					p86967-rs187492239	A/G			

ApoA-I, apolipoprotein A-I; del/D, deletion; HDL-C, high-density lipoprotein cholesterol; SNP, single nucleotide polymorphism; UTR, untranslated region; W, wild type allele for deletion on the RefSeq.
All alleles on reverse strand.
All nine haplotype regions are shown in Figure 7.3.
The composited variants in each region are shown as "SNP Name-SNP ID/Chr12 Position (for novel variants)", and corresponding to 5' to 3' direction.
Detailed single-site associations are shown in Tables B9 and B10.
Detailed haplotype associations are shown in Tables 7.7 and B11.
For each trait, regions with asterisk (*) indicate those regions that included the haplotype window exhibiting the most significant association signal (the smallest global *P*) for that trait.
For each region, the most significant associated haplotype window is shown in **bold**.
SNPs with significant evidence of association with the same trait in both single-site and haplotype analyses (single-site *P* <0.05 and global *P* <0.05) are shown in **bold**.
SNPs with significant evidence of association with different trait in single-site and haplotype analyses (single-site *P* <0.05 and global *P* <0.05) are shown in ***italic bold***.
[a, b] RefSeq *SCARB1*: hg19, NM_005505 (CHIP Bioinformatics).
[c] dbSNP build 139: GRCh37.p10; 10 novel variants were submitted to dbSNP database: http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=KAMBOH (batch ID: SCARB1_AB).
[d] Rare variants of interest with potential effects on lipid traits; see details in Table 7.6.

Table 7.9. Single-site association results of 7 lipid-associated *SCARB1* variants that were observed in US Non-Hispanic Whites (previous study[a]) in African Blacks (this study).

						US Non-Hispanic Whites[a] (n = 623)			African Blacks (n = 788)			
SNP Name[b]	RefSNP ID[c]	Chr12 Position[d]	Location	RegDB Score[e]	Alleles	MA, MAF	β (SE)	*P*	MA, MAF	β (SE)	*P*	Other Assoc Trait(s)[f]
HDL-C												
p28957	rs11057844	125320563	Intron 1	5	G/A	A, 0.1839	-0.0395 (0.0135)	**0.0035**	A, 0.2362	0.3671 (0.2278)	0.1075	
p83884	rs701106	125265636	Intron 12	5	C/T	T, 0.1527	0.0394 (0.0144)	**0.0066**	T, 0.2597	0.2471 (0.2192)	0.2601	ApoA-I
p87927	rs838880	125261593	3' flanking	5	G/A	G, 0.3237	0.0257 (0.0114)	**0.0250**	A, 0.2414	0.0198 (0.2302)	0.9314	
ApoB												
p48969	rs2343394	125300551	Intron 2	5	C/T	T, 0.2850	1.2544 (0.4721)	**0.0082**	T, 0.1898	0.0383 (0.6696)	0.9544	
p49690	rs4765615	125299830	Intron 2	5	G/A	G, 0.4497	1.2493 (0.4518)	**0.0059**	A, 0.4426	0.7771 (0.5178)	0.1338	HDL-C, ApoA-I
p50151	rs2278986	125299369	Intron 3	5	T/C	C, 0.2890	1.1926 (0.4735)	**0.0122**	C, 0.1933	0.1308 (0.6619)	0.8434	
p52556	rs11057820	125296964	Intron 4	5	G/A	G, 0.4871	0.8700 (0.4300)	**0.0436**	A, 0.1000	1.8661 (0.8542)	**0.0292**	

ApoB; apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; MA, minor allele; MAF, minor allele frequency; RegDB, RegulomeDB; SE, standard error; SNP, single nucleotide polymorphism.
All alleles on reverse strand.
HDL-C and ApoB values for US Non-Hispanic Whites were Box-Cox transformed, and adjusted for covariates: sex, age, body mass index, and smoking (past/current/never) for HDL-C; age and smoking for ApoB.
HDL-C and ApoB values for African Blacks were Box-Cox transformed, and adjusted for covariates: sex, age, waist, current smoking (yes/no), and daily walking or biking to work (jobmin) for HDL-C; body mass index and staff status for ApoB.
Nominally significant *P*-values (*P* <0.05) are shown in **bold**.
[a] Data from Niemsiri, V., et al. 2014. *Circ Cardiovasc Genet* **7**: 838-847 (Ref [48]).
[b, d] RefSeq *SCARB1*: hg19, NM_005505 (CHIP Bioinformatics).
[c] dbSNP version 139: GRCh37.p10.
[e] Detailed RegulomeDB (version 1.0) scoring scheme is described in the footnote of Table B12 or can be seen at http://regulome.stanford.edu/help.
[f] Evidence is based on SNPs with MAF ≥5% exhibiting nominally significant association with either HDL-C or ApoA-I (*P* <0.05; Tables B9 and B10) in single-site association results in the current study.

Table 7.10. Significant lipid-associated regions (global *P* <0.05) of *SCARB1* that were observed in US Non-Hispanic Whites (previous study[a]) and African Blacks (this study).

Region #	Consecutive Haplotype Windows in 623 US Non-Hispanic Whites[a]						Consecutive Haplotype Windows in 788 African Blacks					
	Trait	Chr12 Position[b] (Location)		Length (bp)	The Composited Variants from 5' to 3' Direction		Trait	Chr12 Position[b] (Location)		Length (bp)	The Composited Variants from 5' to 3' Direction	
		Start (5')	End (3')		SNP Name[c]-RefSNP ID[d]	Major/Minor Alleles		Start (5')	End (3')		SNP Name[c]-RefSNP ID[d]	Major/Minor Alleles
I	**ApoB**	125300551	125299369	1183	p48969-rs2343394	C/T	**HDL-C**	125300551	125299496	1056	p48969-rs2343394	C/T
		(intron 2)	(intron 3)		p49518-rs144194221	G/A		(intron 2)	(intron 3)		p49537-rs7305310	C/T
					p49690-rs4765615	A/G					p49570delC-rs145376237	W/D
					p49978-rs5891 (p.Val135Ile)	G/A					**p49690-rs4765615**	G/A
					p50151-rs2278986	T/C					p49759-rs146272788	C/T
											p49978-rs5891 (p.Val135Ile)	G/A
											p50024-rs368880622	G/T
							ApoA-I	125299830	125299369	462	**p49690-rs4765615**	G/A
								(intron 2)	(intron 3)		p49759-rs146272788	C/T
											p49978-rs5891 (p.Val135Ile)	G/A
											p50024-rs368880622	G/T
											p50118-rs58710319	C/T
											p50151-rs2278986	T/C
II	**HDL-C**	125269692	125262516	7177	p79828-rs838895	C/G	**HDL-C**	125269692	125267501	2192	**p79828-rs838895**	C/G
		(intron 11)	(exon 13-3' UTR)		p80045-rs838893	G/A		(intron 11)	(intron 11)		p80045-rs838893	G/A
					p83088-rs797729	A/G					p81863-rs185445624	G/A
					p83884-rs701106	C/T					p82019-rs838890	C/T
					p86436-rs10396214 (p.Arg484Trp, isoform 2)	C/T	**HDL-C**	125267501	125267086	416	p82019-rs838890	C/T
					p87004-rs184715678	C/A		(intron 11)	(intron 12)		p82264-rs141545424 (p.Gly501Gly)	C/A
											p82340-rs77483223	G/A
											p82369-rs75446635	G/A
											p82434-rs838889	T/C
							HDL-C	125265636	125262553	3084	***p83884-rs701106***	C/T
								(intron 12)	(exon 13-3' UTR)		p86245-rs188375019	C/T

Table 7.10. (continued)

p86276-rs747155	C/T
p86316-rs701104	G/T
p86481-rs701103 (p.Gly499Arg, isoform 2)	G/A
p86967-rs187492239	A/G

ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; del/D, deletion; HDL-C, high-density lipoprotein cholesterol; UTR, untranslated region; W, wild type allele on RefSeq for deletion.
All alleles on reverse strand.
Results for a US Non-Hispanic White sample were Box-Cox transformed, and adjusted for covariates: sex, age, body mass index, and smoking (past/current/never) for HDL-C; age and smoking for ApoB.
Results for an African Black sample were Box-Cox transformed, and adjusted for covariates: sex, age, waist, current smoking (yes/no), and minutes of walking or biking to work each day (jobmin) for HDL-C; sex and age for ApoA-I.
The composited variants in each region are shown as "SNP Name-SNP ID/Chr12 Position (for novel variants)", and corresponding to 5' to 3' direction.
Location of each region on *SCARB1* gene is shown in Figure 7.4.
SNPs with significant evidence with the same trait in both single-site and haplotype associations (single-site *P* and global *P* <0.05) observed in each population are shown in **bold**.
SNPs with significant evidence with the different trait in single-site and haplotype associations (single-site *P* and global *P* <0.05) in each population are shown in ***italic bold***.
[a.] Data from Niemsiri, V., et al. 2014. *Circ Cardiovasc Genet* **7**: 838-847 (Ref [48]).
[b, c] RefSeq *SCARB1*: hg19, NM_005505 (CHIP Bioinformatics).
[d] dbSNP version 139: GRCh37.p10.

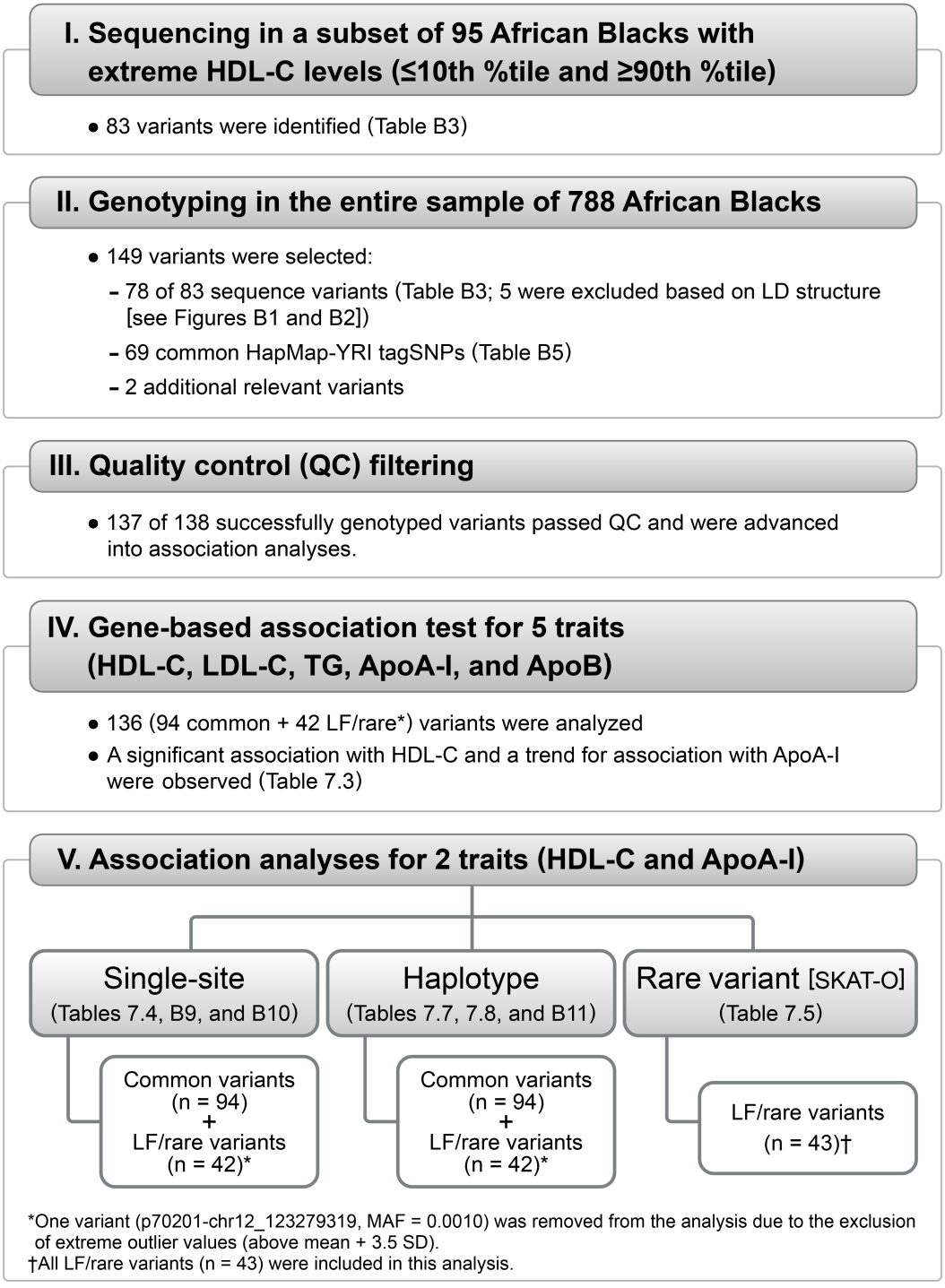

Figure 7.1. Summary of the study design and flow.

Chart presents an overview of the study design and flow, including sequencing and genotyping stages and analysis approaches. ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; LD, linkage disequilibrium; LDL-C, low-density lipoprotein cholesterol; LF, low-frequency; MAF, minor allele frequency; SKAT-O, an optimal sequence kernel association test; SD, standard deviation; SNP, single nucleotide polymorphism; TG, triglycerides; YRI, Yoruba people of Ibadan from Nigeria.

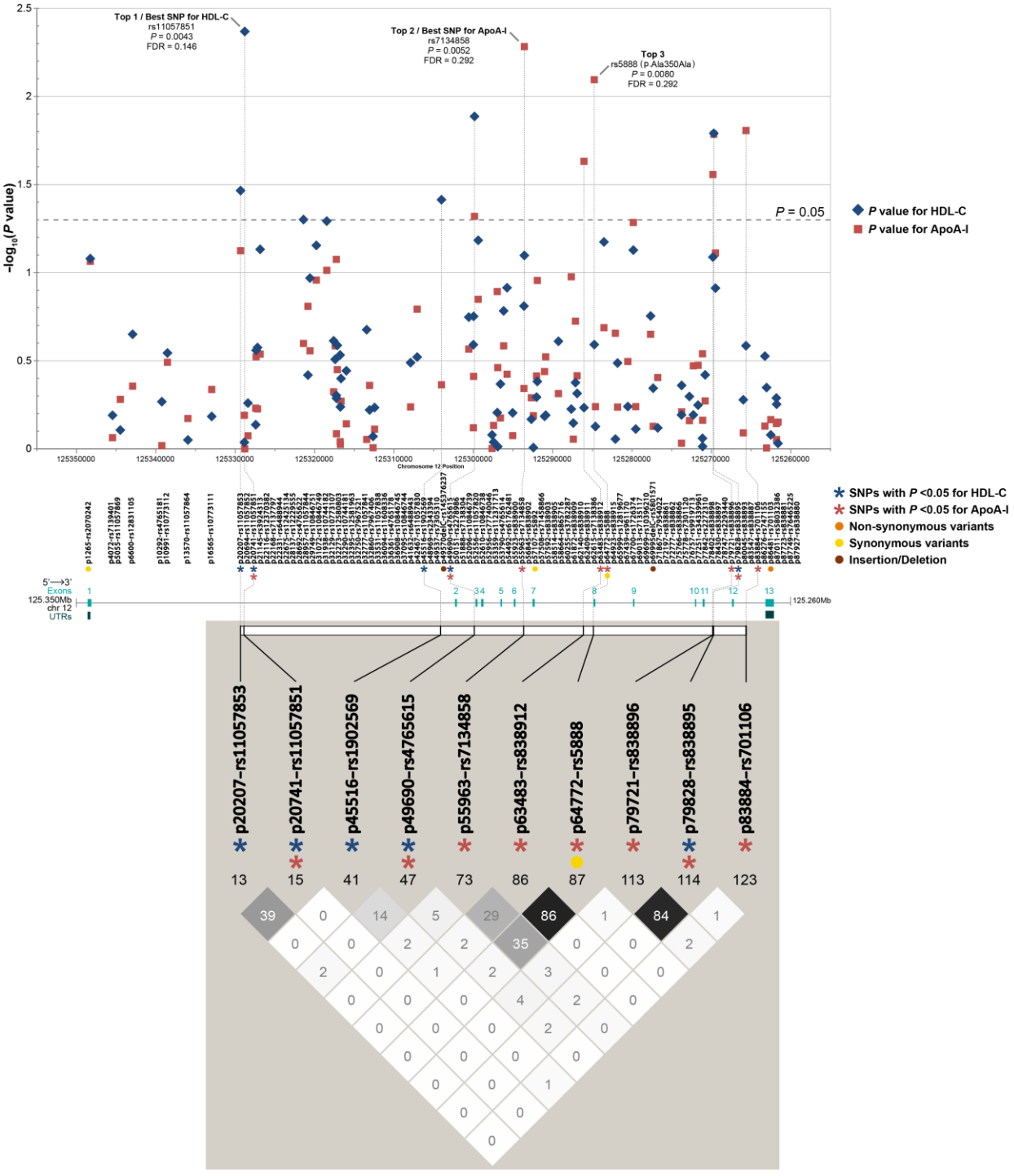
Top 1 / Best SNP for HDL-C
rs11057851
P = 0.0043
FDR = 0.146
Top 2 / Best SNP for ApoA-I
rs7134858
P = 0.0052
FDR = 0.292
Top 3
rs5888 (p.Ala350Ala)
P = 0.0080
FDR = 0.292
P = 0.05
P value for HDL-C
P value for ApoA-I
SNPs with P <0.05 for HDL-C
SNPs with P <0.05 for ApoA-I
Non-synonymous variants
Synonymous variants
Insertion/Deletion
p20207–rs11057853
p20741–rs11057851
p45516–rs1902569
p49690–rs4765615
p55963–rs7134858
p63483–rs838912
p64772–rs5888
p79721–rs838896
p79828–rs838895
p83884–rs701106
13
15
41
47
73
86
87
113
114
123

Figure 7.2. Single-site association *P*-values of 94 *SCARB1* variants with minor allele frequency (MAF) ≥5% for high-density lipoprotein cholesterol (HDL-C) and apolipoprotien A-I (ApoA-I; top), gene structure of *SCARB1* (middle) and linkage disequilibrium (LD) plot of 10 variants associated with HDL-C (n = 5) or ApoA-I (n = 8; *P* <0.05; bottom).

The -$\log_{10}$ *P*-values are in the Y-axis. A total of 94 genotyped variants with MAF ≥5% are on *SCARB1* gene (5'→3'; RefSeq: hg19, NM_005505) in the X-axis. Marker names are shown as "SNP name-SNP ID (dbSNP build 139)". Dash line indicates the significance threshold (*P* = 0.05)**.** Shades and values (r^2 x 100) in each square of LD plot indicate pairwise LD: black indicating complete LD (r^2 = 1), white indicating no LD (r^2 = 0), and shade intensity indicating the degree of LD (r^2 between 0-1). ApoA-I, apolipoprotein A-I; FDR, false discovery rate; HDL-C, high-density lipoprotein cholesterol; MAF, minor allele frequency; SNP, single nucleotide polymorphism; UTR, untranslated region.

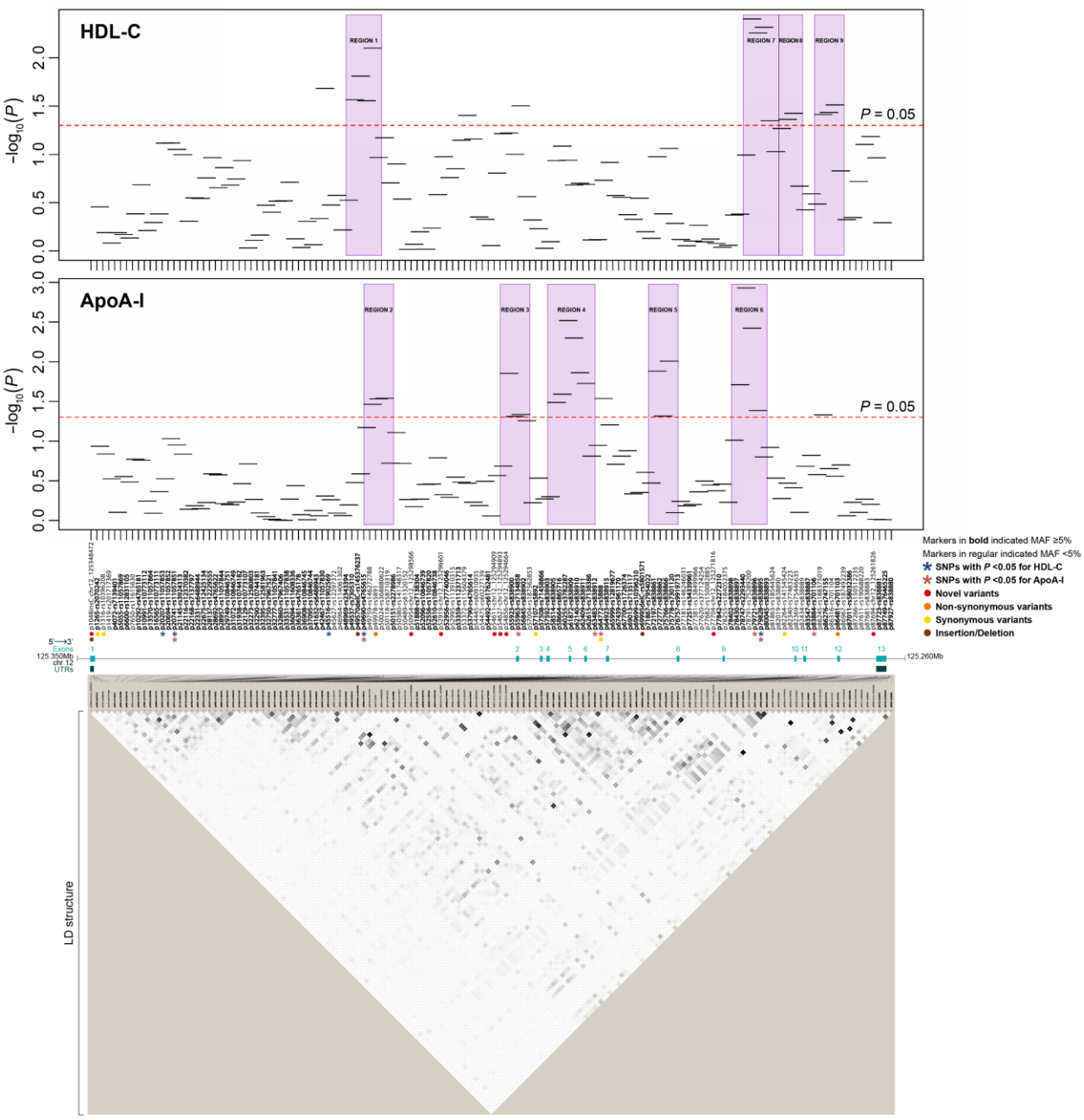

Figure 7.3. Haplotype association plots for high-density lipoprotein cholesterol (HDL-C) and apolipoprotien A-I (ApoA-I; top) and linkage disequilibrium (LD) structure (bottom) of 136 *SCARB1* variants.

The -$\log_{10}$ *P*-values are in the Y-axis. Total 136 genotyped variants are on *SCARB1* gene (5'→3'; RefSeq: hg19, NM_005505) in the X-axis. Marker names are shown as "SNP name-

SNP ID (dbSNP build 139)/chromosome 12 position (for novel variants)". SNPs with MAF ≥5% are shown in **bold**. All 10 novel variants were submitted to dbSNP database: http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=KAMBOH (batch ID: SCARB1_AB) Highlighted areas in the haplotype plots represent significantly associated haplotype regions. Dash line indicates the significance threshold (global P = 0.05). The degree of shades and values (r^2 x 100) in each square of LD plot indicate pairwise LD: black indicating complete LD (r^2 = 1), white indicating no LD (r^2 = 0), and shade intensity indicating the degree of LD (r^2 between 0-1). ApoA-I, apolipoprotein A-I; HDL-C, high-density lipoprotein cholesterol; MAF, minor allele frequency; SNP, single nucleotide polymorphism; UTR, untranslated region.

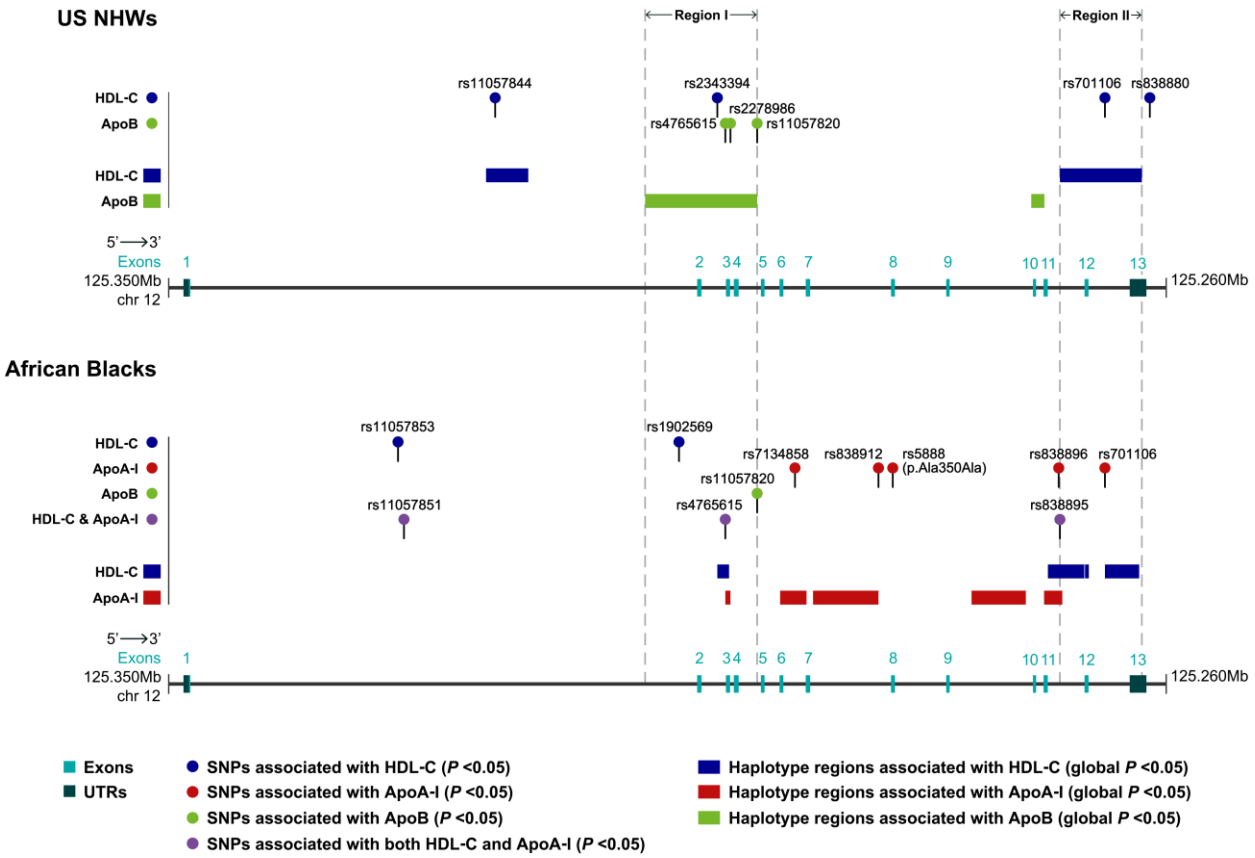

Figure 7.4. Lipid-associated *SCARB1* common variants and haplotype regions identified in US Non-Hispanic Whites (previous study; Ref [48]) and African Blacks (this study).

Lipid-associated variants with MAF ≥5% with *P*-values <0.05 and haplotype regions with global *P*-values <0.05 that were previously identified in US Non-Hispanic Whites (US NHWs; n = 623) are shown in top panel and those identified in African Blacks (n = 788) are shown in bottom panel (see details in **Tables 7.9** and **7.10**). SCARB1 variants and haplotype regions are shown on SCARB1 gene (5'→3'; RefSeq: hg19, NM_005505). All SNP IDs are based on dbSNP build 139. Regions I and II that are defined based on consecutive haplotype windows with evidence of lipid-association in US NHWs (global *P* <0.05; see details in Ref [48]) also show some significant associations in African Blacks (global *P* <0.05; see details in **Tables 7.7** and **7.8**). ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; NHW, Non-Hispanic White; SNP, single nucleotide polymorphism; UTR, untranslated region.

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8.0. GENETIC INFLUENCE OF *ABCA1* VARIANTS ON LIPID TRAITS IN US NON-HISPANIC WHITES

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8.1. ABSTRACT

Background: Abnormal lipoprotein-lipid levels are strongly correlated with the risk of coronary heart disease. ABCA1 is required for high-density lipoprotein cholesterol (HDL-C) formation and cellular cholesterol efflux in lipid metabolism. *ABCA1* mutations cause low HDL-C levels. We aimed to identify common (minor allele frequency [MAF] ≥5%) and low-frequency [LF]/rare (MAF <5%) variants in *ABCA1* and elucidate association with lipid traits in a US Non-Hispanic (NHW) population.

Methods and Results: Sequencing of 50 *ABCA1* exons and exon-intron boundaries in 95 individuals with extreme HDL-C levels identified 404 variants, including 37 novel ones. Subsequently, 250 variants (216 sequence variants and 34 HapMap tagSNPs) were genotyped in the entire 623 NHWs, and 182 variants were examined for associations. Gene-based test revealed the association of *ABCA1* variants with triglycerides (TG) and total cholesterol (TC). Nominal evidence of lipid associations (*P* <0.05) was observed with 26 common variants, of which 17 (16 for TG and 1 for TC) maintained significance after multiple testing correction. Several haplotype windows (n = 60) and regions (n = 16) including multiple sets of LF/rare variants exhibited evidence of lipid-associations.

Conclusions: Our study provides evidence for association of *ABCA1* with TG, suggesting a novel role of *ABCA1* in influencing TG in addition to HDL-C and TC levels in the general population.

8.2. INTRODUCTION

The strong correlation between abnormal lipoprotein-lipid levels including increased low-density cholesterol lipoprotein (LDL-C), decreased high-density cholesterol (HDL-C), and elevated triglycerides (TG) and the risk for coronary heart disease (CHD) [1-3], the leading cause of death worldwide [4], has been well-established. Inter-individual variation in lipid profile is determined by genetic and environmental factors. Genetics plays an important role in affecting lipoprotein-lipid levels with an estimated heritability of 40-80% [5]. Genome-wide association studies (GWASs) have succeeded in identifying common variants (minor allele frequency [MAF] ≥5%) in many genes related to lipid metabolism [6-13]. Nonetheless, the contribution of GWAS-identified variants with modest effect size accounts for a small portion of total genetic variance of lipid phenotypes. It has been suggested that part of the remaining unexplained genetic variance may be explained by low-frequency ([LF], MAF between 1-5%)/rare (MAF<1%) variants [14-16], as evidence from published lipid genetic studies [17-20].

ATP-binding cassette class A1 (ABCA1) is a transmembrane transporter which is highly expressed in liver and macrophages [21]. ABCA1 mediates free cholesterol and phospholipid to lipid-free apolipoprotein (apo) A-I (apoA-I) for pre-β HDL-C particle formation in the first step of reverse cholesterol transport, and it also facilitates cellular efflux of free cholesterol in peripheral tissues [22, 23].

ABCA1 is a 2261-amino acid protein encoded by the *ABCA1* gene (Entrez Gene ID: 19) located on human chromosome 9q31. Genetic mutations in *ABCA1* cause low levels of HDL-C, including familial HDL-C deficiency (MIM: 604091) and Tangiers disease (TD; MIM: 2054000). TD is an autosomal recessive disorder characterized by severe deficiency of HDL-C, hypocholesterolemia, an accumulation of cholesteryl esters (CE) in various tissues, most particularly in macrophage-rich tissues, and an increased incidence of CHD [24]. Disruption of *ABCA1* in TD impairs the removal of cholesterol and phospholipids from cells and subsequently hypercatabolism of apoA-I, leading to tissue deposition of CE and extremely low levels of HDL-C and apoA-I [24]. In addition to markedly reduced HDL-C and apoA-I, decreased LDL-C and increased TG are also observed in TD [24]. Kinetic studies in TD demonstrated that decreased LDL-C and elevated TG levels were attributed to rapid catabolism of abnormal small, cholesterol-poor TG-rich LDL-C particles [25], and reduced lipoprotein lipase activities in lipolysis of TG-rich lipoproteins (i.e., very low-density lipoprotein cholesterol [VLDL-C]) [26], respectively.

ABCA1 knockout mice also display a lipid profile similar to human TD: HDL-C deficiency, markedly low apoA-I, low LDL-C and apoB [27, 28], and high TG [28]. In the absence of *ABCA1* in mice, VLDL formation was reduced due to depleted cholesterol availability, leading to low VLDL-C levels [29]. The hepatic-specific disruption of *ABCA1* enhances VLDL TG production and LDL-C clearance rate, while increased TG secretion and delayed postprandial TG clearance results in low LDL-C and high TG levels [30]. In contrast, mice with overexpression of *ABCA1* show markedly increased HDL-C and apoA-I levels [31]. Whereas, silencing hepatic expression of *ABCA1* reduces large

nascent HDL maturation and increases VLDL TG secretion [32]. Collectively, evidence from human and animal studies indicates the role of *ABCA1* in modulating all major lipoprotein-lipid particles.

In the general population, GWASs have identified associations of common *ABCA1* variants with HDL-C and total cholesterol (TC) [6-12, 33]. Most of the GWAS variants are located in introns with no known functions. Additionally, association of common coding variants in *ABCA1* with lipid traits has also been reported in population-based studies [18, 34-38]. With new sequencing technologies, a large number of LF/rare variants have been discovered. However, little is known about the contribution of LF/rare *ABCA1* variants to lipid phenotypes. To date, a relatively few sequencing studies have demonstrated the association of rare *ABCA1* variants with lipoprotein-lipid levels [17, 18, 39].

In this study, our aim was to identify *ABCA1* sequence variation—common (MAF ≥5%) and LF (MAF between 1-5%)/rare (MAF ≤1%)—and then assess the association between identified *ABCA1* variants and plasma lipid levels in US Non-Hispanic Whites (NHWs). We resequenced the coding regions and exon-intron boundaries of the *ABCA1* gene in 95 individuals with extreme HDL-C levels, followed by genotyping of selected variants in the entire sample of 623 NHWs and evaluating genotype-phenotype association.

8.3. METHODS

8.3.1. Study samples

This study included a total of 623 NHWs (52.65% females), aged 20 to 74 years, from southern Colorado that were originally recruited as part of the San Luis Valley Diabetes Study (SLVDS), as previously described [40]. All study subjects in this study were non-diabetics who had a normal response to a standard oral glucose test. The main characteristics and lipid profile of the entire NHW sample are given in **Table C1**. Informed consent from all study subjects was obtained. The study protocol was approved by the University of Pittsburgh and University of Colorado Denver Institutional Review Boards.

A subset of 95 individuals with extreme HDL-C levels (≥90^{th} or ≤10^{th} percentiles of the distribution) was selected for resequencing. Forty-seven individuals were in the high HDL-C group (≥90^{th} percentile) with mean HDL-C of 77.68 ± 13.32 mg/dL (range: 58-106 md/dL). Forty-eight individuals were in the low HDL-C group (≤10^{th} percentile) with mean HDL-C of 31.81 ± 4.37 mg/dL (range: 20-40 mg/dL). The basic characteristics of 95 resequencing subjects are shown in **Table C2**.

8.3.2. Lipid measurements

At least 8-hour fasting blood samples were collected and immediately placed on ice. Plasma specimens were separated by centrifugation and stored at -80^{o}C before the lipid measurements within 30 days. TC, HDL-C and TG levels were determined by standardized enzymatic assays in the Clinical Research Laboratory at University of Colorado Health Science Center certified by the College of American Pathologists. LDL-C was calculated using the Friedewald formula [41] when TG levels were <400 mg/dL.

8.3.3. DNA sample preparations and sequencing

Genomic DNA was extracted from leukocytes using a standard DNA extraction protocol. Sequencing samples were amplified using specific polymerase chain reaction (PCR) primers, designed by the Primer3 software (Whitehead Institute for Biomedical Research, Whitehead Institute for Biomedical Research, Steve Rozen, Andreas Untergasser, Maido Remm, Triinu Koressaar and Helen Skaletsky, http://primer3.ut.ee/). The *ABCA1* reference sequence (RefSeq) of ~147.15 kb was obtained from NCBI RefSeq database (http://www.ncbi.nlm.nih.gov/, GRCh38: NC_000009.12 [chr9: 104,781,002-104,928,246], NM_005502.3), plus ~1 kb of each 5' and 3' flanking regions. The PCR amplifications produced 96 amplicons with an average size of 1,008 bp (range: 483-1,279 bp) covering a total of 50 exons and its exon-intron boundaries. Total 96 amplicons accounted for ~54% (80.14 kb) of total size of *ABCA1*. PCR protocols and cycling conditions are available upon requests. The sequence primers and amplicon sizes are provided in **Table C3**.

Sequencing reactions were performed according to the manufacturer's protocols. The sequencing was operated on the Applied Biosystems 3730xl DNA Analyzer in the commercial facility (Beckman Coulter Genomics, Danvers, MA, USA). Sequencing calls were analyzed in our laboratory using Variant Reporter (version 1.0, Applied Biosystems, Waltham, MA, USA), and Sequencher (version 4.8, Gene Codes Corporation, Ann Arbor, MI, USA) softwares.

8.3.4. Variant selection for genotyping

We applied the following selection criteria of sequence variants for subsequent genotyping in the entire NHW sample: (1) Tagging single nucleotide polymorphisms (tagSNPs, n = 114) were selected using Haploview Tagger (Broad Institute of MIT and Harvard, Cambridge, MA, http://www.broadinstitute.org/) [42] with MAF ≥4% and an r^2 threshold of 0.80; (2) LF/rare non-exonic variants (MAF <5%) that were carried in at least two individuals (n = 83, including 14 with MAF between 4-5% that were not chosen by Tagger analysis); (3) (LF/rare exonic variants (MAF <5%) that were carried in at least one individual (n = 14); and (4) variants (n = 5) with either low sequencing call rate (<40%) or deviation from Hardy-Weinberg equilibrium (HWE; P <0.001). In order to cover common variants in the entire *ABCA1* gene, we selected additional common tagSNPs (MAF ≥5%, n = 34) from Northern and Western European Ancestry (CEU) HapMap data (Release #27) using Haploview Tagger analysis with an r^2 threshold of 0.80 (**Table C4**; **Figure C1**), irrespective of the LD pattern of common sequence tagSNPs already selected. Thus, a total of 250 variants (216 from sequencing stage

(**Tables C5-C8**), and 34 from HapMap-CEU tagSNPs) were selected for genotyping in a total sample of 623 NHWs.

8.3.5. Genotyping

Selected variants were genotyped using either iPLEX Gold assays (Sequenom, Inc., San Diego, CA, USA) at the Genomics and Proteomics Core Laboratories, University of Pittsburgh (http://www.genetics.pitt.edu/) or TaqMan method (Applied Biosystems, Waltham, MA, USA) in our laboratory. Genotyping reactions were performed following the manufacturers' instructions.

Of 250 selected variants for genotyping, 56 failed genotyping (12 failed assay designs and 44 failed runs) and 11 were removed after checking the genotyping quality controls (QC). The QC-passed variants included those with a success rate ≥90%, a discrepancy rate <1% among the 10% of replication samples, and no deviation from HWE (P <2.73 x 10^{-4} after Bonferroni correction). A total of 183 (182 bi-allelic and 1 tri-allelic) variants were successfully genotyped and passed QC (**Table C9**), of which 182 bi-allelic were proceeded for downstream analyses.

8.3.6. Statistical analysis

Allele and genotype frequencies, including HWE P-values were estimated. Pairwise correlations (r^2) were calculated using Haploview program.

We applied linear regression models to test association of 182 QC-passed genotyped variants with four major lipid traits—HDL-C, LDL-C, TC, and TG—under an

additive model. HDL-C and TG values were Box-Cox transformed to achieve normality. The outliers beyond mean ± 3.5 standard deviation were excluded prior to analyses. The covariates included gender, age, body mass index, and smoking (non-/ex-/current smoker). We used stepwise regression to identify the most parsimonious set of the covariates for each trait and then applied into the model.

Gene-based association test was performed using versatile association test [43] to elucidate the combined effects of *ABCA1* common and LF/rare variant on four lipid traits. A significance threshold for gene-based association was set at a *P*-value of 0.05.

For single-site analysis, each variant was assessed for association with lipid traits. For each trait, common variants (MAF ≥5%, n = 116) were adjusted for multiple testing correction using Benjamini-Hockberg method [44] to control the false discovery rate (FDR). We considered a single-site *P*-value of <0.05 for suggestive evidence of association and an FDR value (*q*-value) of <0.20 for statistical significance, as applied previously [45]. Because of low statistical power for LF/rare variants (MAF <5%, n = 66), those with a single-site *P*-value <0.05 were interpreted separately. For the common TC- and TG-associated variants, we additionally performed conditional analyses to identify independent signals. We used tagger analysis with r^2 ≥0.50 capturing the top associated variants for each bin (4 bins for TC and 11 bins for TG) and included in the conditional analysis.

Haplotype association analysis of 182 variants was carried out with a fixed sliding window, using four variants per window and one overlapping variant. A global *P*-value of <0.05 was considered significant for haplotypes with frequency >1% in each window.

Rare variant analysis was performed using an optimal sequence kernel association test (SKAT-O) [46] to examine the effect of segregating LF/rare variants. LF/rare variants were tested at three different MAF thresholds: <5% (n = 66), ≤2% (n = 40), and ≤1% (n = 20). A SKAT-O significant threshold was set at <0.05.

Gene-based association test was performed using the VEGAS program (http://gump.qimr.edu.au/VEGAS/) [43]; all other analyses were conducted using the R statistical software (http://www.r-project.org/).

8.3.7. Replication study

To replicate single-site associations, we used 744 European Americans (EAs; 61.42% females), aged 45-74 years, as part of the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) study. In brief, the Heart SCORE study is a longitudinal cohort study comprised of ~2000 individuals from Pittsburgh, Pennsylvania [47]. The replication sample is based on the first consecutive 744 EAs who provided DNA samples after obtaining informed consent and were successfully genotyped with <2% missing data. The basic characteristics of 744 EAs are given in **Table C1**.

The Heart SCORE sample was genotyped for 49,094 selected variants (including *ABCA1* variants, n = 138) using Illumina CVD BeadChip. Single-site associations for 11 common *ABCA1* variants associated with lipid traits in 623 NHWs from SLVDS was examined in the replication sample. All 11 variants had genotype call rate >95% and did not deviate from HWE (P <1 x 10^{-6}).

The replication analyses were performed under additive linear regression model using the R statistical software. For each trait, significant covariates identified by

stepwise regression were added to the model. The covariates included gender, age, education, body mass index, smoking, drinking, history of health conditions (diabetes, hypertension), and current medications (anti-hypertensive and lipid-lowering). Lipid phenotypes required Box-Cox transformation to achieve normality prior further analyses. Meta-analysis using the discovery and replication data on selected variants was performed using METAL software (http://www.sph.umich.edu/csg/abecasis/Metal/) [48].

8.4. RESULTS

8.4.1. Identification of *ABCA1* sequence variants

By sequencing 95 individuals with extreme HDL-C levels, we identified 404 variants (400 bi-allelic and 4 tri-allelic), of which 37 were novel (36 bi-allelic and 1 tri-allelic; already submitted in dbSNP database: http://www.ncbi.nlm.nih.gov/projects/SNP/snp_viewTable.cgi?h=KAMBOH (batch IDs: ABCA1_EA and ABCA1_EA+AB) (**Tables C5** and **C6**). The majority of these variants (n = 377) were single nucleotide variations (SNVs) and the rest (n = 27) were short insertion and deletion variations (indels) of 1-21 bp in length.

Of 404 variants, 356 were located in introns including 3 within splice sites (defined as ± 20 bp from the start or end of an exon), 34 were located in exons (21 coding: 11 non-synonymous and 10 synonymous; **Table C7**), and the remaining were

located in flanking regions (**Figure C2**). Among ten 5' flanking variants, five (p252insTGTTT/insTGTTTGTTT-chr9_104928904, rs2980083, rs16905745, rs2246298, and rs2740483) were located in the putative promoter region [49]. One variant (rs10991377) in exon 50-3'-untranslated region (UTR) was close to a binding-site of miRNA340-5p (TargetScan version 6.2).

Tagger analysis with an r^2 threshold of 0.80 for 239 common alleles (MAF ≥5%) identified 93 bins (**Table C8**) and 22 haplotype blocks (using the definition of Gabriel, et al [50]) with sizes ranging from 0-20 kb (**Figure C3**).

Of 169 LF/rare variants (MAF between 1-5%, n = 102; MAF ≤1%, n = 67), 31 (including rs9282543 [p.Val399Ala]) were present only in the high HDL-C group; whereas 45 (including rs143299210 [p.Gly572Gly], rs145582736 [p.Gly815Gly], rs76881554 [p.Ser1181Phe], rs141980942 [p.Ile1802Ile] and p122751-chr9_104806405 [p.Glu1434Lys]) were present only in the low HDL-C group. In the high HDL-C group, 45 of 47 individuals carried at least one LF/rare allele (range: 1-24). While, in the low HDL-C group, 45 of 48 individuals carried at least one LF/rare allele (range: 1-32). There was no difference in the numbers of individuals carrying at least one LF/rare alleles between the two extreme HDL-C groups (P = 0.664).

Of 37 novel variants (31 SNVs and 6 indels; **Table C6**), 35 had MAF <5% (MAF between 1-5%, n = 13; MAF ≤1%, n = 22). Most were located in introns (n=33), two were in 3' flanking region, one (p122751-chr9_104806405 [p.Glu1434Lys]) was in exon 31, and the last (p252insTGTTTGTTT/TGTTT-chr9_104928904) was in a putative promoter region [49]. Among these 37 novel variants, 15 (including p122751-

chr9_104806405 [p.Glu1434Lys]) were present only in the high HDL-C group, whereas eight were present only in the low HDL-C group.

8.4.2. Genotyping of *ABCA1* variants

Of the total 250 selected variants (216 sequence variants, plus 34 common HapMap-CEU tagSNPs) for genotyping in the entire sample of 623 NHWs, 183 (182 bi-allelic and 1 tri-allelic) were successfully genotyped and passed QC (**Tables C9**, **C10**; **Figures C4**, **C5**).

All 182 bi-allelic successfully genotyped variants (176 SNVs and 6 indels) were evaluated for association with four major lipid traits (HDL-C, LDL-C, TC, and TG; **Table 8.1**). The majority of these 182 variants are already known (n = 173). Among 16 (out of total 37) novel variants selected for genotyping, nine (7 SNVs and 2 indels) were successfully genotyped. One hundred and sixteen of 182 variants had MAF≥5%, including two known coding variants (rs2066716 [p.Thr1427Thr] and rs2230808 [p.Lys1587Arg]). The rest had MAF <5% (MAF between 1-5%, n = 46; MAF≤1%, n = 20), including 10 coding variants (9 known: rs9282543 [p.Val399Ala], rs143299210 [p.Gly592Gly], rs20667181 [p.Val771Met], rs138880920 [p.Lys776Asn], rs145582736 [p.Glu815Gly], rs33918808 [p.Glu1172Asp], rs76881554 [p.Ser1181Phe], rs41277763 [p. Thr1512Thr], rs9282537 [p.Gly2061Gly]; 1 novel: p122751-chr9_104806405 [p.Glu1434Lys]). Furthermore, 115 of 182 genotyped variants were located in introns, 20 in exons (9 non-synonymous, 3 synonymous, and 8 exons-UTRs), and the remaining seven in flanking regions (**Figure C6**).

8.4.3. Associations of *ABCA1* variants with lipid traits

Gene-based association

Gene-based association analysis revealed significant association between 182 *ABCA1* genotyped variants and TG (*P* = 0.0108; best SNP: rs117745266, MAF = 0.0142; **Table 8.2**). A trend toward association with TC was also observed (*P* = 0.1630). No gene-based association was detected with HDL-C or LDL-C.

Association of common variants

Main study

Single-site analysis results showed nominal association (*P* <0.05) of 26 variants (MAF ≤5%) with at least one lipid trait (21 with TG, 5 with TC, 5 with LDL-C, and 2 with HDL-C), of which 6 were associated with more than one trait (**Tables C11-C14**). After multiple testing correction by controlling FDR, 17 association signals (16 for TG and 1 for TC) remained significant at FDR <0.20 (**Table 8.3**; **Figure 8.1**).

The top signal for TG was observed with rs2066716 [p.Thr1427Thr] located in exon 31 (β = 0.01169, *P* = 0.0016, FDR = 0.098).

The best SNP for TC was rs4743763 located in intron 14 (β = 9.1145, *P* = 0.0005, FDR = 0.057). This SNP was also associated with LDL-C (β = 5.1763, *P* = 0.039, FDR = 0.691) and TG (β = 0.0524, *P* = 0.0418, FDR = 0.231).

The lead SNP for LDL-C was rs3831227 located in intron 33 (β = 6.8557, *P* = 0.0113, FDR = 0.691), which also showed association with TC (β = 7.8474,

P = 0.0058, FDR = 0.335). For HDL-C, the best SNP was rs2777795 located in intron 1 (β = -0.0396, *P* = 0.0237, FDR = 0.883, which was also associated with TG (β = 0.0874, *P* = 0.0200, FDR = 0.154).

Based on the LD structure of 16 TG-associated variants with FDR significance (FDR <0.20; **Figure 8.1**), three (rs4149297, rs4149314, and rs55993392) were in strong LD (r^2 ≥0.80, range: 0.84-0.96) and 12 were in moderate LD (r^2 between 0.40-0.80) as follows: rs2777795, rs2254708, and rs2575876 (r^2 = 0.52-0.55); rs3905001 and rs3758294 (r^2 = 0.63); rs4149297 and rs6479283 (r^2 = 0.45); rs2066716 and rs62566031 (r^2 = 0.49); rs4149329, rs4149337, and rs2066882 (r^2 = 0.43-0.74).

To identify independent association among TG- (n = 21) and TC- (n = 5) associated variants, we performed conditional analysis. The conditional results indicate that 7 signals for TG (conditional *P* = 0.1020-0.0009; **Figure 8.2**) and 2 signals for TC (conditional *P* = 0.0571-0.0145; **Figure 8.3**) are likely to be independent.

Replication study

Replication of total 26 lipid-associated *ABCA1* variants in 623 NHWs from SLVDS listed in **Table 8.3** was sought in an independent sample of 744 EAs from the Heart SCORE cohort that was previously genotyped for 49,094 variants using Illumina CVD BeadChip. Eleven of the 26 lipid-associated *ABCA1* variants were represented on the Illumina CVD BeadChip. As shown in **Table 8.4**, five (rs2777795, rs3905001, rs2575876, rs10120087, and rs2066716 [p.Thr1427Thr]) of the TG-associated variants in 623 NHWs exhibited the same trend of associations in the replication sample (although none of them achieved nominal significance at *P* = 0.05) as reflected in the improved meta-analysis *P*-values (meta-*P* = 0.0162-0.0016). Furthermore, one SNP (rs12338782)

that was associated with TC (*P* = 0.0367) and LDL-C (*P* = 0.0474) in the NHW SLVDS sample was also replicated in the Heart SCORE sample (*P* = 0.0275 for TC and *P* = 0.0378 for LDL-C).

Association of haplotypes

Haplotype association analysis revealed 60 haplotype windows significantly associated (global *P* <0.05) with at least one lipid trait (47 with TG, 21 with TC, 12 with LDL-C, and 1 with HDL-C), of which 15 were associated with more than one trait (**Tables C15-C17**; **Figure C7**). The most number of haplotype windows with significant evidence of association (global *P* <0.05) was found with TG (n = 47), of which the best signal was detected in window #169 spanning between intron 48 and intron 49 (global *P* = 0.0005). This window contained rs2777797 that yielded FDR-significant association with TG (*P* = 0.0117, FDR = 0.136) in single-site analysis.

There were 20 and 12 haplotype windows significantly association with TC, and LDL-C, respectively. The window #118 spanning in intron 23 showed the strongest signal for TC (global *P* = 0.0008) and LDL-C (global *P* = 0.0025). This window contained rs12338782 that was associated with TC (*P* = 0.0367, FDR = 0.820) and LDL-C (*P* = 0.0474, FDR = 0.691) in single-site analysis. There was only one haplotype window (#63) associated with HDL-C (global *P* = 0.0345); this window did not contain any HDL-C associated variants observed in single-site analysis.

Using consecutive haplotype windows with significant associations (global *P* <0.05), we were able to identify 16 regions significantly association with TG (n = 9), TC (n = 5), and LDL-C (n = 4; **Figure 8.4**; **Table C18**). Of nine regions (regions: 2, 3, 5, 7, 9, 11, 12, 13, 15) significantly associated with TG, region 15 spanning between intron

45 and exon 50-3' UTR exhibited the strongest signal. This region harbored 13 variants (rs80237807, p141039ins4-chr9_104788117, rs9282537 [p.Gly2061Gly], rs4149337, rs142157628, rs2066882, rs2777797, rs62566031, rs1331924, rs117745266, rs2482433, rs4149338, and rs41432545), of which three (rs4149337, rs2066882, and rs2777797) were FDR-significantly associated with TG in single-site analysis.

Five regions (regions: 1, 8, 10, 14, 16) were significantly associated with TC. Four regions (regions: 1, 4, 6, 10) were significantly associated with LDL-C. Region 10 spanning between intron 23 and intron 24 yielded the best signal for both TC and LDL-C. This region harbored seven variants (rs12338782, rs58866705, rs9299383, rs2297402, rs13292447, rs33918808 [p.Glu1172Asp], rs2297401), including rs12338782, as of the single-site results, associated with both TC (*P* = 0.0367, FDR = 0.820) and LDL-C (*P* = 0.0474, FDR = 0.691).

Association of low-frequency/rare variants

We used SKAT-O test to analyze the lipid association of 66 LF/rare variants, which were categorized in three groups on the basis of MAF thresholds: <5% (n = 66), ≤2% (n = 40), and ≤1% (n = 20). A set of 40 LF/rare variants with MAF ≤2% yielded nominal association with TC (*P* = 0.0437; **Table 8.5**). A trend toward association (*P* <0.20) for the group of 66 LF/rare variants with MAF≤5% was also observed with TC (*P* = 0.1356) and TG (*P* = 0.1694). No association of LF/rare variants with either HDL-C or LDL-C was detected at any MAF thresholds.

To further evaluate the effects of LF/rare functional variants on lipid traits, we performed SKAT-O test for 13 LF/rare exonic variants (7 non-synonymous, 3 synonymous, and 3 exons-UTRs). SKAT-O revealed that a group of seven rare exonic

variants (MAF ≤1%, range: 0.008-0.0057) was marginally associated with HDL-C (*P* = 0.0552), LDL-C (*P* = 0.0749) and TC (*P* = 0.0868; **Table 8.5**). Following the SKAT-O results, we individually examined these seven LF/rare exonic variants and found two of them were associated with HDL-C, LDL-C or TC levels (**Table 8.6**). One was a non-synonymous variant in exon 17 (rs145582736 [p.Glu815Gly], MAF = 0.0008) associated with large reduction in HDL-C (β = -0.6219, *P* = 0.0009). Another variant was located in exon 50-3' UTR (rs41432545, exon 50, MAF = 0.0008) having an elevated effect on LDL-C (β = 86.3036, *P* = 0.0312) and TC (β = 95.4413, *P* = 0.0228).

8.4.4. Functional prediction of identified *ABCA1* variants

We used the RegulomeDB database (version 1.1, Stanford University, CA, http://regulomedb.org/) [51] to determine the regulatory function for all 436 variants (404 identified by sequencing and 32 common HapMap-CEU tagSNPs). The majority (n = 321) of these 436 variants had RegulomeDB scores from 1b to 6, likely to be implicated in gene regulation (**Tables C19** and **C20**). However, a small portion (n = 24) had significant genetic regulatory functions (scores <3), of which three were strong expression quantitative trait loci (rs2437821, score 1b; rs1800977 and rs2487050, score 1f).

Twenty-one of total 26 lipid-associated variants observed in single-site analysis, had regulatory information (scores 2b-6). Of 17 FDR-significant variants, two (rs4149297, score 2b; rs3905001, score 3a) were likely to affect transcription factor binding and motif.

Among 37 novel variants, 24 variants had supportive evidence for regulatory functions (scores 2b-6). Two novel variants (p1902-chr9_104927254 and p2167-chr9_104926989) in intron 1 were likely to have significant regulatory roles with score 2b.

8.5. DISCUSSION

We sequenced 50 exons and exon-intron boundaries of *ABCA1* in 95 NHWs with extreme HDL-C levels and identified 404 variants. Subsequently, we selected 250 variants (216 from sequencing stage and 34 from HapMap-CEU tagSNPs) for genotyping in the entire sample of 623 NHWs. Ultimately, 182 bi-allelic variants that successfully genotyped and passed QC were evaluated for association with four major lipid traits using single-site, haplotype and rare variant analyses.

8.5.1. Association of common and LF/rare *ABCA1* variants with lipid traits

Initial gene-based analysis revealed a significant association between 182 *ABCA1* variants and TG (P = 0.0180), and also a trend association with TC (P = 0.1630; **Table 8.2**). Single-site analysis identified nominal evidence for association of 26 common variants (P <0.05): 21 with TG, 5 with TC, 5 with LDL-C, and 2 with HDL-C (**Table 8.3**;

Figure 8.1), of which 17 (16 with TG and 1 with TC) maintained significance after controlling FDR (FDR <0.20).

We successfully replicated one variant (rs12338782 in intron 23) associated with both TC and LDL-C in an independent sample of 744 EAs (P = 0.0024 for TC and P = 0.0041 for LDL-C; **Table 8.4**). Additionally, five TG-associated variants showed similar trend of association in the replication sample and also reached significance threshold in the meta-analysis; notably, an exonic variant (rs2066716 [p.Thr1427Thr] in exon 31 (meta-P = 0.0088).

Majority of the published genetic studies have revealed the association of common *ABCA1* variants with HDL-C and/or TC [6-12, 33]. However, in our study, we have found stronger association of common *ABCA1* variants with TG as compared to HDL-C or TC. Previously, some studies have also indicated the association of common *ABCA1* variants with TG. One study identified two variants (rs2575875 and rs4149272) having elevated effects on postprandial TG and TG-rich lipoprotein levels in healthy Spanish men [52]. Another study reported one variant (rs2230808 [p.Lys1578Arg]) promoting TG levels in healthy Greek women [38]. In contrast, a large cohort study of African Americans demonstrated two additional variants (rs2515602 and rs1800977) associated with lower TG [53]. Furthermore, association of common *ABCA1* variants with TG has been shown in subjects with extreme HDL-C and/or CHD [34, 35, 54, 55]. Thus, our findings together with published reports support the role of common *ABCA1* variants in affecting TG levels.

In fact, elevated TG and by-products of TG (i.e., remnant lipoprotein particles) are shown to be involved in the development of atherosclerosis [1, 2, 56]. Moreover,

among individuals experiencing premature CHD, high TG is commonly seen in conjunction with low HDL-C, although the mechanism behind this inverse relationship has not been completely understood. Additionally, this inverse relationship between TG and HDL-C levels has been seen in humans with *ABCA1* mutations (i.e., TD) [24] as well as in *ABCA1* transgenic animals [27, 28, 31]. Furthermore, two variants (rs2777795 and rs2575876) in the current study were found to be jointly associated with increased TG and decreased HDL-C, reflecting the plausible involvement of *ABCA1* in the inverse correlation between TG and HDL-C. Altogether, these data provide additional insights into the physiological functions of *ABCA1* in TG metabolism and the mechanistic connection between TG (including TG sub-fractions/by-products) and HDL-C in relation to CHD.

In agreement with single-site analysis, haplotype analysis identified 60 haplotype windows associated with at least one lipid trait (**Tables C15-C17**; **Figure C7**). The most significant association signals were found with TG in 47 haplotype windows, of which windows #169 exhibited the best signal for overall haplotypes and for TG (global P = 0.0005). Notably, 45 of total 60 associated haplotype windows contained 23 associated variants with lipid traits detected in single-site analysis. Likewise, 13 of 16 associated regions contained 18 single-site associated variants (1-3 variants per region). These observations indicate that most single-site associated variants reported in the current study substantially influence the association signals of haplotype windows and regions, implying the causal/functional *ABCA1* variants related to lipid metabolism might exist in these regions. Thus, these associated windows/regions are candidates for future deep-resequencing in order to identify the real causal/functional *ABCA1* variants.

SKAT-O analysis revealed a significant association of a set of LF/rare variants with MAF ≤2% (n = 40) with TC (*P* = 0.0437; **Table 8.5**). A trend toward association of a set of LF/rare variants with MA F≤5% (n = 66; *P* <0.20) with TG, as well as a borderline association of a set of rare exonic variants with MAF≤1% (n = 7; *P* <0.10) with HDL-C, LDL-C, and TC were also detected in the present study. Moreover, two known rare exonic variants (rs145582736 [p.Glu815Gly] and rs41432545) appeared to have large effects on HDL-C, LDL-C and TC at the extreme levels (above or under the 10^{th} percentile; **Table 8.6**). So, our findings suggest the contribution of LF/rare variants to lipoprotein-lipid levels.

Based on the RegulomeDB information in determining the regulatory function of total 436 variants identified in the current study, a small number (n = 24) were supported by strong regulatory evidence (scores <3). Of 26 single-site associated variants, only one variant in intron 8 (rs4149297) was likely to have potentially regulatory function (score 2a). Because the majority of the identified variants, including lipid-associated variants are non-coding with unknown functions, future functional experiments are needed to gain biological insights of *ABCA1* variants.

8.5.2. Comparing to *ABCA1* variants with previously reported lipid-association

Several common GWAS-identified *ABCA1* variants with previously reported associations with HDL-C and/or TC (i.e., rs18003025 [10, 12], rs3847303 [8], rs3890182 [7], rs4149268 [6], and rs4149274 [6]) were not identified in our study. These GWAS-identified variants are located in introns and were not part of exons and exon-intron junctions sequenced in this study. However, using the HapMap-CEU tagSNPs database

(**Table C4**; **Figure C1**), one GWAS variant (rs18003025) [10, 12] with reduced effect on HDL-C and TC was in strong LD (bin 31; r^2 = 0.94) with rs2575876 that was associated with decreased HDL-C as well as increased TG in our NHW sample. So, the association of rs18003025 was replicated in the current study with the same effect through it proxy SNP, rs18003025.

Seven non-synonymous coding *ABCA1* variants (rs2230805 [p.Arg219Lys], rs2066718 [p.Val771Met], rs138880920 [p.Lys776Asn], rs2066715 [p.Val825Ile], rs2066714 [p.Ile883Met], rs33918808 [p.Glu1172Asp], and rs2230808 [p.Lys1587Arg]) with previous evidence of lipid-association [17, 18, 34-38, 57] were identified in our sequencing stage (**Table C7**) and four of them (rs2066718 [p.Val771Met], MAF = 0.0287; rs138880920 [p.Lys776Asn], MAF = 0.0041; rs33918808 [p.Glu1172Asp], MAF = 0.0265; rs2230808 [p.Lys1587Arg], MAF = 0.2327) were successfully genotyped in our entire sample. However, none were associated with lipid traits in our NHW sample (**Tables C11-C14**).

Four lipid-associated variants in this study (rs3905001 with TG; rs2575876 with HDL-C and TG; rs10120087 with TG; rs4743763 with TC, LDL-C, and TG; **Table 8.3**) have been previously reported to be associated with lipid traits. The first three variants showed suggestive association with HDL-C in a meta-analysis of four large EA cohorts [58]. In addition, rs2575876 was also found to be associated with TC/esterified cholesterol ratios in a Finnish population [11]. The last variant was associated with HDL-C among African Americans [33]. To our knowledge, we report novel associations between the remaining 22 *ABCA1* variants and lipid traits.

Comparing the results of multiple ethnic groups, we notice inconsistent patterns of associations (i.e., differences in trait-associations, effect alleles, minor allele frequencies, and directional effects). This could be explained by differences in genetic structures as well as the interplay between gene-gene, and gene-environment among study populations. Therefore, understanding the complete *ABCA1* genetic architecture across various populations is essential for refining association signals in order to discover the causal/functional *ABCA1* variants related to lipid traits.

8.6. STUDY LIMITATIONS

The current study has some limitations. First, since we sequenced only exons and exon-intron boundaries of *ABCA1* gene, we may have missed potential/novel regulatory variants in introns and flaking regions of the gene. Second, our positive findings need to be replicated in larger set of independent samples for confirmation. Third, we did not explore the functions of lipid-associated *ABCA1* variants. Further functional studies are required to identify causative variants.

8.7. CONCLUSIONS

In this study, we successfully identified common and LF/rare variants, including 37 novel ones in *ABCA1* by sequencing individuals with extreme HDL-C levels. Our association results of *ABCA1* variants with four major lipid traits (HDL-C, LDL-C, TC, and TG) showed the most evidence of significant association with TG in multiple analyses. To our best knowledge, this is the first comprehensive study suggesting that *ABCA1* variants contribute to TG variation in the general population. In addition to 26 common *ABCA1* variants, several haplotype windows and regions including multiple sets of LF/rare variants of *ABCA1* also exhibited evidence of association with lipoprotein-lipid levels. Future investigation to gain a fully understanding of the role(s) of *ABCA1* in the regulation of lipoprotein-lipid levels is warranted, which may uncover new/potential therapeutic interventions for CHD and lipid-related diseases.

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Disclosures

None.

Table 8.1. Characteristics of 182 bi-allelic *ABCA1* genotyped variants.

	Total N (%)	MAF ≥5% n (%)	MAF between 1-5% n (%)	MAF ≤1% n (%)
Total Variants	**182 (100.00)**	**116 (63.74)**	**46 (25.27)**	**20 (10.99)**
By Known/Novel[a]				
Known	**173 (95.05)**	**116 (63.74)**	**44 (24.17)**	**13 (7.14)**
- SNVs	169	112	44	13
- Short Indels	4	4		
Novel[a]	**9 (4.95)**		**2 (1.10)**	**7 (3.85)**
- SNVs	7		2	5
- Short Indels	2			2
By Locations				
5' flanking	5	5		
5' UTR-Exons	2	1	1	
Exons	12	2	4	6
Introns	155	103	40	12
3' UTR-Exon	6	4	1	1
3' flanking	2	1		1
By Amino Acid Changes	**12**	**2**	**4**	**6**
Non-synonymous	8	1	2	5
Synonymous	4	1	2	1

Indels, insertion and deletion variations; MAF, minor allele frequency; SNV, single nucleotide variant; UTR, untranslated region.

[a] dbSNP build 141: GRCh38; 37 novel variants were submitted to dbSNP database: http://www.ncbi.nlm.nih.gov/projects/SNP/snp_viewTable.cgi?h=KAMBOH (batch IDs: ABCA1_EA and ABCA1_EA+AB).

Table 8.2. Result of gene-based association analysis.

Trait	No of Variants	Test Statistics	*P*	Best SNP		
				SNP Name[a]-RefSNP ID[b]	MAF	*P*
HDL-C	182	187.28	0.4010	p103375-rs145582736 [p.Glu815Gly]	0.0008	0.0009
LDL-C	182	194.10	0.3320	p103375-rs145582736 [p.Glu815Gly]	0.0008	0.0007
TC	182	222.16	0.1630	p98255-rs4743763	0.2771	0.0005
TG	181[c]	302.81	**0.0108**	p143860-rs117745266	0.0142	0.0015

MAF, minor allele frequency; SNP, single nucleotide polymorphism.
Significant gene-based *P*-values (*P* <0.05) are shown in **bold**.
[a] RefSeq *ABCA1:* NCBI GRCh38, NC_000009.12, NM_005502.3.
[b] dbSNP build 141: GRCh38.
[c] One rare variant (p103375-rs145582736 [p.Glu815Gly], MAF = 0.0008) were excluded due to missing phenotype values.

Table 8.3. Significant single-site association (*P* <0.05) of *ABCA1* common variants (MAF ≥5%).

SNP Name[a]	RefSNP ID[b]	Chr9 Position[a]	Location	Amino Acid Change	RegDB Score[c]	Major/ Minor Alleles	MAF	β	SE	*P*	FDR	Other Trait(s) (Effect)
HDL-C												
p19072	rs2777795	104910084	Intron 1		5	C/T	0.1088	-0.0396	0.0175	**0.0237**	0.883	TG (↑)
p25698	rs2575876	104903458	Intron 2		4	C/T	0.2681	-0.0249	0.0120	0.0392	0.883	TG (↑)
LDL-C												
p212	rs10991419	104928944	5' flanking		5	G/A	0.0963	-8.4891	3.9391	0.0316	0.691	
p44418	rs11789603	104884738	Intron 3		7	G/A	0.1071	7.1477	3.6020	0.0477	0.691	
p98255	rs4743763	104830901	Intron 14		5	T/A	0.2771	5.1763	2.5024	0.0390	0.691	TC (↑), TG (↑),
p110778	rs12338782	104818378	Intron 23		7	G/A	0.0730	-8.3051	4.1798	0.0474	0.691	TC (↓)
p126865 delCT	rs3831227	104802291	Intron 33		5	[-/CT]	0.2382	6.8557	2.6984	**0.0113**	0.691	TC (↑)
TC												
p89896	rs2472386	104839260	Intron 9		5	C/T	0.3522	5.4813	2.4007	0.0228	0.820	
p98255	rs4743763	104830901	Intron 14		5	T/A	0.2771	9.1145	2.6026	**0.0005**	**0.057**	LDL-C (↑), TG (↑)
p108439	rs55993392	104820717	Intron 20		4	G/C	0.0997	7.7176	3.9067	0.0487	0.820	TG (↑)
p110778	rs12338782	104818378	Intron 23		7	G/A	0.0730	-9.2189	4.4027	0.0367	0.820	LDL-C (↓)
p126865 delCT	rs3831227	104802291	Intron 33		5	[-/CT]	0.2382	7.8474	2.8330	0.0058	0.335	LDL-C (↑)
TG												
p8315	rs2472510	104920841	Intron 1		5	A/C	0.2653	-0.0539	0.0258	0.0371	0.231	
p9464	rs67348902	104919692	Intron 1		3a	C/T	0.0797	0.0888	0.0429	0.0390	0.231	
p19072	rs2777795	104910084	Intron 1		5	C/T	0.1088	0.0874	0.0375	0.0200	**0.154**	HDL-C (↓)
p23373	rs3905001	104905783	Intron 1		3a	C/G	0.2932	-0.0618	0.0254	0.0151	**0.146**	
p24290	rs2254708	104904866	Intron 1		5	A/G	0.1742	0.0869	0.0300	0.0039	**0.098**	
p25698	rs2575876	104903458	Intron 2		4	C/T	0.2681	0.0742	0.0260	0.0045	**0.098**	HDL-C (↓)
p26622	rs3758294	104902534	Intron 2		6	A/G	0.2118	-0.0684	0.0288	0.0178	**0.148**	
p30287	rs10120087	104898869	Intron 2		5	G/T	0.0985	-0.0847	0.0377	0.0251	**0.182**	
p84376	rs4149295	104844780	Intron 8		4	A/T	0.1145	0.0993	0.0361	0.0061	**0.098**	
p87333	rs4149297	104841823	Intron 8		2b	T/C	0.0998	0.0983	0.0375	0.0090	**0.116**	
p89636	rs6479283	104839520	Intron 9		6	G/A	0.1797	0.0684	0.0287	0.0176	**0.148**	
p98255	rs4743763	104830901	Intron 14		5	T/A	0.2771	0.0524	0.0257	0.0418	0.231	LDL-C (↑), TC (↑)
p107086	rs4149314	104822070	Intron 19		5	A/G	0.0898	0.1178	0.0388	0.0025	**0.098**	
p108439	rs55993392	104820717	Intron 20		4	G/C	0.0997	0.1043	0.0378	0.0060	**0.098**	TC (↑)
p122732	rs2066716	104806424	Exon 31	Thr1427Thr	7	G/A	0.0972	0.1169	0.0368	**0.0016**	**0.098**	
p126971	rs2297404	104802185	Intron 33		5	G/C	0.0831	-0.0831	0.0403	0.0399	0.231	
p136692	rs4149329	104792464	Intron 42		6	T/A	0.1288	-0.0856	0.0345	0.0134	**0.142**	
p139711	rs4149335	104789445	Intron 44		6	A/T	0.1073	-0.0796	0.0369	0.0316	0.216	
p142619	rs4149337	104786537	Intron 47		7	T/C	0.0691	-0.1237	0.0448	0.0060	**0.098**	
p142975	rs2066882	104786181	Intron 48		6	A/G	0.0909	-0.1094	0.0403	0.0068	**0.098**	
p143062	rs2777797	104786094	Intron 48		7	T/G	0.1084	0.0974	0.0385	0.0117	**0.136**	

del, deletion; FDR, false discovery rate; MAF, minor allele frequency; RegDB, RegulomeDB; SE, standard error; SNP, single nucleotide polymorphism.
↓, decreased; ↑, increased.
All alleles on reverse strand.
For each trait, the most significant *P*-value (the smallest *P*) is shown in **bold**.
FDRs that passed a threshold of 0.20 are shown in **bold**.
[a] RefSeq *ABCA1*: NCBI GRCh38, NC_000009.12, NM_005502.3.
[b] dbSNP build 141: GRCh38.
[c] The RegulomeDB (version 1.1) scoring scheme represented as following: **score 1a**, expression quantitative trait loci (eQTL) + transcription factor (TF) binding + matched TF motif + matched DNase Footprint + DNase peak; **score 1b**, eQTL + TF binding + any motif + DNase Footprint + DNase peak; **score 1c**, eQTL + TF binding + matched TF motif + DNase peak; **score 1d**, eQTL + TF binding + any motif + DNase peak; **score 1e**, eQTL + TF binding + matched TF motif; **score 1f**, eQTL + TF binding / DNase peak, **score 2a**, TF binding + matched TF motif + matched DNase Footprint + DNase peak; **score 2b**, TF binding + any motif + DNase Footprint + DNase peak; **score 2c**, TF binding + matched TF motif + DNase peak; **score 3a**, TF binding + any motif + DNase peak; **score 3b**, TF binding + matched TF motif; **score 4**,TF binding + DNase peak; **score 5**, TF binding or DNase peak; **score 6**, others; **score 7**, no data, or can be seen at http://regulome.stanford.edu/help. See detailed functional assignments in Table C20.

Table 8.4. Results of single-site association analysis in the replication study and meta-analysis for 11 common *ABCA1* variants (MAF ≥5%).

						Single-site Analysis								Meta-analysis		
						Main Study (623 NHWs from SLVDS)				Replication Study (744 EAs from Heart SCORE Study)						
SNP Name[a]	RefSNP ID[b]	Chr9 Position[a]	Location (Amino Acid Change)	RegDB Score[c]	Major/ Minor Alleles	HWE *P*	MAF	β	*P*	HWE *P*	MAF	β	*P*	Direction (NHWs,EAs)	Z	*P*
HDL-C																
p19072	rs2777795	104910084	Intron 1	5	C/T	0.3627	0.1088	-0.0396	**0.0237**	0.2268	0.1304	-0.0007	0.3085	-,-	-2.2830	**0.0224**
p23373	rs3905001	104905783	Intron 1	3a	C/G	0.8073	0.2932	0.0138	0.2427	0.3846	0.2598	1.11E-05	0.9840	-,-	-0.8080	0.4190
p25698	rs2575876	104903458	Intron 2	4	C/T	0.3746	0.2681	-0.0249	**0.0392**	0.3900	0.2655	-7.32E-05	0.8940	-,-	-1.4970	0.1343
p30287	rs10120087	104898869	Intron 2	5	G/T	0.8379	0.0985	0.0099	0.5742	0.2003	0.1129	-0.0005	0.4877	-,+	-0.1280	0.8980
p44418	rs11789603	104884738	Intron 3	7	G/A	1	0.1071	-0.0047	0.7841	0.1919	0.1075	-0.0003	0.6548	-,-	-0.5140	0.6070
p84376	rs4149295	104844780	Intron 8	4	A/T	0.9220	0.1145	-0.0178	0.2922	0.2287	0.1317	0.0004	0.5407	+,+	1.1640	0.2443
p87333	rs4149297	104841823	Intron 8	2b	T/C	0.5483	0.0998	0.0057	0.7428	0.1748	0.0968	0.0005	0.5319	-,-	-0.6820	0.4954
p110778	rs12338782	104818378	Intron 23	7	G/A	0.0342	0.0730	0.0128	0.5209	0.1219	0.0652	0.0010	0.3263	+,+	1.1570	0.2474
p122732	rs2066716	104806424	Exon 31 (Thr1427Thr)	7	G/A	1	0.0972	-0.0048	0.7798	0.1716	0.0948	-0.0006	0.4555	-,-	-0.7380	0.4606
p142619	rs4149337	104786537	Intron 47	7	T/C	0.6746	0.0691	0.0109	0.6005	0.0921	0.0484	-0.0002	0.8611	+,-	-0.2270	0.8205
p142975	rs2066882	104786181	Intron 48	6	A/G	0.0331	0.0909	0.0055	0.7688	0.1348	0.0727	-0.0003	0.7216	+,-	0.0620	0.9506
LDL-C																
p19072	rs2777795	104910084	Intron 1	5	C/T	0.3627	0.1088	-1.2279	0.7426	0.2268	0.1304	0.1037	0.6112	+,+	0.5960	0.5510
p23373	rs3905001	104905783	Intron 1	3a	C/G	0.8073	0.2932	-3.5826	0.1510	0.3846	0.2598	-0.0716	0.6477	-,-	-1.3110	0.1900
p25698	rs2575876	104903458	Intron 2	4	C/T	0.3746	0.2681	1.6751	0.5129	0.3900	0.2655	0.0303	0.8467	+,+	0.5860	0.5578
p30287	rs10120087	104898869	Intron 2	5	G/T	0.8379	0.0985	0.1507	0.9677	0.2003	0.1129	-0.5483	**0.0102**	+,+	1.9150	0.0555
p44418	rs11789603	104884738	Intron 3	7	G/A	1	0.1071	7.1477	**0.0477**	0.1919	0.1075	-0.1521	0.4872	+,+	1.8550	0.0637
p84376	rs4149295	104844780	Intron 8	4	A/T	0.9220	0.1145	2.2833	0.5249	0.2287	0.1317	-0.1293	0.5201	+,-	0.0410	0.9676
p87333	rs4149297	104841823	Intron 8	2b	T/C	0.5483	0.0998	2.1503	0.5613	0.1748	0.0968	-0.0513	0.8249	-,-	-0.5570	0.5777
p110778	rs12338782	104818378	Intron 23	7	G/A	0.0342	0.0730	-8.3051	**0.0474**	0.1219	0.0652	-0.5769	**0.0378**	+,+	2.8720	**0.0041**
p122732	rs2066716	104806424	Exon 31 (Thr1427Thr)	7	G/A	1	0.0972	5.3006	0.1493	0.1716	0.0948	-0.0475	0.8445	+,+	1.1230	0.2615
p142619	rs4149337	104786537	Intron 47	7	T/C	0.6746	0.0691	3.3204	0.4574	0.0921	0.0484	0.0910	0.7730	-,-	-0.7160	0.4738
p142975	rs2066882	104786181	Intron 48	6	A/G	0.0331	0.0909	0.0397	0.9921	0.1348	0.0727	0.0511	0.8492	-,-	-0.1460	0.8837
TC																
p19072	rs2777795	104910084	Intron 1	5	C/T	0.3627	0.1088	-2.6388	0.4987	0.2268	0.1304	0.0136	0.8378	+,-	-0.3090	0.7574
p23373	rs3905001	104905783	Intron 1	3a	C/G	0.8073	0.2932	-2.8388	0.2781	0.3846	0.2598	-0.0116	0.8218	+,+	0.9020	0.3671
p25698	rs2575876	104903458	Intron 2	4	C/T	0.3746	0.2681	3.5467	0.1851	0.3900	0.2655	-0.0344	0.5035	-,+	0.4080	0.6830
p30287	rs10120087	104898869	Intron 2	5	G/T	0.8379	0.0985	-0.0337	0.9931	0.2003	0.1129	-0.1306	0.0613	-,-	-1.3800	0.1676
p44418	rs11789603	104884738	Intron 3	7	G/A	1	0.1071	3.4606	0.3605	0.1919	0.1075	-0.0440	0.5396	-,+	0.1700	0.8648

Table 8.4. (continued)

p84376	rs4149295	104844780	Intron 8	4	A/T	0.9220	0.1145	3.2766	0.3852	0.2287	0.1317	-0.0751	0.2516	-,-	-1.4310	0.1523
p87333	rs4149297	104841823	Intron 8	2b	T/C	0.5483	0.0998	4.8025	0.2146	0.1748	0.0968	-0.0225	0.7649	+,-	-0.6230	0.5334
p110778	rs12338782	104818378	Intron 23	7	G/A	0.0342	0.0730	-9.2189	**0.0367**	0.1219	0.0652	-0.2005	**0.0275**	-,-	-3.0370	**0.0024**
p122732	rs2066716	104806424	Exon 31 (Thr1427Thr)	7	G/A	1	0.0972	7.0027	0.0682	0.1716	0.0948	0.0188	0.8128	+,+	1.4120	0.1580
p142619	rs4149337	104786537	Intron 47	7	T/C	0.6746	0.0691	-1.0826	0.8168	0.0921	0.0484	0.0131	0.8985	-,+	0.0640	0.9493
p142975	rs2066882	104786181	Intron 48	6	A/G	0.0331	0.0909	-4.1583	0.3211	0.1348	0.0727	0.0147	0.8673	-,+	0.5510	0.5815
TG																
p19072	rs2777795	104910084	Intron 1	5	C/T	0.3627	0.1088	0.0874	**0.0200**[d]	0.2268	0.1304	0.0034	0.1290	+,+	2.6940	**0.0071**
p23373	rs3905001	104905783	Intron 1	3a	C/G	0.8073	0.2932	-0.0618	**0.0151**[d]	0.3846	0.2598	-0.0021	0.2192	+,+	2.5510	**0.0107**
p25698	rs2575876	104903458	Intron 2	4	C/T	0.3746	0.2681	0.0742	**0.0045**[d]	0.3900	0.2655	0.0028	0.0946	+,+	3.1570	**0.0016**
p30287	rs10120087	104898869	Intron 2	5	G/T	0.8379	0.0985	-0.0847	**0.0251**[d]	0.2003	0.1129	-0.0028	0.2283	-,-	-2.4050	**0.0162**
p44418	rs11789603	104884738	Intron 3	7	G/A	1	0.1071	-0.0193	0.5997	0.1919	0.1075	-0.0003	0.8942	-,-	-0.4540	0.6499
p84376	rs4149295	104844780	Intron 8	4	A/T	0.9220	0.1145	0.0993	**0.0061**[d]	0.2287	0.1317	0.0035	0.1072	+,-	-0.6770	0.4983
p87333	rs4149297	104841823	Intron 8	2b	T/C	0.5483	0.0998	0.0983	**0.0090**[d]	0.1748	0.0968	4.56E-05	0.9855	-,-	-1.7870	0.0740
p110778	rs12338782	104818378	Intron 23	7	G/A	0.0342	0.0730	-0.0581	0.1701	0.1219	0.0652	2.74E-06	0.9993	+,-	-0.9310	0.3520
p122732	rs2066716	104806424	Exon 31 (Thr1427Thr)	7	G/A	1	0.0972	0.1169	**0.0016**[d]	0.1716	0.0948	0.0017	0.5186	+,+	2.6210	**0.0088**
p142619	rs4149337	104786537	Intron 47	7	T/C	0.6746	0.0691	-0.1237	**0.0060**[d]	0.0921	0.0484	0.0031	0.3822	-,+	1.2250	0.2205
p142975	rs2066882	104786181	Intron 48	6	A/G	0.0331	0.0909	-0.1094	**0.0068**[d]	0.1348	0.0727	0.0069	**0.0214**	-,+	0.1490	0.8814

EA, European American; Heart SCORE Study, the Heart Strategies Concentrating on Risk Evaluation Study; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; NHW, Non-Hispanic White; RegDB, RegulomeDB; SLVDS, the San Luis Valley Diabetes Study; SNP, single nucleotide polymorphism.
All alleles on reverse strand.
Significant *P*-values (*P* <0.05; meta-*P* <0.05) are shown in **bold**.
[a] RefSeq *ABCA1*: NCBI GRCh38, NC_000009.12, NM_005502.3.
[b] dbSNP build 141:GRCh38.
[c] Detailed RegulomeDB (version 1.1) scoring scheme is described in the footnote of Table 8.3 or can be seen at http://regulome.stanford.edu/help.
[d] Single-site *P*-values for each variant in NHWs after controlling false discovery rate (FDR) remained significant (*P* <0.05 and FDR <0.20); see details in Table 8.3.

Table 8.5. Result of SKAT-O analysis for low-frequency/rare variants (MAF <5%).

MAF	No of Variants	No of Samples, with/without LF/rare Variants	SKAT-O *P*			
			HDL-C	LDL-C	TC	TG[a]
All LF/rare variants (N = 66)						
≤0.01	20	90/533	0.4195	0.2281	0.4303	0.7217
≤0.02	40	211/412	0.3650	0.1648	**0.0437**	0.5864
<0.05	66	429/194	0.3157	0.4218	0.1356	0.1694
Only LF/rare exonic variants (N = 13)						
≤0.01	7	21/602	0.0552	0.0726	0.0868	0.8460
≤0.02	10	63/560	0.2829	0.3649	0.2123	0.7719
<0.05	13	147/476	0.3947	0.7734	0.4640	0.8271

MAF, minor allele frequency; LF, low-frequency; SKAT-O, an optimal sequence kernel association test.
Significant *P*-values (*P* <0.05) are shown in **bold**.
[a] For TG, one rare variant (p103375-rs145582736 [p.Glu815Gly], MAF = 0.0008) was excluded from this analysis due to missing phenotype values.

Table 8.6. Characteristics and effects of rare *ABCA1* variants (MAF <1%) of interest.

SNP Name[a]	RefSNP ID[b]	Chr9 Position[a]	Location	Amino Acid Change	RegDB Score[c]	Major/ Minor Alleles	MAF	GT	GT Count (Carrier Freq)	Adjusted Mean ± SD (mg/dL)	β	SE	*P*[d]	Other Trait (Effect)
HDL-C														
p103375	rs145582736	104825781	Exon 17	Glu815Gly	5	A/G	0.0008	AA	619	50.60 ± 14.00	-0.6219	0.1855	0.0009	
								AG	1 (0.16)	27.09 ± NA				
LDL-C														
p146494	rs41432545	104782662	Exon 50-3' UTR		7	T/A	0.0008	TT	617	136.82 ± 40.83	86.3036	39.9695	0.0312	TC (↑)
								TA	1 (0.16)	222.64 ± NA				
TC														
p146494	rs41432545	104782662	Exon 50-3' UTR		7	T/A	0.0008	TT	617	216.82 ± 43.59	95.4113	41.7998	0.0228	LDL-C (↑)
								TA	1 (0.16)	311.39 ± NA				

GT, genotype; MAF, minor allele frequency; NA, not analyzed; RegDB, RegulomeDB; SD, standard deviation; SE, standard error; SNP, single nucleotide polymorphism; UTR, untranslated region.
↑, increased.
All alleles on reverse stand.
[a] RefSeq *ABCA1*: NCBI GRCh38, NC_000009.12, NM_005502.3.
[b] dbSNP build 141:GRCh38.
[c] Detailed RegulomeDB (version 1.1) scoring scheme is described in the footnote of Table 8.3 or can be seen at http://regulome.stanford.edu/help.
[d] Detailed single-site association results are shown in Tables C11-C13.
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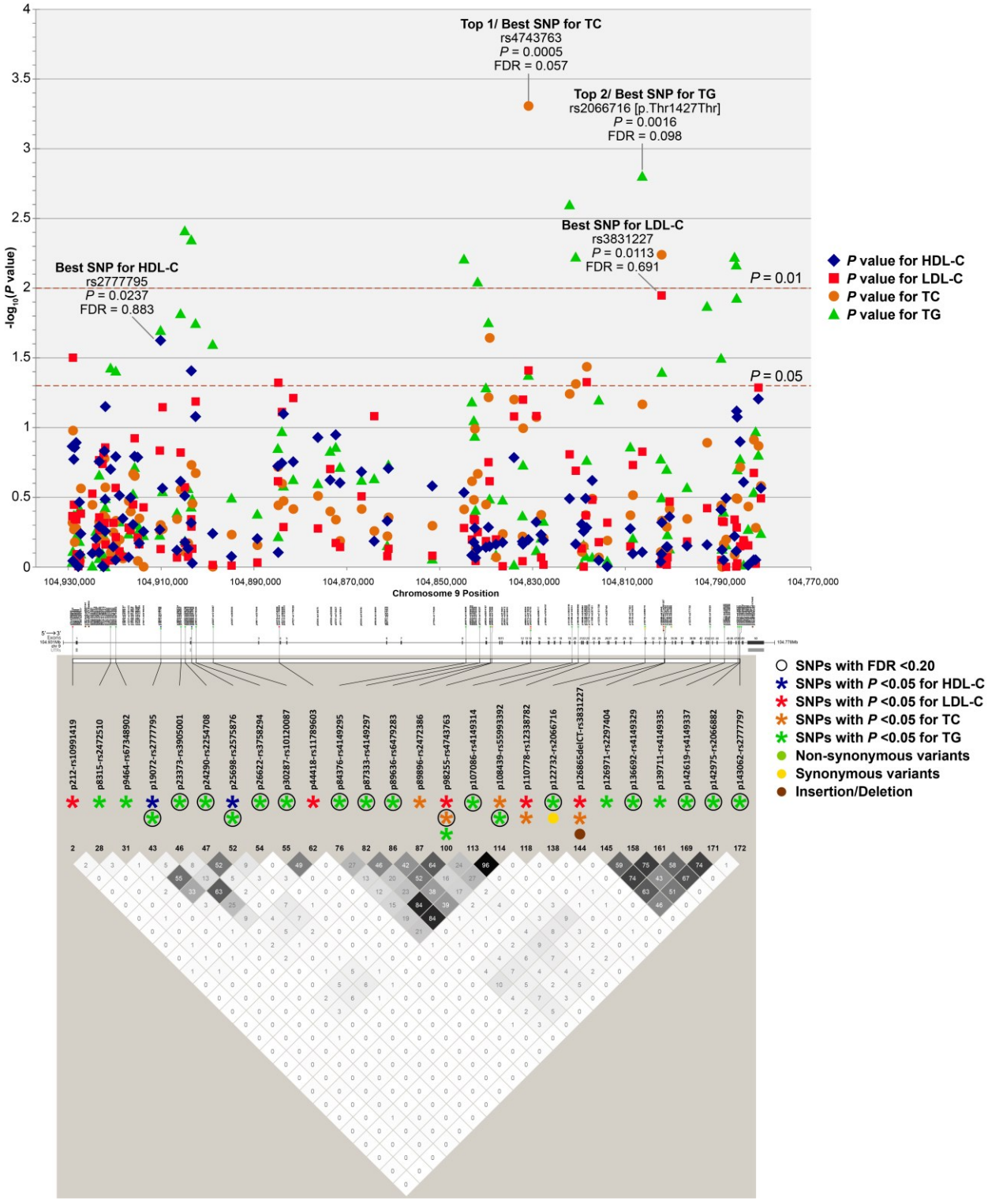
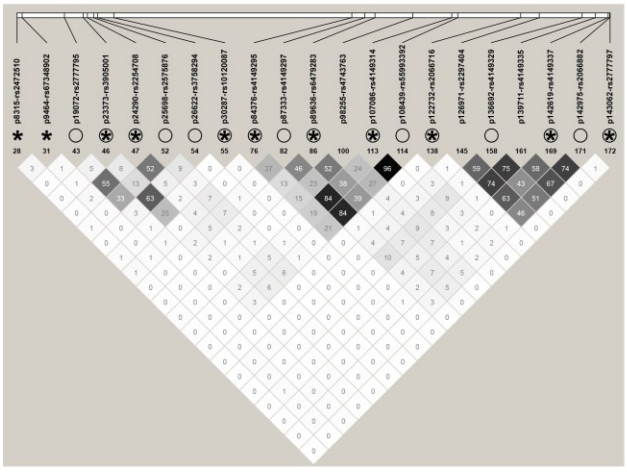

Top 1/ Best SNP for TC
rs4743763
P = 0.0005
FDR = 0.057
Top 2/ Best SNP for TG
rs2066716 [p.Thr1427Thr]
P = 0.0016
FDR = 0.098
Best SNP for LDL-C
rs3831227
P = 0.0113
FDR = 0.691
Best SNP for HDL-C
rs2777795
P = 0.0237
FDR = 0.883
P = 0.01
P = 0.05
-log10(P value)
Chromosome 9 Position
P value for HDL-C
P value for LDL-C
P value for TC
P value for TG
SNPs with FDR <0.20
SNPs with P <0.05 for HDL-C
SNPs with P <0.05 for LDL-C
SNPs with P <0.05 for TC
SNPs with P <0.05 for TG
Non-synonymous variants
Synonymous variants
Insertion/Deletion

Figure 8.1. Single site association *P*-values of 116 *ABCA1* variants with MAF ≥5% for 4 lipid traits (top), gene structure of *ABCA1* (middle) and linkage disequilibrium (LD) plot of 26 lipid-associated variants (bottom).

The $-\log_{10}$ *P*-values are presented in the Y-axis. A total of 116 variants are on *ABCA1* gene (5'→3'; RefSeq: NCBI GRCh38, NC_000009.12, NM_005502.3) in the X-axis. Marker names are shown as "SNP name-SNP ID (dbSNP build 141)". Dash line indicates the significance threshold (*P* = 0.05)**.** Shades and values (r^2 x 100) in each square of LD plot indicate pairwise LD: black indicating complete LD (r^2 = 1), white indicating no LD (r^2 = 0), and shade intensity indicating the degree of LD (r^2 between 0-1). FDR, false discovery rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MAF, minor allele frequency; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglycerides.



★ Associated variants with the best *P*-value iin each bin that were chosen for conditional analysis

○ Associated variants with FDR <0.20

List of 11 common TG-associated *ABCA1* variants chosen for conditional analysis and tagged SNPs using Tagger analysis with r^2 ≥0.50

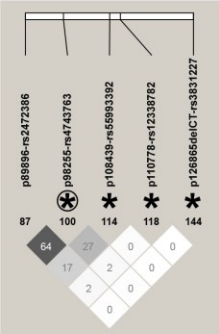
Bin #	Chosen SNPs	Alleles Captured
1	p8315-rs2472510	
2	p9464-rs67348902	
3	p23373-rs3905001	p26622-rs3758294
4	p24290-rs2254708	p19072-rs2777795, p25698-rs2575876
5	p30287-rs10120087	
6	p84376-rs4149295	
7	p89636-rs6479283	p98255-rs4743763
8	p107086-rs4149314	p87333-rs4149297, p108439-rs55993392
9	p122732-rs2066716	
10	p142619-rs4149337	p126971-rs2297404, p136692-rs4149329, p139711-rs4149335, p142975-rs2066882
11	p143062-rs2777797	

Results of single-site and conditional analyses for 11 common TG-associated *ABCA1* variants

			Unconditional Analysis		Conditional Analysis, conditioned on SNP 2 (P_{SNP1}|SNP2)										
					SNP 1										
		SNP Name-SNP ID	Single-site *P*	FDR	p8315-rs2472510	p9464-rs67348902	p23373-rs3905001	p24290-rs2254708	p30287-rs10120087	p84376-rs4149295	p89636-rs6479283	p107086-rs4149314	p122732-rs2066716	p142619-rs4149337	p143062-rs2777797
Conditional Analysis, conditioned on SNP 1 (P_{SNP2}|SNP1)	SNP 2	p8315-rs2472510	0.0371	0.2308		0.0848	0.0167	0.0054	0.0223	0.0135	0.0212	0.0031	0.0019	0.0059	0.0112
		p9464-rs67348902	0.0390	0.2308	0.0668		0.0221	0.0041	0.0246	0.0075	0.0144	0.0014	0.0009	0.0079	0.0127
		p23373-rs3905001	0.0151	**0.1464**	0.0581	0.0610		0.0124	0.4050	0.0031	0.0356	0.0020	0.0036	0.0035	0.0154
		p24290-rs2254708	0.0039	**0.0982**	0.0793	0.1020	0.1160		0.0694	0.0323	0.0460	0.0101	0.0011	0.0051	0.0131
		p30287-rs10120087	0.0251	**0.1822**	0.0855	0.0769	0.0991	0.0109		0.0076	0.0353	0.0055	0.0030	0.0046	0.0090
		p84376-rs4149295	0.0061	**0.0982**	0.0224	0.0428	0.0163	0.0143	0.0811		0.2000	0.0764	0.0028	0.0073	0.0209
		p89636-rs6479283	0.0176	**0.1476**	0.0392	0.0579	0.0794	0.0079	0.1590	0.0174		0.0250	0.0121	0.0005	0.0071
		p107086-rs4149314	0.0025	**0.0982**	0.0553	0.1110	0.2290	0.0822	0.1160	0.0482	0.5170		0.0005	0.0262	0.0423
		p122732-rs2066716	0.0016	**0.0982**	0.0478	0.0464	0.0215	0.0020	0.0853	0.0051	0.0415	0.0016		0.0112	0.0028
		p142619-rs4149337	0.0060	**0.0982**	0.0374	0.0808	0.0084	0.0031	0.0149	0.0123	0.0013	0.0011	0.0029		0.0274
		p143062-rs2777797	0.0117	**0.1361**	0.0119	0.0205	0.0154	0.0026	0.0320	0.0091	0.0101	0.0014	0.0024	0.0105	

Figure 8.2. Linkage disequilibrium (LD) plot for 21 common TG-associated *ABCA1* variants (*P* <0.05; upper left), a list of 11 TG-associated tagSNPs chosen for conditional analysis (upper right), including the results of single-site and conditional analyses (bottom).

The best TG-associated variant from each of the 11 bins were chosen by Tagger analysis with r^2 ≥0.50 capturing a total of 21 common TG-associated *ABCA1* variants (MAF ≥5%). Marker names are shown as "SNP name-SNP ID (dbSNP build 141)". Shades and values (r^2 x 100) in each square of LD plot indicate pairwise LD: black indicating complete LD (r^2 = 1), white indicating no LD (r^2 = 0), and shade intensity indicating the degree of LD (r^2 between 0-1). False discovery rate (FDR) <0.20 are shown in bold. MAF, minor allele frequency; SNP, single nucleotide polymorphism; TG, triglycerides.



List of 4 common TC-associated *ABCA1* variants chosen for conditional analysis and tagged SNPs using Tagger analysis with r^2 ≥0.50

Bin #	Chosen SNPs	Alleles Captured
1	p98255-rs4743763	p89896-rs2472386
2	p108439-rs55993392	
3	p110778-rs12338782	
4	p126865delCT-rs3831227	

✱ Associated variants with the best *P*-value iin each bin that were chosen for conditional analysis

○ Associated variants with FDR <0.20

Results of single-site and conditional analyses for 4 common TC-associated ABCA1 variants

			Unconditional Analysis		Conditional Analysis, conditioned on SNP2 (P_{SNP1}|SNP2)			
					SNP 1			
		SNP Name-SNP ID	Single-site *P*	FDR	p98255-rs4743763	p108439-rs55993392	p110778-rs12338782	p126865delCT-rs3831227
Conditional Analysis, conditioned on SNP 1 (P_{SNP2}|SNP1)	SNP 2	p98255-rs4743763	0.0005	**0.0575**		0.9130	0.1370	0.0091
		p108439-rs55993392	0.0487	0.8204	0.0032		0.0729	0.0104
		p110778-rs12338782	0.0367	0.8204	0.0002	0.0262		0.0145
		p126865delCT-rs3831227	0.0058	0.3350	0.0007	0.0527	0.0571	

Figure 8.3. Linkage disequilibrium (LD) plot for 5 common TC-associated *ABCA1* variants (*P* <0.05; upper left), a list of 4 TC-associated tagSNPs chosen for conditional analysis (upper right), including the results of single-site and conditional analyses (bottom).

The best TC-associated variant from each of the 4 bins were chosen by Tagger analysis with r^2 ≥0.50 capturing a total of 5 common TC-associated *ABCA1* variants (MAF ≥5%). Marker names are shown as "SNP name-SNP ID (dbSNP build 141)". Shades and values (r^2 x 100) in each square of LD plot indicate pairwise LD: black indicating complete LD (r^2 = 1), white indicating no LD (r^2 = 0), and shade intensity indicating the degree of LD (r^2 between 0-1). False discovery rate (FDR) <0.20 are shown in **bold**. MAF, minor allele frequency; SNP, single nucleotide polymorphism; TC, total cholesterol.

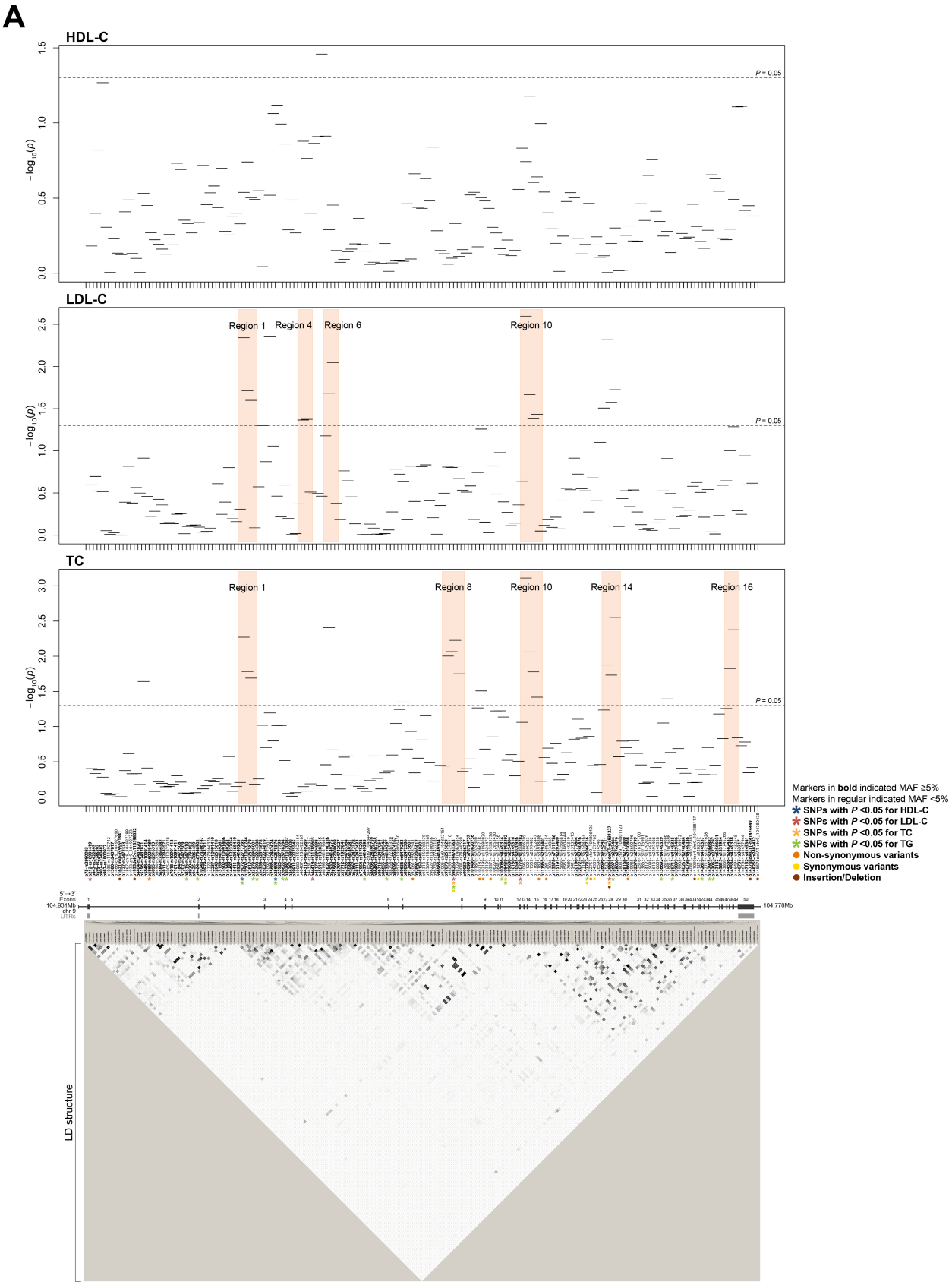

A
HDL-C
LDL-C
TC
P = 0.05
Region 1
Region 4
Region 6
Region 8
Region 10
Region 14
Region 16
Markers in bold indicated MAF ≥5%
Markers in regular indicated MAF <5%
SNPs with P <0.05 for HDL-C
SNPs with P <0.05 for LDL-C
SNPs with P <0.05 for TC
SNPs with P <0.05 for TG
Non-synonymous variants
Synonymous variants
Insertion/Deletion
LD structure

Figure 8.4. (continued)

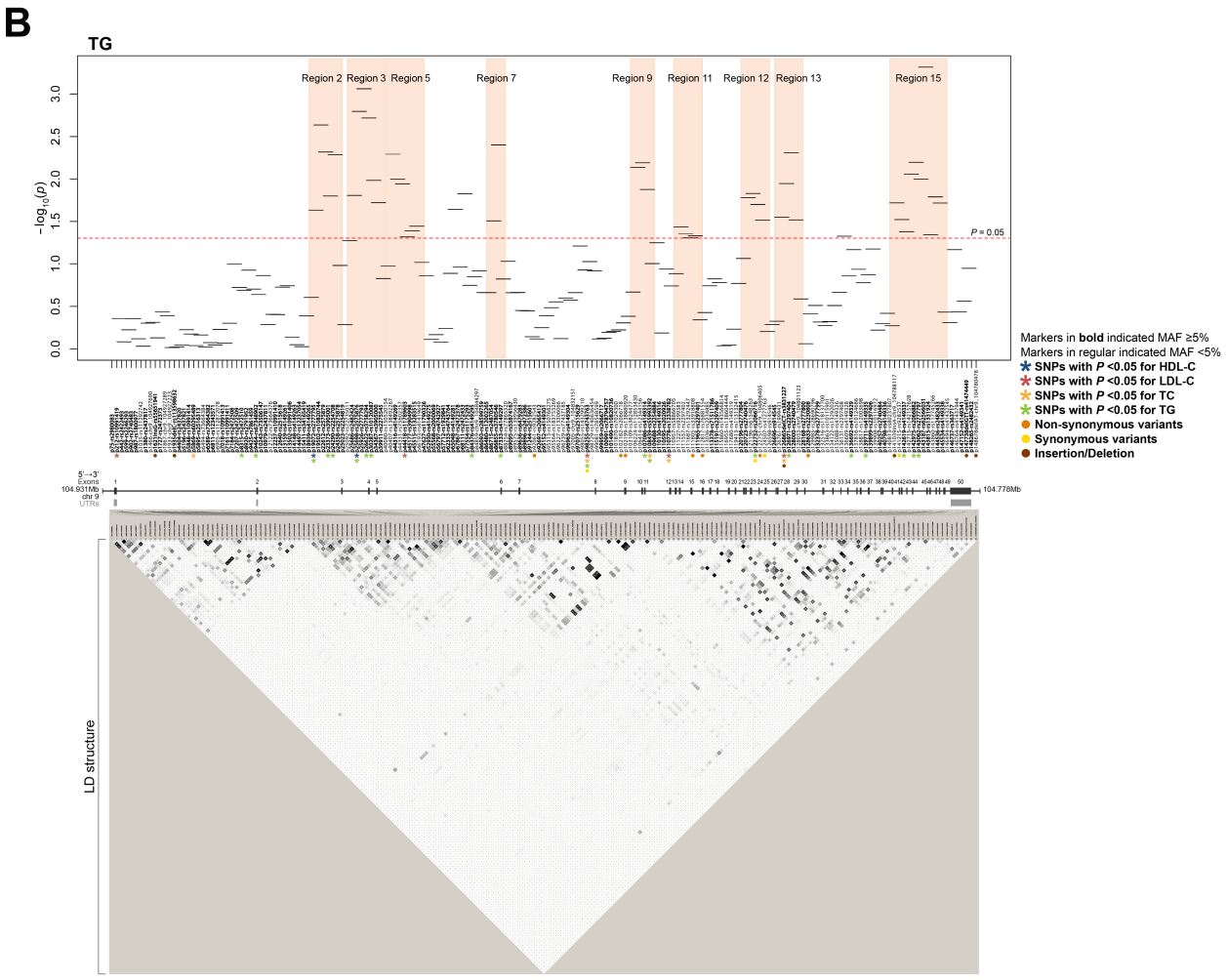


Figure 8.4. Haplotype association plots (top) for HDL-C, LDL-C, and TC (A) and for TG (B), including gene structure of *ABCA1* (middle), and linkage disequilibrium (LD) plots (bottom).

The -$\log_{10}$ *P*-values are presented in the Y-axis. All variants are on *ABCA1* gene (5'→3'; RefSeq: NCBI GRCh38, NC_000009.12, NM_005502.3) in the X-axis. The plots include 182 variants for HDL-C, LDL-C, and TC (**Figure 8.4A**) and 181 variants for TG (**Figure 8.4B**; after excluding p103375-rs145582736 [p.Glu815Gly] due to missing phenotypic values). Marker names are shown as "SNP name-SNP ID (dbSNP build 141)/chromosome 9 position (for novel variants)". SNPs with MAF ≥5% are shown in **bold**. All 37 novel variants identified in this study have been submitted to dbSNP:

http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=KAMBOH (Batch IDs: ABCA1_EA and ABCA1_EA+AB). Highlighted areas in the haplotype plots represent significantly associated haplotype regions. Dash line indicates the significance threshold (global P = 0.05). The degree of shades and values (r^2 x 100) in each square of LD plot indicate pairwise LD: black indicating complete LD (r^2 = 1), white indicating no LD (r^2 = 0), and shade intensity indicating the degree of LD (r^2 between 0-1). HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MAF, minor allele frequency; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglycerides.

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9.0. CONCLUSIONS

9.1. SUMMARY OF MAIN RESULTS

In this study, we successfully achieved the study aims by identifying common (minor allele frequency [MAF] ≥5%) and rare (MAF <5%) variants in the scavenger receptor class B type 1 (*SCARB1*) and ATP-binding cassette transporter A1 (*ABCA1*) genes, and by determining the contribution of variations in these two lipid genes to major lipid traits in two ethnic groups (Non-Hispanic Whites [NHWs] and African Blacks [ABs]). While the detailed results are presented in previous chapters: **Chapter 6.0** (*SCARB1* in NHWs), **Chapter 7.0** (*SCARB1* in ABs), and **Chapter 8.0** (*ABCA1* in NHWs), below we summarize the main findings.

In the sequencing stage, a total of 105 *SCARB1* variants were identified in NHWs (n = 44) and ABs (n = 83), of which 22 were shared between both populations. A total of 404 *ABCA1* variants were identified in NHWs. Of all identified sequence variants, 58 were novel, including 21 in *SCARB1* (11 in NHWs, and 10 in ABs) and 37 in *ABCA1*.

Following the genotyping of selected bi-allelic variants, the quality control-passed variants (69 *SCARB1* in NHWs, 137 *SCARB1* in ABs, and 182 *ABCA1* in NHWs) were tested for associations using various analyses.

The gene-based association tests showed evidence of association of *SCARB1* with high-density lipoprotein cholesterol (HDL-C) and/or apolipoprotein B (apoB) and *ABCA1* with triglycerides (TG). Consistent with the gene-based association results, single-site analyses also revealed nominal associations (P <0.05) of 15 *SCARB1* variants with HDL-C and/or apoB in at least one population, and of 21 *ABCA1* variants with TG in NHWs. After adjustment for multiple testing using false discovery rate (FDR), four *SCARB1* variants remained significant (FDR <0.20), including three associated with apoB in NHWs, and one associated with HDL-C in ABs. In addition, 16 *ABCA1* variants associated with TG in NHWs also remained significant (FDR <0.20). Moreover, haplotype association analyses identified several significant haplotype windows and regions (global P <0.05) in *SCARB1* associated with HDL-C/apoB in NHWs and with HDL-C in ABs. Similarly, haplotype analyses also identified multiple significant haplotype windows and regions (global P <0.05) in *ABCA1* associated with TG. Furthermore, a set of 17 and 20 rare *SCARB1* variants with MAF ≤1% was found to be associated (P <0.05) with apoB in NHWs and with HDL-C in ABs, respectively. Whereas a set of 23 rare *ABCA1* variants with MAF ≤1% was associated with total cholesterol in NHWs.

In summary, our findings demonstrate the association of common (MAF ≥5%) and rare (MAF <5%) variants in the *SCARB1* and *ABCA1* genes with lipoprotein-lipid levels in the general population, which further strengthen the genetic contribution of these two genes in the regulation of lipid metabolism and levels in humans. In addition, our results also suggest that the *SCARB1* and *ABCA1* genes may be good candidates for targeted therapeutic development.

9.2. STUDY LIMITATIONS

Although we have successfully identified a number of common and rare variants, including novel ones, by resequencing the *SCARB1* and *ABCA1* genes in extreme HDL-C groups, we possibly have missed some relevant variants, particularly those located in large introns, since we primarily focused on exons and exon-intron boundaries (*SCARB1* and *ABCA1*) and small introns (*ABCA1*) and also some very rare ones due the small size of our sequencing samples (n = 95 from each ethnic group). Moreover, we could have missed some associations due to the moderate size of our study samples (<800 subjects in each ethnic group).

Despite these limitations, our study has identified a number of common and rare variants, including haplotypes and regions in *SCARB1 and ABCA1*, associated with various lipid traits. Replication of our novel findings in independent larger samples is warranted for validation. Additionally, the majority of associated variants identified in our study did not show strong regulatory functions (scores <3) based on evidence from RegulomeDB, suggesting that these variants may be linked to other functional variants or need to further assessed by additional functional studies. Interestingly, the comparison of the results of *SCARB1* associations between NHWs and ABs has revealed that the majority of the associated signals tend to be ethnic-specific. This observation suggests that a comprehensive analysis of these two lipid genes in various

populations is necessary in order to identify the causal/functional variants in different ethnic backgrounds.

9.3. PUBLIC HEALTH PERSPECTIVES

Coronary heart disease (CHD) is a major public health burden because it is the leading cause of death globally. It is now well established that lipoprotein-lipid levels are major risk factors for CHD and play important roles in CHD development. Lipoprotein-lipid levels are determined by genetic and environment factors. However, genetic factors only explain a small portion of the inter-individual variations in lipid levels in humans. In the current study, we have identified new associations of common and rare *SCARB1* and *ABCA1* variants, including some haplotypes, with major lipid traits. These findings are likely to further improve our understanding of the genetic contribution to lipoprotein-lipid variation in the general population and eventually to lead to the risk prediction for and better management of CHD. In addition, the progress made in understanding the genetic basis of lipoprotein-lipid levels also provides the biological insights into the regulation of lipid metabolism, which in turn may lead to developments of new interventions or treatments for CHD and various lipid-related diseases.

APPENDIX A

SUPPLEMENTAL MATERIAL FOR
CHAPTER 6.0.
GENETIC INFLUENCE OF *SCARB1* VARIANTS
ON LIPID TRAITS IN US NON-HISPANIC WHITES

http://d-scholarship.pitt.edu/25673/1/APPENDIX_A.pdf

Impact of Genetic Variants in Human Scavenger Receptor Class B Type I (*SCARB1*) on Plasma Lipid Traits

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The supplemental material can be seen at http://circgenetics.ahajournals.org/content/7/6/838/suppl/DC1

APPENDIX B

SUPPLEMENTAL MATERIAL FOR
CHAPTER 7.0.
GENETIC INFLUENCE OF *SCARB1* VARIANTS
ON LIPID TRAITS IN AFRICAN BLACKS

http://d-scholarship.pitt.edu/25673/2/APPENDIX_B.pdf

APPENDIX C

SUPPLEMENTAL MATERIAL FOR
CHAPTER 8.0.
GENETIC INFLUENCE OF *ABCA1* VARIANTS
ON LIPID TRAITS IN US NON-HISPANIC WHITES

http://d-scholarship.pitt.edu/25673/3/APPENDIX_C.pdf

APPENDIX D

RESOURCES FOR THE FULL-SIZED FIGURES

CHAPTER 6.0. GENETIC INFLUENCE OF *SCARB1* VARIANTS TO LIPID TRATIS IN US NON-HISPANIC WHITES

Figure 6.1. Plot of single-site *P*-values of 39 common *SCARB1* variants and linkage disequilibrium structure of 7 common variants associated with HDL-C and ApoB in US Non-Hispanic Whites.
Available at http://d-scholarship.pitt.edu/25673/5/Figure_6.1.tif

Figure 6.2. Haplotype association plots for HDL-C and ApoB and linkage disequilibrium structure of 69 *SCARB1* variants in US Non-Hispanic Whites.
Available at http://d-scholarship.pitt.edu/25673/6/Figure_6.2.tif

Figure A1. Linkage disequilibrium structure of 81 *SCARB1* common HapMap-CEU tagSNPs.
Available at http://d-scholarship.pitt.edu/25673/7/Figure_A1.jpg

Figure A2. Linkage disequilibrium structure of 44 *SCARB1* sequence variants.
Available at http://d-scholarship.pitt.edu/25673/8/Figure_A2.jpg

Figure A3. Linkage disequilibrium structure of 69 *SCARB1* genotyped variants.
Available at http://d-scholarship.pitt.edu/25673/9/Figure_A3.jpg

CHAPTER 7.0. GENETIC INFLUENCE OF *SCARB1* VARIANTS TO LIPID TRATIS IN AFRICAN BLACKS

Figure 7.2. Plot of single-site *P*-values of 94 common *SCARB1* variants and linkage disequilibrium structure of 10 common variants associated with HDL-C and/or ApoA-I in African Blacks.

Available at http://d-scholarship.pitt.edu/25673/10/Figure_7.2.tif

Figure 7.3. Haplotype association plots for HDL-C and ApoA-I and linkage disequilibrium structure of 136 *SCARB1* variants in African Blacks.

Available at http://d-scholarship.pitt.edu/25673/11/Figure_7.3.tif

Figure B1. Linkage disequilibrium structure of 83 *SCARB1* sequence variants.

Available at http://d-scholarship.pitt.edu/25673/12/Figure_B1.jpg

Figure B2. Linkage disequilibrium structure of 32 *SCARB1* common sequence variants.

Available at http://d-scholarship.pitt.edu/25673/13/Figure_B2.jpg

Figure B3. Linkage disequilibrium structure of 108 *SCARB1* common HapMap-YRI tagSNPs.

Available at http://d-scholarship.pitt.edu/25673/14/Figure_B3.pdf

Figure B4. Linkage disequilibrium structure of 137 *SCARB1* genotyped variants.

Available at http://d-scholarship.pitt.edu/25673/15/Figure_B4.pdf

CHAPTER 8.0. GENETIC INFLUENCE OF *ABCA1* VARIANTS TO LIPID TRATIS IN US NON-HISPANIC WHITES

Figure 8.1. Plot of single-site *P*-values of 116 common *ABCA1* variants and linkage disequilibrium structure of 26 common variants associated with HDL-C, LDL-C, TC, and TG in US Non-Hispanic Whites.

Available at http://d-scholarship.pitt.edu/25673/16/Figure_8.1.jpg

Figure 8.2. Single-site association and conditional analyses results and linkage disequilibrium structure of 11 *ABCA1* common tagSNPs associated with TG in US Non-Hispanic Whites.

Available at http://d-scholarship.pitt.edu/25673/17/Figure_8.2.pdf

Figure 8.3. Single-site association and conditional analyses results and linkage disequilibrium structure of 4 *ABCA1* common tagSNPs associated with TC in US Non-Hispanic Whites.

Available at http://d-scholarship.pitt.edu/25673/18/Figure_8.3.pdf

Figure 8.4. Haplotype association plots for HDL-C, LDL-C, TC (A) and TG (B), and linkage disequilibrium structure of 182 bi-allelic *ABCA1* variants in US Non-Hispanic Whites.

Available at http://d-scholarship.pitt.edu/25673/19/Figure_8.4A.pdf

Available at http://d-scholarship.pitt.edu/25673/20/Figure_8.4B.pdf

Figure C1. Linkage disequilibrium structure of 217 *ABCA1* common HapMap-CEU tagSNPs.

Available at http://d-scholarship.pitt.edu/25673/21/Figure_C1.pdf

Figure C3. Linkage disequilibrium structure of 239 *ABCA1* common sequence alleles.

Available at http://d-scholarship.pitt.edu/25673/22/Figure_C3.jpg

Figure C4. Linkage disequilibrium structure of 183 *ABCA1* genotyped variants.

Available at http://d-scholarship.pitt.edu/25673/23/Figure_C4.jpg

Figure C5. Linkage disequilibrium structure for 116 common *ABCA1* genotyped variants.

Available at http://d-scholarship.pitt.edu/25673/24/Figure_C5.jpg

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