

LIPOPROTEIN LIPASE GENE SEQUENCING AND PLASMA LIPID PROFILE

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Dilek Pirim

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This thesis was presented

by

Dilek Pirim

It was defended on

December 8, 2010

and approved by

F. Yeşim Demirci, M.D., Assistant Professor, Human Genetics
Graduate School of Public Health, University of Pittsburgh

Clareann H. Bunker, Ph.D., Associate Professor, Epidemiology,
Graduate School of Public Health, University of Pittsburgh

Thesis Director: M. Ilyas Kamboh, Ph.D, Professor and Department Chair
Human Genetics, Graduate School of Public Health
University of Pittsburgh

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Dilek Pirim, M.S.

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In the United States, coronary heart disease (CHD) is the most common cause of death and number one killer of American males and females. Several epidemiological studies have identified risk factors for CHD, like low high-density lipoprotein cholesterol (HDL-C), elevated total cholesterol and low-density lipoprotein (LDL) cholesterol, and high triglycerides (TGs), but underlying genetic variations that cause predisposition to these traits still remain unclear. Lipoprotein lipase (*LPL*) is one of the major genes involved in lipid metabolism and its gene sequence variation has already been reported to be associated with the risk of CHD and risk of other complex diseases like dyslipidemia, type 2 diabetes, essential hypertension, and Alzheimer's disease. Unraveling the unknown genetic variation in the *LPL* gene in relation to HDL-C and correlated lipid traits is critically important for public health because identification of genetic markers may lead to promising future public health interventions, like prognostic tools and therapeutic approaches to alleviate the burden of CHD in the U.S. In this study, we investigated the role of common and rare variation in *LPL* by resequencing individuals having extremely low (n=48) and high (n=47) HDL-C levels selected from a population-based non-Hispanic white (NHW) sample of 623 individuals. A total of 179 variants were identified in 95 individuals by resequencing the entire *LPL* gene, including 91 uncommon or rare variants [minor allele frequency (MAF) <0.05] and 88 common variants (MAF \geq 0.05). Of the 91 relatively uncommon or rare variants, 21 were present only in the low-HDL group and 25 were present

only in the high HDL-C group. Overall, the prevalence of uncommon or rare variants was higher in the high HDL-C than the low HDL-C group. Thirty two of the 88 common variants demonstrated significant association (P-value <0.05) between the high and low HDL-C groups. We also examined 12 common variants (MAF \geq 0.05) in the total NHW sample and identified 7 variants to be significantly associated with lipid levels.

In conclusion, our comprehensive resequencing of the LPL gene confirms that both common and rare variants in this gene are associated with interindividual variation in plasma lipid profile.

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1.0 BACKGROUND AND SIGNIFICANCE

1.1 CORONARY HEART DISEASE

1.1.1 Public health importance of coronary heart disease

In the United States, coronary heart disease (CHD) is the most common cause of death and major killer of American males and females; one of every six deaths is caused by CHD (Coronary Heart Disease) according to the National Vital Statistics in 2009 (Heron et al. 2009). Based on the Framingham Heart Study (FHS) in 2001 more than half of the total cardiovascular events were due to the CHD in females and males under age 75 (Lloyd-Jones et al. 2010; American Heart Association 2010). In 2006, almost 425,000 (224K males and 201K females) people died from CHD and the CHD death rate was 134.9 per 100,000 (176.3 for white males, 206.4 for black males, 101.5 for white females and 130.0 for black females (Lloyd-Jones et al. 2009). American Heart Association computation based latest available mortality data show that in every twenty five seconds an American will experience a coronary event and it will cause death of one person in about every minute. The same data computes that about 81% of Americans die of CHD are at age 65 or above (Lloyd-Jones et al. 2010). According to the data of National Health and Nutrition Examination Survey (NHANES) between 2003 and 2006, the prevalence of CHD in the United States was 17,600,000 for adults age 20 and older. It means 7.9 percent (9.1 percent for males and

7.0 percent for females) of American adults age 20 and older have CHD (Lloyd-Jones et al. 2010). The incidence is different between females and males; males at 40 and older have high risk of developing CHD than females at the same age. The percentages for lifetime risk of developing CHD are 48.6 % for males and 31.7% for women at age 40 (Ulrich et al. 1999). The latest available data of the American Heart Association estimates that the direct and indirect cost of CHD is \$177.1 billion for 2009 (Lloyd-Jones et al. 2010).

1.1.2 Risk factors of coronary heart disease

The risk factors for CHD are classified into two categories according to their causative and quantitative contributions to CHD. Low level serum high-density lipoprotein (HDL) cholesterol, elevated serum total and low-density lipoprotein (LDL) cholesterol, elevated blood pressure, cigarette smoking, diabetes mellitus and advancing age are the major and independent risk factors. Several studies, including Framingham Heart Study, have investigated the quantitative relationship between these factors and CHD. The major and independent risk factors contribute the total risk of a person independently and total risk calculation can be done by summing of the risk imparted by each of the major risk factors. Second category includes predisposing and conditional risk factors that are associated with independent risk factors for CHD. Obesity, physical activity, ethnic characteristics, psychosocial factors, family history of premature CHD are predisposing risk factors and elevated serum triglycerides, small LDL particles, elevated serum homocysteine, elevated serum lipoprotein (a), prothrombotic factors and inflammatory markers (eg. C-reactive protein) are conditional risk factors for CHD (Grundy et al. 1999). After nine years follow up, the data of the three prospective cohort studies includes both sexes and a spectrum of adult ages shows that 87% to 100% of fatal CHD cases had prior exposure to at least

one clinically elevated major CHD risk factor which are cholesterol ≥ 240 mg/dL [≥ 6.22 mmol/L], arterial blood pressure $\geq 140/190$ mm Hg, medication use for cholesterol and hypertension, high cholesterol, cigarette smoking or clinical diabetes; 64% to 100% of these fatal CHD cases had prior exposure to two or more major CHD risk factors at higher than favorable levels (Greenland et al. 2003). The mortality data, prevalence and incidence of CHD show that male gender and blacks has higher risk of developing a CHD (Keil et al. 1989; Cooper et al. 1992; Johnson et al. 1986).

Since family history is an important risk factor for CHD, several studies imply the importance of genetic factors in the pathogenesis of CHD due to their aggregation in families with combination of lifestyle and common environment (Jannotti et al. 2000; Tiret et al. 2002; Breslow et al. 2001). The heritability of these risk factors has been investigated in many twin studies to understand the role of shared genes and shared environment. These results strength the importance of the genetic factors in predisposition to CHD (Hans et al. 1997; Marenberg et al. 1994; Chen et al. 2009; Austin et al. 1987). Most of the known genetic variation that affects the risk of CHD underlay in the genes that have an important role in lipid metabolism, including the lipoprotein lipase (*LPL*) gene. These variations may result a change in the gene function and cause changes in the lipid levels. This emphasizes the significance of the genetic studies for unraveling the genetic variation in candidate genes that may affect the lipid levels and contribute to the etiology of CHD.

1.2 HIGH DENSITY LIPOPROTEIN (HDL)

High density lipoprotein (HDL) is the smallest and densest lipoprotein particle in the circulation which consists of several distinct subclasses that vary in size, shape, density (1.063 to 1.21g/ml), surface charge and composition (Rye et al. 2009; Tsompanidi et al. 2009). The other major groups of lipoproteins, in order of largest to smallest according to their lipid content, are chylomicrons, very low density lipoprotein (VLDL), intermediate-density low protein (IDL), and low density lipoprotein (LDL). They are responsible to carry the lipids like cholesterol and triglyceride (TG) in the circulation. HDL particles compose by a hydrophobic core of cholesterol esters and a small amount of triglycerides (TGs); is surrounded by phospholipids, free cholesterol and apolipoproteins (apos) (Rye et al. 2009; Florentin et al. 2008). HDL may have a discoidal or spherical shape depending on the lipid composition. Mature HDL is in spherical shape and contains 45-55% apolipoproteins, 26-32% phospholipids, 15-20% esterified cholesterol, 3-5% free cholesterol and about 5% triglycerides (Tsompanidi et al. 2009). The major apolipoprotein particles of HDL are apoAI and apoAII and the minor apolipoproteins are apoIV, apoCI/CII/CIII, apoD, apoE, apoJ, apoK and apoM (Florentin et al. 2008). HDL particles also include liposoluble, antioxidants, and several enzymes like paraoxonase-I (PONI), platelet-activating factor acetylhydrolase (PAF-AH) and glutathione phospholipid peroxidase (Florentine et al. 2008). As a major component of HDL, apoAI has a role in biogenesis and functions of HDL (Tsompanidi et al. 2009).

Although, high blood cholesterol is a major risk factor for CHD, cholesterol which is carried in HDL particles is called ‘good’ cholesterol and it is beneficial for a number of reasons (Toth, 2005; Genest et al. 1999). American Heart Association considers low high density lipoprotein (HDL-C) as a major risk factor for heart disease if it is less than 40 mg/dL for men and less than 50 mg/dL for women; HDL-C levels of 60 mg/dL and above is considered as being protective against heart disease.

1.2.1 HDL-Metabolism

Unlike the other lipoprotein particles, HDL promotes the reverse cholesterol transport (RCT), carries the excess cholesterol from non-hepatic cells (such as macrophages) to the liver and steroidogenic organs so cholesterol can be able to use for the synthesis of lipoproteins, bile acids, Vitamin D, and steroid hormones (Genest et al. 1999; Stein et al.1999; Von Eckardstein et al 2001). There are three different pathways that reverse cholesterol transport can take place. First, LDL receptors on liver recognize the multiple copies of apoE particles on mature HDL particles so they can be taken up by liver (Bruce et al. 1998). Second, scavenger receptor class B1 (SR-B1) mediates the selective uptake of the high-density lipoprotein cholesteryl esters (HDL-CE) (Acton et al. 1996). Third, cholesteryl ester transfer protein (CETP) transfers the cholesteryl esters from HDL to the TG rich lipoprotein particles, and then they can be taken by the liver via LDL receptors (Bruce et al., 1998)

1.2.2 The antiatherogenic role of HDL particles

The antiatherogenic role of HDL particles is mainly related its function in reverse cholesterol transport pathway which removes cholesterol from peripheral cells (macrophages in the artery wall) (Rye et al. 2009; Florentin et al. 2008). Besides this function, HDL has anti-thrombotic and anti-inflammatory properties that contribute to its atheroprotective role (Von Eckardstein et al. 2001; Genest et al. 1991). It also prevents atherosclerotic lesion progression by inhibiting LDL oxidation, promoting endothelial repair, inhibiting the binding of monocytes to the endothelium and improving endothelial function (Rye et al. 2009; Assmann et al. 1993; Rust et al.1999)

1.2.3 HDL cholesterol and CHD risk

Lipid levels and their role in development of CHD have been studied since the early 1950s (Barr et al. 1951). Despite the fact that HDLs carry about 20% of the total plasma cholesterol, several clinical and epidemiological studies have demonstrated the inverse relationship between HDL-C and CHD (Gordon et al. 1989, Castelli et al. 1986, Castelli 1977). This was reported as a 1 mg/dL increase of HDL-C levels is associated with a 2-3% decreased CHD risk (Gordon et al. 1989). It is worth noting that although the direct relationship between the CHD risk and low HDL-C is clearly identified in many studies, this does not reflect a causal relationship due to other factors that affect CHD risk as well as HDL-C levels (Vergeer et al. 2010). The risk factors for CHD that also cause low level of HDL-C are: male gender, smoking, obesity, hypertriglyceridemia, insulin resistance, physical inactivity, systemic inflammation, and low socioeconomic status (Vergeer et al. 2010).

1.2.4 Effects of genetic factors on HDL-C levels

Since the inverse relationship between HDL-C levels and CHD have been confirmed in various epidemiological studies, interest has focused on the role of genetic factors influencing HDL-C concentration (Weissglas-Volkov et al. 2010). Based on family and twin studies, the heritability of HDL-C levels is estimated up to 80 % (Kronenberg et al. 2002; Perusse et al., 1997; Wang and Paigen 2005; Goode et al. 2007). This means, in addition to environmental factors, genetic factors play an important role in affecting the variation of plasma HDL-C levels.

The genetic inheritance of low and high HDL-C levels can be monogenic, polygenic, or can be determined by interactions between several genes and their interactions with environmental factors. Most of the researchers conduct studies by using case/controls, families, and unascertained populations to understand the role of major genes in lipid metabolism influencing HDL-C variations (Weissglas-Volkov et al. 2010). In this aspect, the mutations in the *APOA1*, *ABCA1*, *LCAT*, and *LPL* genes have been associated with low HDL-C levels and they cause monogenic disorders as apoAI deficiency, Tangier disease, familial lecithin-cholesterol-acyl transferase (LCAT) deficiency (FLD), and lipoprotein lipase (LPL) deficiency (Type I hyperlipoproteinemia or familial chylomicronemia), respectively. However, mutations in the *CETP* and *HL* genes have been associated with high HDL-C levels (Weissglas-Volkov et al. 2010).

The approaches to identify the susceptibility genes in complex polygenic diseases fall into two categories: candidate gene studies and genome wide association studies (GWAS). The candidate gene studies using resequencing and association techniques have identified several variants in some of the genes that have a known important function in HDL metabolism. Among

these genes (*CETP*, *LIPC*, *LPL*, *LIPG*, *LCAT*, *ABCA1*, *APOA1*, *APOC3*, *APOC5*, *APOE*, *SR-BI*, and *PONI*), the common variants that have been identified in *LPL*, *CETP*, and *LIPC* are the most significantly associated with HDL-C levels (Weissglas-Volkov et al. 2010, Sviridov et al. 2007).

There are ten GWASs published on HDL-C levels (Willer et al. 2008, Kathiresan et al. 2008a, Kooner et al. 2008; Wallace et al. 2008; Kathiresan et al. 2007; Aulchenko et al., 2009, Chasman et al., 2008; Sabatti et al. 2009; Ridker et al. 2009, Heid et al. 2008). These results are consistent with the previous association studies and the strongest association signals were observed for SNPs in the *LPL*, *CETP*, and *LIPC* genes. However, these GWASs also identified new candidate genes influencing HDL-C levels which need further studies to understand their functional importance in the HDL metabolism.

1.3 LIPOPROTEIN LIPASE

The lipoprotein lipase (LPL protein; *LPL* gene) is located on chromosome 8p22 in humans (Sparkes et al. 1987). The National Center for Biotechnology Information (NCBI) reference nucleotide sequence is NC_00008.10 (<http://www.ncbi.nlm.gov/sites/entrez>). It is a member of the TG lipase gene family; the other genes in the same family are hepatic lipase (HL), pancreatic lipase (PL), and endothelial lipase (EL). The *LPL* gene comprises 10 exons spanning 30 kb (Deeb and Peng 1989). The gene encodes 475 amino acids and it becomes a mature protein of 448 residues after cleavage of a 27-amino-acid signal peptide (Wion et al 1987). 5'- untranslated region, the signal peptide and the first two amino acids are encoded by the first exons. The rest of the 9 exons encode the remaining 446 amino acids. The 10th exon is the largest exon which encodes the long 3'-untranslated region of 1,948 nucleotides. Figure 1 shows the gene structure

and the basic promoter elements of the *LPL* gene which are located within 101bp upstream of the transcription starting site (Wang et al 2009).

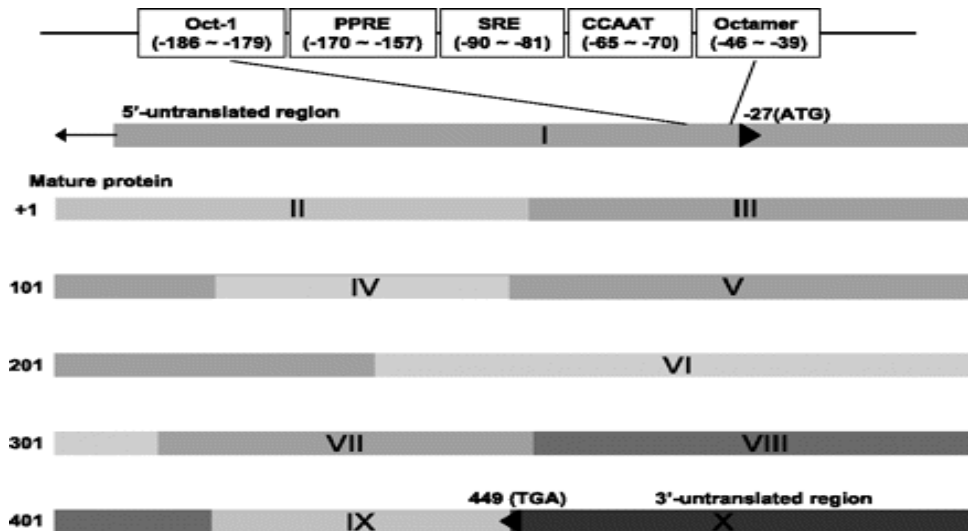


Figure 1. Lipoprotein lipase gene structure with promoter elements. (From Wang et al 2009, "Am Physiol Soc, used with permission")

1.3.1 *LPL* gene regulation

The expression and regulation of *LPL* is regulated at transcriptional, posttranscriptional, translational and posttranslational levels in a tissue specific manner. Hormonal levels and nutritional regulations can also affect the expression of *LPL* by interacting proteins or directly modify the regulation of *LPL* (Wang et al. 2009). Variations in *LPL* gene sequence, expression and regulation are important because they may influence its activity and also its contribution to disease so screening the *LPL* gene in people who have different level of HDL-C levels may explain how *LPL* variants affect HDL-C levels and CHD. HDL-C levels may be regulated in three different ways due to plasma LPL activity (Weissglas-Volkov 2010). First, some of the phospholipids and apolipoproteins are shed and transferred to the HDL particles during the hydrolysis of TG-rich lipoproteins (Klos et al. 2007). Second, LPL limits the CETP-mediated

HDL-C reduction by decreasing plasma TG because the exchange of cholesterol for TG from HDL is modulated by CETP due to the amount of VLDL particles (Rye et al. 2009). Third, HDL lipid composition is altered by LPL enzyme activity because affects the catabolic rate of apoA1 so they can be more rapidly cleared from the circulation (Barter et al. 2003).

1.4 LIPOPROTEIN LIPASE ENZYME

In 1943, Paul Hahn first observed clearance of postprandial lipidemic plasma heparin injection (Hahn 1943). After this finding, more studies focused on understanding the mechanism of so called heparin releasable ‘clearing factor’ and it was revealed that this factor is a lipolytic enzyme which has high activity against the TG component of plasma lipoproteins. The enzyme was called LPL, after discovering its role on breaking down plasma triglycerides of TG-rich lipoproteins which are VLDL and chylomicrons (Tsutsumi 2003). LPL is a major rate-limiting enzyme responsible for the hydrolysis of TG-rich particles circulating in the bloodstream, so it has a central role in overall lipid metabolism and transport. ApoCII is required for activation of LPL and there are several factors such as apoCIII, apoE, high salt conditions and fatty acids that inhibit LPL activity (Murthy et al. 1996). It is synthesized by parenchymal cells and then transferred to the luminal surface of endothelial cells where it is anchored to the surface of the cells by heparan sulfate proteoglycans (Goldberg et al. 1996).

LPL enzyme activity has been detected in several extrahepatic tissues and cells, including adipose tissues which it is predominantly found and in other tissues such as heart, skeletal muscle, lung, lactating mammary gland, brain, kidney, and macrophages (Kirchgessner et al. 1989). LPL enzyme activity has been also identified in differentiated macrophages, placenta, spleen,

pancreatic β -cells and steroidogenic tissue, but not in liver of adult animals. Due to tissue-specific activity of LPL, high levels of hepatic messenger RNA and enzyme activities are shown during suckling period in lactating mammary glands (Semenkovich et al. 1989, Yacoub et al. 1952; Merkel et al. 1998a). The high level LPL activity in adipose tissue is seen after a meal consistent with the fat storage function of adipose tissue and its level is increased by fasting and exercise training in skeletal muscle (Merkel et al. 2002).

The LPL enzyme is catalytically active in its dimer nascent form which is composed of 55 kDa subunits and it shows a head-to-toe configuration by noncovalent interactions (Zhang et al. 2005). Maturation of nascent LPL starts in the endoplasmic reticulum depending the activity of lipase maturation factor 1 (Peterfly et al. 2007). According to the three-dimensional structure of LPL, it has been shown that LPL is organized into two structurally distinct domains; a larger N-terminal domain (residues 1-312) and a smaller C-terminal domain (residues 312-448) connected by a flexible peptide (Yang et al. 1989, Bengtsson-Olivecrona et al. 1986, Lookene et al. 1993) N-terminal domain includes catalytic site covered by a lipid-binding lid and catalytic center consists of three amino acids: Ser132, Asp156, and His241. Interaction of LPL with lipoprotein substrates occurs in the C-domain and it results a conformational change that allows enzyme to conduct its catalytic function by opening of the lid in the N-terminal domain (Santamarina-Fojo et al. 1994).

There are four distinct physiological activities of LPL that have been identified in several studies which all influence the plasma lipoprotein profile and the cellular metabolism of fatty acids and lipids (Preiss-Landl et al 2002). First and major activity of LPL is its hydrolyzing function of TG-rich lipoproteins (chylomicron and VLDL) to provide fatty acids to underlying tissues (Figure 2). Second, LPL has uncatalytic activity called 'bridging function' which allow enzyme to interact with lipoproteins and anchoring them to the vessel wall so it facilitate

triglyceride hydrolysis and lipoprotein uptake (Merkel et al. 1998b, Merkel et al. 2002). Third, LPL has a role in the uptake of lipoproteins by acting as a ligand for the LDL receptors, VLDL receptors, megalin, and LDL receptor related protein (Medh et al. 1996, Takahashi et al. 1995, Kounnas et al. 1993, Beisiegel et al. 1991). Fourth, LPL facilitates the selective uptake of lipoproteins associated lipids (Merkel et al. 2002; Seo et al. 2000) and lipophilic vitamins such as vitamin A and vitamin E (Preiss-Landl et al. 2002; Sattler et al. 1996; Van Bennekum et al. 1999).

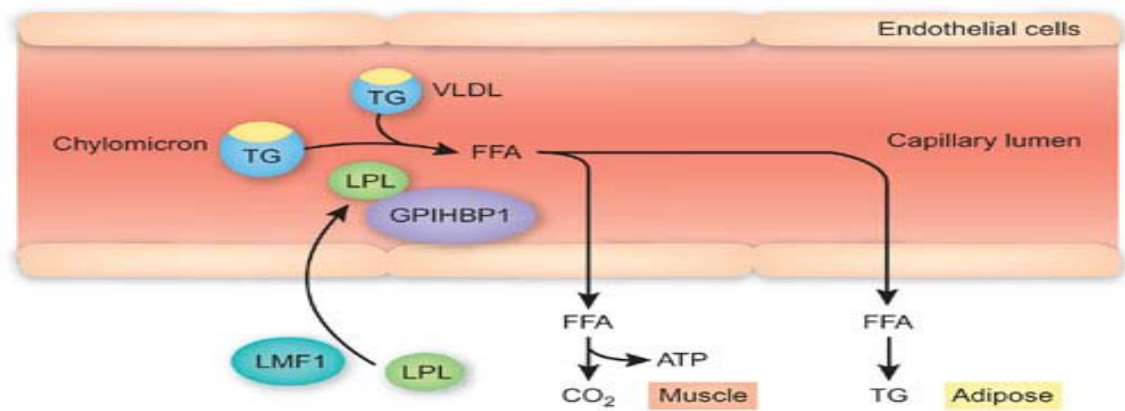


Figure 2. LPL activity in fatty acid transport to muscle and adipose tissues (Attie et al. 2010, used with permission).

1.4.1 Effects of Lipoprotein Lipase activity on lipid levels

Lipoprotein lipase is the major enzyme in lipid metabolism so its catalytic function and bridging function are both critically important for humans to maintain the lipid levels favorably. Due to its essential role in regulation of lipid metabolism, it is a candidate susceptibility gene for influencing CHD risk. Several studies show variations in the human *LPL* gene that have a role in the disease's etiology due to changes in *LPL* gene sequence, expression and regulation (Xie et al. 2010). Havel

and Gordon discovered LPL deficiency in 1960 and then the gene was cloned in 1991 followed by identification of several mutations (Havel et al. 1960, Henderson et al. 1991). Clinically abnormal lipid levels have been associated with CHD, atherosclerosis and obesity in individuals who have a number of functional DNA sequence variations in their *LPL* gene (Murthy et al. 1996; Reymer et al. 1995; Brunzell et al. 1995; Wiebusch et al. 1992). LPL deficiency, known as Type 1 hyperlipoproteinemia or familial chylomicronemia (MIM 238600), is a rare autosomal recessive disorder (1/1,000,000) caused by defects in the *LPL* gene and it is correlated with severe hypertriglyceridemia due to chylomicronemia and VLDL accumulation with very low levels of LDL-C and HDL-C levels (<20mg/dL). LPL deficiency is characterized with hepatosplenomegaly, xanthomas, acute pancreatitis and recurrent episodes of abdominal pain (Klos et al. 2007). Several studies have shown that individuals with LPL deficiency are compound heterozygotes and most of the disease causing mutations occur predominantly in exons 4, 5 and 6 of the *LPL* gene. The disease causing mutations usually result catalytically inactive LPL enzyme that is being degraded within the cell and cause little or no postheparin LPL activity (Wang et al. 2009).

1.5 LIPOPROTEIN LIPASE GENE SEQUENCE VARIATION

LPL is a candidate gene for influencing the risk of CHD due to its central role in lipid metabolism. Hypertriglyceridemia, which is the main clinical feature of LPL deficiency and dysfunction may be also a risk factor in the pathogenesis of CHD as well as dyslipidemia, type 2 diabetes (T2D), essential hypertension (EH), and Alzheimer's disease (Xie et al. 2010). It is thought to be a common biological basis for these complex diseases that structure, expression and

function of the *LPL* gene may have a role in that. This explains importance of understanding the *LPL* gene sequence variation and its association between specific phenotypes. Initially, 9.7kb of *LPL* sequence, about one-third of the *LPL* gene, was sequenced in 71 individuals, that identified 88 variable sites (79 were single nucleotide substitutions and 9 were insertion-deletion) (Nickerson et al. 1998). The average nucleotide diversity across the region, spanning from 3' end of intron 3 to the 5' end of intron 9, was found to be 0.2 %, which is almost one variant in every 500bp (Nickerson et al. 1998). Nickerson et al. (1998) sequenced a total of 9,734bp including 8,736bp in non-coding region and 9,98bp in coding region; 81 variants were identified in non-coding region and 7 variants were identified in coding region. Most of the variable sites detected in the coding region were silent and third base substitutions except one variable site that leads to stop codon and premature truncation of the protein in position 9040C>G (447Ser→Ter) and three amino-acid substitutions were observed in the coding region in positions 2849A>G (291Asn→Ser), 6176 G>A (370Val→Met), and 6203A>G (379Thr→Ala) (Nickerson et al. 1998). In another study, sequence variants were identified by resequencing all 10 exons and introns/flanking regions of the human *LPL* gene in 95 subjects, and 24 variants were identified including 9 in coding and 15 in non-coding regions (Morabia et al. 2002). Three of the 24 variants were found to be associated with amino acid changes; 291Asn291→Ser (N291S) in exon 6 and 447Ser→Ter (S447X) in exon 9 and 9Asp→Asn (D9N) in exon 2 (Morabia et al. 2002). In addition to these common coding variants, almost 100 naturally occurring mutation have been described in *LPL* gene: 61 missense mutation, 12 nonsense mutations, 10 frameshift mutations or small insertion/deletions, 3 gross mutations, 8 splicing mutations and 4 promoter variants (Merkel et al. 2002). Ser447X is the only variant associated with increased LPL activity and found in up to 20% in the general population (Merkel et al. 2002). The most common *LPL* coding variants with

their locations and percentages of carriers in related populations are briefly described in the Table 1.

Table 1. Most common *LPL* variants

<i>LPL</i> Variant	Location	Carrier frequencies (%) in populations
Ser447X	Exon 9	20% in Caucasians (Humphries et al. 1998, Wittrup et al. 1999)
Asp9Asn	Exon 2	1.5% in Caucasians (Wittrup et al. 1999)
Asn291Ser	Exon 6	2-5% in Caucasians (Wittekoek et al. 1998)
-93T→G	Promotor	76.4% in South African Blacks and 1.7% in Caucasians (Ehrenborg et al. 1997)

1.6 *LPL* POLYMORPHISMS ASSOCIATED WITH PLASMA HDL-C LEVELS AND CORONARY HEART DISEASE

Several studies have investigated the association of *LPL* polymorphisms with HDL-C levels (Boes et al. 2009). Seven variants have shown significant association with HDL-C levels. Two of them are the common variants (Asp9Asn, N291S) that result amino acid changes and lead to decrease in enzymatic activity and so they have been projected to reduce HDL-C levels (Wittrup et al. 1999, Zhang et al. 1996; Maily et al. 1995). While D9N (rs1801177) results in 3.2 mg/dl decrease in HDL-C levels and 20% increase in TG levels, Asn291Ser (rs268) leads to 4.6mg/dl decrease in HDL-C levels (Wittrup et al. 1999). The D9N variant is in near-complete linkage disequilibrium with the promoter variant (T93G), which may cause decreased LPL activity due to lower promoter activity but the role of the promoter variant is not yet clear (Merkel et al. 2002). The other amino acid substitution, Gly188Glu, decreases HDL-C levels (10mg/dl) and it is most

frequent in French Canadians in Quebec (Wittrup et al. 1999; Merkel et al. 2002). It is the major mutation that results nonfunctional LPL protein and has the strongest link with increased risk of CHD among all *LPL* variants (Merkel et al. 2002). The *HindIII* (rs320) polymorphism is located in intron 8 of the *LPL* gene and is in strong linkage disequilibrium with the Ser447X variant (Humphries et al. 1998). The *HindIII* polymorphism has been estimated to increase HDL-C levels up to 5.5mg/dl (Senti et al. 2001; Holmer et al. 2000; Corella et al. 2002; Ukkola et al. 2001; Radha et al. 2006, Ahn et al. 1993). Ser447X (rs328) is the other common variant that cause stop codon and it is also associated with increased HDL-C levels in several studies (Wittrup et al. 1999; Nettleton et al. 2007; Lee et al. 2004; Komurcu-Bayrak et al. 2007; Pallaud et al. 2001; Constanza et al. 2005; Kathiresan et al. 2008b; Talmud et al. 2002). Two single nucleotide polymorphisms (SNPs), rs326 and rs13702, have been identified to be in a strong LD with rs320 so they are also associated with HDL-C levels (Boes et al. 2009; Klos et al. 2006).

Genome wide association (GWA) studies have also identified many SNPs associated with HDL-C levels. To our knowledge, seven GWA studies have found association signals in the *LPL* gene with HDL-C levels (Boes et al. 2010). The reported SNPs with the lowest p-values are rs2083637 (Aulchenko et al. 2009), rs10503669 (Willer et al. 2008), rs331 (Chasman et al. 2008), rs328 (Kathiresan et al. 2008b), rs17482753 (Heid et al. 2008), rs17411031 (Wallace et al. 2008), and rs326 (Kooner et al. 2008).

1.7 SPECIFIC AIMS

The objective of this study is to evaluate the role of common and rare genetic variation in the *LPL* gene in relation to HDL-C and correlated lipid traits in a non-Hispanic white (NHW) sample (n=623). Since *LPL* is a biological candidate gene for HDL-C levels, its common and/or rare variation is hypothesized to contribute to the variation in HDL-C levels. The following are the three aims of our study.

Aim 1: Resequence the *LPL* gene in a subset of phenotypically determined samples having HDL-C in the upper (n=47) and lower (n=48) 5th percentile derived from a NHW sample of 623 individuals to identify both rare (allele frequency <0.01) and common variants (allele frequency ≥ 0.05).

Aim 2: Screen the tagSNPs of common variants of the *LPL* gene in the total NHW sample (n=623).

Aim 3: Evaluate the association of both rare and common *LPL* variants identified in Aim 1 and Aim 2 with HDL-C and correlated lipids (total cholesterol, triglycerides, LDL-C) levels in the NHW sample.

2.0 SUBJECTS AND METHODS

2.1 SUBJECTS

The study samples comprised 623 non-Hispanic whites (NHWs) derived from the San Luis Valley Diabetes Study in Colorado. The main features of the study subjects are depicted in Table 2. All subjects used in this current study were non-diabetics and a more detailed description of the sample population is given in Razagghi et al. (2000) and Demirci et al. (2010). Total fasting serum cholesterol was determined by using esterase-oxidase method, total HDL-C and TG were measured using enzymatic methods and LDL-C was calculated by the Friedewald equation when triglycerides levels were less than 400mg/dl (4.5 mmol/l) (Harris et al. 1998).

DNA was extracted from buffy coat using standard DNA extraction procedures.

Table 2. Population characteristic data (mean±SD) of entire NHW (n=623)

Variable	Men (n=295)	Women (n=328)
Age (years)	52.9 ± 0.6	52.4 ± 0.6
BMI (kg/m ²)	26.2 ± 0.20	24.8 ± 0.2
LDL (mg/dl)	139.8± 2.0	134.7± 2.0
HDL-C (mg/dl)	43.9 ± 0.6	56.3 ± 0.7
Triglycerides (mg/dl)	147.6 ± 4.1	128.2 ± 2.9
Total Cholesterol (mg/dl)	213.7 ± 2.2	217.7 ± 2.1

A total of 95 NHW individuals whose serum HDL-C in the upper 5th (n=47) and in the lower 5th (n=48) percentiles were selected for resequencing of the entire *LPL* gene. Of the selected 95 individuals, 47 were females (23 with high HDL-C levels and 24 with low HDL-C levels) and 48 were (24 with high HDL-C levels and 24 with low HDL-C levels) males. A summary of the selected sample of 95 individuals is presented in Table 3.

Table 3. Population characteristic data (mean±SD) of the subset of population used for DNA sequencing

Variable	Total n=95		p-value
	High HDL(n=47)	Low HDL(n=48)	
Age (years)	55.45 ± 9.8	53.03 ± 10.54	0.25
Sex (M/F)	24/23	24/24	0.92
BMI (kg/m ²)	23.17 ± 3.17	27.35 ± 3.90	<0.001
LDL (mg/dl)	126.84 ± 46.95	136.95 ± 41.28	0.28
HDL-C (mg/dl)	77.68 ± 13.32	31.81 ± 4.37	<0.001
Triglycerides (mg/dl)	114.09 ± 60.88	240.21±153.22	<0.001
Total Cholesterol (mg/dl)	227.34 ± 51.76	208.81 ± 44.65	0.06

2.2 DNA SEQUENCING

The *LPL* gene is located on chromosome 8p22 and comprises 10 exons. Figure 3 depicts the location of the *LPL* gene on chromosome 8 with neighboring genes and indicates their orientations. The accession number for DNA reference sequence for *LPL* gene used in PCR and resequencing is NC_00008.10; it is derived from Genbank in NCBI site (<http://www.ncbi.nlm.nih.gov>). A total of 37 overlapping resequencing amplicons were sequenced in both directions and the PCR primers that were used to produce these overlapping

amplicons are given in Table 4. These amplicons cover the entire LPL gene (27,993 bp) as well as 1,196 bp in the 5' flanking region and 1kb in the 3' flanking region resulting into a total of 30,189 bp genomic fragment. Although we design most of the primers by using Primer 3 software (<http://frodo.wi.mit.edu/primer3/>), we also used a subset of primers from a previous study (Nickerson et al. 1998). That sequenced only a portion of the targeted region (9.7kb), starting 3' end of intron 3 to 5' end of intron 9.

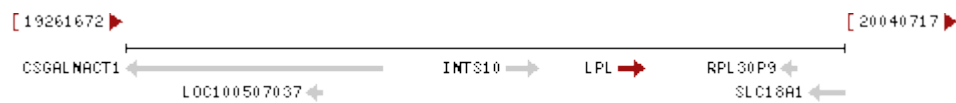


Figure 3. *LPL* and neighboring genes on chromosome 8.

Table 4. *LPL* Polymerase chain reaction (PCR) primers

Amp. #	PCR Amp. (bp)	Forward Primer	Reverse Primer	Internal Sequencing Primer
1	822	5'-GGGTTGGGGATACACTTCAT-3'	5'-TGTTTTCCAAGGAGGGAAAAG-3'	
2	722	5'-TGATCCATCTTGCCAATGTT-3'	5'-AGGGCTTTGCTCTCCATCT-3'	
3	851	5'-GAAAGCTGCCCACTTCTAGC-3'	5'-GTACTTTTCCACCCCGACA-3'	
4	703	5'-TGACCTGCAGTCACCTCTCT-3'	5'-GCTCTCTATGCTGCTGTTGC-3'	
5	800	5'-GGGGCCAAATGAGAATGTC-3'	5'-AGTTGGCTCCTACCATCTTC-3'	
6	1177	5'-GGATCAGTTTGAAAACACTGGA -3'	5'-CATTTTGTATGGCTGGAACAT-3'	
7	1112	5'-TGCCTTATGCCAGATTGTTC-3'	5'-TTGAATGAAGGGCTGTTGAG-3'	
8	1147	5'-ATACCACTTCTGGCTTGGATT-3'	5'-ACTGATGTGGTCGATTTGGT-3'	
9	1056	5'-AGCTGCATGTTAGAGAAGTCAA-3'	5'-CCAAACTTCAGTCAGCTCTCC-3'	
10	1093	5'-CTGCCCAATAGCAATCACAG-3'	5'-CAATGGGTAAACACTCCAAGA-3'	
11	1113	5'-TCTTGGTGGATGAATGGAT-3'	5'-ATTACCAGTGTGAGCCATCG-3'	
12	1022	5'-GCCATAGGAGTGGGAACAGT -3'	5'-ACTGGAGGGTTGCTTGATTT-3'	
13	787	5'-TCGAAAACACTTCAGAAAACAAA-3'	5'-AGTAAATGGAGGCCAGAGA-3'	
14	1090	5'-CTGCGAGGTTGGTAAAGGAT-3'	5'-CCTGCCTGTGCTGAAAATA-3'	
15	1053	5'-TGTGATAAAATCTCAAATTCCTAAA-3'	5'-TCCTACAGTGGCTGACATTTTT-3'	
16	1019	5'-AGGGAGGGCTTCAGTTCAG-3'	5'-TTCACAATGGGAACCCTGTA-3'	
17	1049	5'-AACCCGATTTTCTGCCTTA-3'	5'-TGAATGCCCCAGAAAATA-3'	
18	1084	5'-AGAGTTGGGTGCCAAAACCTT-3'	5'-GGGTATATATTTTCCCATTATCC-3'	
19	691	5'-AACCAGGTAATTGGAAGTAAAAA-3'	5'-ACAGTCTGCCAAAAATAAAACT-3'	
20	1061	5'-TGTTTACGGAAAAGTGAACAAA-3'	5'-GGGGCTTCTGCATACTCAA-3'	
21 *	475	5'-GGCCAAATGTGTATATGAAAAC-3'	5'-CCATGACTGTAGAATAGGAGC-3'	
22 *	1783	5'-AGAGGACTTGGAGGTAATATT-3'	5'-GACTCCTTGGTTTCTTATTTA-3'	5'-ATGTTACTGGAACAGAAGATG-3' 5'-CTGGTCCACATCTGGGTAAA-3'
23 *	1229	5'-AGGCTGGAGACTGTTGTAAAT-3'	5'-CTCAGGTTTCCATCTGGATTC-3'	5'-CTATCAACTCTGTTATGGTGGC-3'
24	708	5'-CCCTCTATGTGCTCATGCAA-3'	5'-TGGGGCCACTGTCTTTAAT-3'	
25	1169	5'-GGAATGGTCGAAAATGAGA-3'	5'-AAGGAAAGGCAGCAGGACTA-3'	

Table 4 Continued				
26	915	5'-CCACGCCCAACTAATTC-3'	5'-CCTAGAAAATGCAGACCTTGAA-3'	
27	1057	5'-TGTTTGGCCTTCTGATTG-3'	5'-CATGGTGAGACCCTGTGC-3'	
28	755	5'-AGTAAGAAGTCCATGACAAAAGTGT -3'	5'-TTTCCTGGGTTTCCTACAAT-3'	
29*	1881	5'-CATCAATTACAGTCGTACCTAT-3'	5'-TCAGCTTTAGCCCAGAATGC-3'	5'-GAGCAGTCTTATGTTACTGGGC-3'
30*	794	5'-TCATTGCAGAAAGGAAAGG-3'	5'-AATTCAGAACAGGAGTAGTG-3'	
31	874	5'-TGCCTCTTTCCTACCTGACC-3'	5'-ATTTTTGTAAAGGACGAAAAACAT-3'	
32	1072	5'-AAAAACATGCCTATTAGGAAAAG-3'	5'-CGCATCTGAACATTCTCTGTC-3'	
33	1078	5'-CGGCCCTAGATGCAGTTTA-3'	5'-AGATTGCCCCAGTTTCTGAG-3'	
34	1049	5'-AGAAGTCATTGGCCAGTC-3'	5'-GCTGAGGATTACAGGCTCATT-3'	
35	1046	5'-ACTTGGAGAGGGACGAAGAA-3'	5'-TCACAACCCAAATCCAGAAA-3'	
36	1044	5'-GCATAATTCGGAAGGGAAAA-3'	5'-TTATCAAGGCAACCCAAAGC-3'	
37	754	5'-GCCTGCATAAAGTACACAGGA-3'	5'-CTTCTCCACATCCTCAGCAA-3'	

*Primers from the study of Nickerson et al. (1998)

The GeneAMP® PCR System 9700 thermal cycler with a heated lid (Applied Biosystems, Foster City, CA) was used for performing the polymerase chain reaction (PCR). The PCR reactions and cycling conditions are presented in Table 5. Gel electrophoresis by using 96-well pre-cast agarose E-Gel® 96 2% with SYBR® Safe (Invitrogen Corporation, Carlsbad, CA) was performed following amplification of each of the PCR fragments to check the success of the reaction. Reamplification was done for some of the samples that were failed in the initial amplification and regular 2% agarose gel with ethidium bromide (2µl) was performed after PCR reaction of this subset of reamplified samples to confirm the amplification.

All the amplified samples were sent to a commercial sequencing lab where automated sequencing and capillary electrophoresis were performed on ABI 3730x1DNA Analyzers (Genomic Services of Beckman Coulter, Danvers, MA). Sequencing data received from the commercial lab was analyzed in our lab by Variant Reporter version 1.0 (Applied Biosystems) and Sequencher version 4.8 (Gene Codes Corporation, Ann Arbor, MI).

Table 5. PCR reaction and cycling conditions

PCR Reaction (Total volume 25 μ L)		PCR conditions
DNA	3.0 μ L	1. 95° C for 5 minutes
dH2O	12.25-13.75 μ L	2. 95° C for 45 seconds
10x BufferGold	2.5 μ L	3. 58-60° C for 45 seconds
MgCl ₂ (25 mM)	1-3.5 μ L	4. 72° C for 1 minute
dNTPs (1.25mM)	3.8 μ L	- Repeat steps 2-4 for 40
Forward Primer (20mM)	0.4 μ L	cycles
Forward Primer (20mM)	0.4 μ L	5. 72° C for 10 minutes
AmpliTaqGold (5U/ μ L)	0.15 μ L	6. Cool to 4° C

2.3 GENOTYPING

While the sequencing was underway, we used the Hapmap database and Haploview program to determine the number of common tagSNPs that cover the entire gene and ~1kb flanking regions in whites. Seventeen tagSNPs were identified in Haploview ($MAE \leq 4\%$, $r^2 \geq 0.7$) using SNP genotype data of CEU population (Utah residents with Northern and Western European ancestry) (www.hapmap.org). To date, we have genotyped 12 of them using TaqMan SNP genotyping assays. Table 6 lists the 48 variants that were captured by genotyping of 12 tagSNPs; 12 tagSNPs are highlighted in yellow. Table 7 lists the assay IDs of the genotyped 12 SNPs in the entire NHW sample with their refSNP IDs.

Table 6. Tagger results using SNP genotype data of CEU population provided from HapMap (MAF \geq 4%, $r^2 \geq$ 0.7)

<i>Bin</i>	<i>Variants captured</i>
1	rs3916027, rs295, rs297, rs291, rs13702, rs326, rs327, rs331, rs320 (HindIII), rs301
2	rs264, rs3779787, rs271, rs256, rs255, rs3779788, rs263
3	rs11570891, rs15285, rs3735964, rs1059611, rs12679834, rs328 (Ser447X), rs325
4	rs312, rs4922115, rs11570892, rs316, rs4921684, rs330
5	rs10099160, rs319, rs3200218
6	rs17410577, rs13266204
7	rs258, rs253
8	rs1534649, rs10104051
9	rs270
10	rs281
11	rs9644636
12	rs285
13	rs248
14	rs249
15	rs3289
16	rs283
17	rs343

Twelve screened variants are highlighted in yellow.

Table 7. TaqMan SNP Genotyping Assays

Assay ID	<i>LPL</i> refSNP IDs	Location	Position	Taqman Assay Type
C__27500004_10	rs3779787	Intron 1	2335	Pre-made
C__11856397_10	rs13266204	Intron 1	4424	Pre-made
C__9642884_10	rs1534649	Intron 1	4060	Pre-made
C__12104326_10	rs249	Intron 4	15425	Pre-made
C__1842993_10	rs253	Intron 4	15836	Pre-made
C__12104296_20	rs264	Intron 5	17599	Pre-made
C__1842996_10	rs270	Intron 6	18095	Pre-made
C__12104268_10	rs283	Intron 6	19517	Pre-made
C__12104236_10	rs312	Intron 7	22416	Pre-made
C__1843006_20	rs327	Intron 8	23955	Pre-made
C__8804467_10	rs3289	Exon 10 3'UTR	27611	Pre-made
C__8804485_10	rs1059611	Exon 10 3'UTR	28982	Pre-made

384-well plates containing dried whole genome amplified DNA was used in TaqMan genotyping. PCR amplification was performed using a PTC-200 MJThermal Cycler (Biorad) or a GeneAmp 9700 (Applied Biosystems). After thermal cycling, Real-Time PCR system (ABI Prism 7900HT Sequence Detection Systems) was used for endpoint fluorescence reading of the plates. TaqMan reaction and thermal cycler conditions are given in Table 8. The remaining common SNPs are currently being genotyped by the medium-throughput Sequenom IPLEX genotyping assays.

Table 8. TaqMan reaction and thermal cycler conditions

TaqMan Reaction (total volume of 5 μ L)		PCR Cycling Conditions
dH ₂ O	2.43	1. 95° C for 10 minutes 2. 95° C for 15 seconds 3. 60° C for 1 minute -repeat steps 2-3 50x
TaqMan Master Mix	2.50 μ L	
TaqMan Assay Mix	0.06 μ L	

2.4 STATISTICAL METHODS

Allele and genotype frequencies were determined by direct counting. Concordance of the genotype distribution to Hardy-Weinberg equilibrium was tested using a χ^2 goodness-of-fit test for each variant. The variants identified by sequencing were analyzed by using Haploview to determine allele frequencies and their distributions among high and low HDL groups and their LD patterns. In the subset of sample that was used for sequencing, the χ^2 test was used to compare the allele frequencies between the low and high HDL groups. For those SNPs that were genotyped in the entire sample, linear regression was performed to test for the effects of genotypes on the means of plasma lipoprotein lipid levels. The HDL-C and TG levels were transformed using normal log transformation to reduce the effects of non-normality. The significant covariates were identified using stepwise regression in both directions. The additive and dominant models were used for data analysis. The covariates included in the final model were sex, age, BMI and smoking. The R statistical software package (version 2.3.1, <http://www.r-project.org>) and Statistical Analysis Software (SAS) were used to perform all computations. A P-value of less than 0.05 under one of these models was considered as suggestive evidence of association and a P-value between 0.05 and 0.1 was considered as marginally significant ($0.05 \leq P \leq 0.1$).

3.0 RESULTS

3.1 DNA RESEQUENCING

A total of 179 variants, single base substitutions or indels plus a microsatellite (tetranucleotide repeat marker) in intron 6, were identified in our study by complete resequencing of the *LPL* gene in 95 American NHW individuals falling in the upper (n=47) and lower (n=48) 5th percentile of HDL-C distribution. Table 9 shows a summary of the *LPL* variants identified in our NHW population sample.

Of the 179 variants identified, 105 were found to be already reported in the Chip Bioinformatics which currently uses dbSNP build 130. Among these 179 variants, 88 had a MAF ≥ 0.05 , 54 had a MAF 1-5% and 37 had MAF ≤ 0.01 . Seventeen of those variants were insertions or deletions, and remaining 162 were single nucleotide substitutions. One hundred forty two variants were located in the introns (including the microsatellite), 7 were located in the 3' flanking region, 8 were located in the 5' flanking region and 23 were located in the exons. Of the 23 exonic variants, 3 resulted in non-synonymous changes; aspartate⁹->asparagine (D9N) in exon 2, asparagine²⁹¹->serine (N291S) in exon 6, and serine⁴⁴⁷->stop codon in exon 9, and 3 resulted in synonymous changes; valine¹⁰⁸->valine in exon 3, glutamic acid¹¹⁸->glutamic acid in exon 4 and threonine³⁶¹->threonine in exon 8.

The remaining 17 exonic variants were located in the last exon 10 that codes 3'UTR region (Table 9). Of the 17 insertions and deletions, only one of the insertions was located in the exonic region but it was in 3'UTR and so did not affect the protein sequence. The range of indels sizes was 1-20 base except a 697-base deletion identified in intron 2.

We identified 74 variants that are not reported in the dbSNP build 130; 22 of these new identified variants had $MAF \geq 0.05$, 52 had $MAF < 0.05$ and 33 had $MAF \leq 0.01$. Of 74 new identified variants, 4 were located in 5' flanking region, 62 were in introns, 5 were in 3'UTR and 3 were in the 3' flanking region.

Table 9. *LPL* variants identified in our study for the NHW population

<i>LPL</i> variant	Alleles	Location	refSNP ID	Amino Acid Change	MAF	Call Rate %
208	T>C	5' flanking	rs1470186		0.016	98.90
351	C>A	5' flanking			0.005	98.90
428	G>A	5' flanking	rs73667465		0.016	98.90
549	C>T	5' flanking	rs17091742		0.016	98.90
958	G>A	5' flanking			0.005	95.80
1088	G>T	5' flanking			0.005	97.90
1090	T>G	5' flanking	rs1800590 (-T93G)		0.011	98.90
1130	G>C	5' flanking			0.005	97.90
2335	G>T	Intron 1	rs3779787		0.122	98.90
2913	T>C	Intron 1			0.005	95.80
3558	G>A	Intron 1	rs34309063		0.247	100.00
3964	G>C	Intron 1	rs17410577		0.239	98.90
4060	G>T	Intron 1	rs1534649		0.430	97.90
4424	A>G	Intron 1	rs13266204		0.263	97.90
4621	C>G	Intron 1			0.021	100.00
4948	C>G	Intron 1	rs6997330		0.022	97.90
5094	C>G	Intron 1			0.005	97.90
5107	C>T	Intron 1			0.005	96.80
5118	A>T	Intron 1			0.022	95.80
5200	C>T	Intron 1			0.006	91.60
5531	G>A	Intron 1	rs1031045		0.016	96.80
5772	A>G	Intron 1	rs60633545		0.016	98.90
5949	T>G	Intron 1			0.137	100.00
6383	G>T	Intron 1			0.005	100.00
6435	G>C	Intron 1			0.005	97.90
6477	T>C	Intron 1			0.005	98.90
6553	C>T	Intron 1	rs59254395		0.016	100.00
6554	A>G	Intron 1	rs56043715		0.016	100.00
6821	C>T	Intron 1	rs10104051		0.426	98.90
7130	T>C	Intron 1	rs28615996		0.021	73.70
7131	T>G	Intron 1			0.007	75.80
7313	G>A	Intron 1	rs28645722		0.016	98.90
7388	C>G	Intron 1	rs28575919		0.016	100.00

Table 9(Continued)

7503	T>C	Intron 1	rs6999612		0.016	100.00
7512	C>T	Intron 1	rs3779788		0.121	100.00
7556	T>C	Intron 1	rs59811201		0.016	89.50
8221	A>C	Intron 1	rs7000460		0.018	84.20
8250	G>A	Intron 1	rs59630933		0.019	97.90
8415	T>A	Intron 1	rs56321069		0.183	100.00
8467	C>T	Intron 1			0.005	100.00
8516	delG	Intron 1			0.011	50.50
9015	A>G	Intron 1	rs28445964		0.021	68.40
9024	T>C	Intron 1			0.008	98.90
9130	T>A	Intron 1	rs13252357		0.005	98.90
9411	A>C	Intron 1	rs28689946		0.016	98.90
9418	G>A	Intron 1	rs28582042		0.016	98.90
9589	C>T	Intron 1			0.016	98.90
9696	G>T	Intron 1	rs73667468		0.016	97.90
9914	T>G	Intron 1	rs73667469		0.016	100.00
10127	G>A	Exon 2 (non-synonymous)	rs1801177(D9N)	Aspartate9> Asparagine	0.016	100.00
10632	C>T	Intron 2			0.005	94.70
10912	A>G	Intron 2			0.022	94.70
10987	C>A	Intron 2			0.122	96.80
11050	T>C	Intron 2	rs7016529		0.016	96.80
11090	C>G	Intron 2	rs8176337		0.190	98.90
11228	T>C	Intron 2			0.005	96.80
11574	G>A	Intron 2	rs34123038		0.049	100.00
11600	G>C	Intron 2			0.011	100.00
11760	A>C	Intron 2	rs73667470		0.016	100.00
11888_11889	insA	Intron 2			0.011	95.80
12224_12920	del697	Intron 2			0.005	98.90
12449	G>A	Intron 2			0.117	97.90
12484	C>A	Intron 2			0.048	97.90
12550	G>A	Intron 2			0.118	95.80
12810_12829	dup20	Intron 2			0.126	95.80
12853_12854	Ins16	Intron 2			0.055	95.80
12861_12864	del4	Intron 2			0.060	95.80
12878_12889	del12	Intron 2			0.005	100.00

Table 9(Continued)

12884_12887	del4	Intron 2			0.016	100.00
13003	G>T	Intron 2			0.053	100.00
13639	G>A	Intron 2			0.011	98.90
13854	G>A	Exon 3 (synonymous)	rs1121923(V108V)	Valine108> Valine	0.016	100.00
14114	T>C	Intron 3	rs73667472		0.080	100.00
15206	C>A	Intron 3	rs343		0.042	100.00
15245	G>A	Exon 4 (synonymous)	rs248(E118E)	Glutamic acid118> Glutamic acid	0.047	100.00
15425	T>C	Intron 4	rs249		0.095	98.90
15449_15450	Ins2	Intron 4			0.063	98.90
15653	delA	Intron 4			0.410	92.60
15836	C>T	Intron 4	rs253		0.410	100.00
16316	C>G	Intron 5	rs254		0.074	97.90
16320	T>C	Intron 5	rs255		0.105	96.80
16386	C>T	Intron 5	rs256		0.118	97.90
16442	G>C	Intron 5			0.005	97.90
16563	T>A	Intron 5			0.016	93.70
16671	G>C	Intron 5	rs258		0.409	100.00
17231	C>T	Intron 5	rs263		0.135	100.00
17476	A>C	Intron 5			0.005	95.80
17599	G>A	Intron 5	rs264		0.116	60.00
17948	A>G	Exon 6 (non-synonymous)	rs268 (N291S)	Asparagine291> Serine	0.033	92.60
18065	T>G	Intron 6			0.009	91.60
18086	T>G	Intron 6	rs269		0.119	96.80
18095	C>A	Intron 6	rs270		0.201	100.00
18121	G>A	Intron 6	rs271		0.109	100.00
18297	A>C	Intron 6			0.005	97.90
18395_18396	InsT	Intron 6			0.121	100.00
18462	T>G	Intron 6			0.005	98.90
18621	C>T	Intron 6			0.005	100.00
18708	T>C	Intron 6	rs276		0.021	95.80
18822	T>C	Intron 6	rs277		0.232	97.90
18942	G>A	Intron 6	rs278		0.211	97.90
19442	A>T	Intron 6	rs281		0.242	95.80

Table 9(Continued)

19445	C>G	Intron 6	rs282		0.129	96.800
19517	C>T	Intron 6	rs283		0.161	100.00
19608	C>T	Intron 6	rs285 (PvuII)		0.407	97.90
19675	A>T	Intron 6	rs286		0.060	95.80
19815	G>A	Intron 6			0.005	97.90
19975	A>G	Intron 6	rs287		0.172	100.00
20038	T>C	Intron 6	rs289		0.159	100.00
20080	C>T	Intron 6			0.005	100.00
20271	T>C	Intron 6	rs291		0.163	98.90
20363	A>T	Intron 6			0.053	100.00
20505_20506	insA	Intron 6			0.163	100.00
20544	T>C	Intron 6	rs294		0.080	100.00
20657	A>C	Intron 6	rs295		0.163	98.90
20663	G>A	Intron 6	rs296		0.005	97.90
20790	T>C	Intron 6	rs297		0.163	91.60
21125_21128	del4	Intron 6			0.005	87.40
21353	T>C	Intron 7	rs301		0.172	86.30
21780	T>G	Intron 7	rs304		0.103	86.30
21820	A>G	Intron 7	rs305		0.108	97.90
21895	T>G	Intron 7	rs308		0.012	94.70
21965	C>T	Intron 7	rs310		0.006	93.70
22044_22047	del4	Intron 7			0.075	92.60
22416	G>C	Intron 7	rs312		0.072	100.00
22461	G>A	Intron 7	rs314		0.180	92.60
22514	T>C	Intron 7	rs315		0.011	92.60
22855	C>A	Exon 8 (synonymous)	rs316 (T361T)	Threonine361-> Threonine	0.079	88.40
23190_23191	del2	Intron 8			0.500	86.30
23192	G>T	Intron 8			0.068	88.40
23388	C>G	Intron 8	rs318		0.018	90.50
23395	A>C	Intron 8	rs319		0.293	93.70
23496	T>G	Intron 8	rs320 (HindIII)		0.185	94.70
23573	T>C	Intron 8			0.017	95.80
23636	A>C	Intron 8	rs322		0.170	98.90
23747	T>C	Intron 8	rs325		0.067	98.90
23858	A>G	Intron 8	rs326		0.206	100.00

Table 9(Continued)						
23955	T>G	Intron 8	rs327		0.192	90.50
24143	C>G	Exon 9 (non-synonymous)	rs328 (Ser447X)	Serine447-> Stop codon	0.005	100.00
24505	A>G	Intron 9	rs329		0.016	98.90
24573	T>C	Intron 9			0.005	100.00
24815	G>A	Intron 9	rs330		0.111	100.00
24824	G>A	Intron 9	rs331		0.122	100.00
24852	T>C	Intron 9	rs12679834		0.080	100.00
24899	C>T	Intron 9			0.016	100.00
25005	A>G	Intron 9			0.005	100.00
25049	G>A	Intron 9			0.063	98.90
25320	C>T	Intron 9			0.005	95.80
25335	C>T	Intron 9			0.079	98.90
25352	A>C	Intron 9			0.079	91.60
25844	T>G	Intron 9			0.074	95.80
26201	T>G	Intron 9			0.077	96.80
26234	T>G	Intron 9	rs10099160		0.282	98.90
27000	C>T	Intron 9			0.029	98.90
27160	T>A	Intron 9			0.027	98.90
27229	C>T	Intron 9	rs11570891		0.082	97.90
27249	G>A	Exon 10-3' UTR	rs4922115		0.085	98.90
27611	T>C	Exon 10-3' UTR	rs3289		0.016	95.80
27688	C>T	Exon 10-3' UTR			0.005	95.80
27783	A>T	Exon 10-3' UTR			0.011	95.80
28036	A>G	Exon 10-3' UTR	rs11570892		0.101	100.00
28067	A>T	Exon 10-3' UTR	rs3208305		0.203	100.00
28093	C>T	Exon 10-3' UTR	rs1803924		0.082	100.00
28382	C>T	Exon 10-3' UTR	rs1059507		0.110	100.00
28407	C>A	Exon 10-3' UTR			0.026	100.00
28464	C>A	Exon 10-3' UTR	rs3735964		0.079	100.00
28490	A>G	Exon 10-3' UTR	rs3200218		0.279	100.00
28524	C>T	Exon 10-3' UTR			0.005	100.00
28911	T>C	Exon 10-3' UTR	rs13702		0.200	100.00
28982	T>C	Exon 10-3' UTR	rs1059611		0.079	97.90
29046_29047	Ins2	Exon 10-3' UTR			0.079	100.00
29086	C>T	Exon 10-3' UTR	rs15285		0.200	100.00
29088	C>A	Exon 10-3' UTR	rs3866471		0.105	100.00

Table 9(Continued)

29287	G>A	3' flanking	rs3916027		0.184	100.00
29315	T>G	3' flanking	rs9644636		0.339	100.00
29474	C>T	3' flanking			0.026	98.90
29487	T>A	3' flanking	rs4921683		0.105	98.90
29547	C>T	3' flanking	rs4921684		0.105	98.90
29557_29558	InsA	3' flanking			0.005	98.90
29716	T>C	3' flanking			0.026	95.80

3.1.1 *LPL* Annotated Sequence

Figure 4 depicts the variants identified in *LPL* within a color FASTA representation of the annotated reference sequence adapted and modified from the CHIP Bioinformatics database (<http://snpper.chip.org>). The color code for the reference sequence is as follows: green for 5' and 3' flanking regions, grey for introns, black for exons. Capital letters are used to show coding bases and small letters show the bases in the UTR and flanking regions. The variants identified in this study also listed in dbSNP build 130 are shown in blue font with refSNP ID; the variants that are identified in only our study are shown in red font. The 197 variants which were already reported in public databases but not identified in this study are shown in grey font. The small deletions and the insertions identified in our study are shown by highlighting the region in yellow. We identified a large deletion in intron 2 which was not reported before in any public databases and it is highlighted in *italics*.

	1	2	3	4	5
	1234567890	1234567890	1234567890	1234567890	1234567890
19,839,852	ataaat	atagtagatt	ggaggttctg	atltgatgag	ccagtttctc
19,839,898	agccataaac	tgagaggggg	ttggggatac	acttcattgt	ccttcctggc
19,839,948	taatgtaaat	cccttatatt	taaaaagata	tttaaaagta	ttccaagcat
19,840,008	tttggcagaa	aagcatagta	tctaattgta	tttttttctt	atltttatgtg
19,840,058	catgcctctt	atccatttaa	aaatagcttt	actgacctat	aatttacaca
19,840,108	ctatataa	ctcccattga	aagtgcataa	ttctgttctt	tttagtata
19,840,158	ttacagagtt	gtgcagcatt	agcataatgt	aatctagaac	attgtcatca
19,840,208	acta c ccca	aatctctatt	cttcccttcc	cctattaatt	accagcccc
19,840,258	aggcaagcac	tgatctactt	ttggtctcta	tggatttctc	tatttctgga
19,840,308	cactttaaat	ggaatcatac	aatatgtgtc	ttttggcact	atcttcttct
19,840,358	acttatcata	actcaatagc	gcttttagatt	atltgacctc	gatgttctgc
19,840,408	ctgtgaacat	aaaatattat	ccttgcattc	cttgatgagt	ttgaggattg
19,840,458	agaataa	gcatgagaca	aaaattagaa	actagttaga	gcaagtaggc
19,840,508	ttttctccat	cacataagct	gatccatctt	gccaatgtta	aaacaccaga
19,840,558	ttgtacaagc	acaagctggg	acgcaatgtg	tgtccctcta	ttccctacatt
19,840,608	gactttgcgg	gggtggggat	ggggtgcggg	gtgagtgagg	gaggactgca
19,840,658	agtgcacaa	aggattctgc	aaaagagagg	tgtattaaag	tgccgatcaa
19,840,708	atgtaattta	acagctaaac	tttccctcct	tggaaaacag	gtgattgttg
19,840,758	agtatttaac	gtgaatcgat	gtaaacctgt	gtttggtctt	tagacagggg
19,840,808	gccccggggt	agagtggaa	cccttaagct	aagcgaacag	gagcctaaca
19,840,858	aagcaatttt	ttccgtctgc	ctttccccc	ttctctctgt	ggcagggctc
19,840,908	atcctcatta	ctgtttgtct	aaaagtttag	aagtgaattt	aggtccctcc
19,840,958	ccccaaatta	tgattttata	gccaataggt	gatgaggttt	atlttgcata
19,841,008	ttccagtcac	ataagcagcc	ttggcgtgaa	aacagtgta	gactcgattc
19,841,058	cccctcttcc	ttctctcaa	gggaaagctg	cccacttcta	gctgcctctg
19,841,108	catccccttt	aaagggcgac	ttgctcagcg	ccaaaccgag	gctccagccc
19,841,158	tctccagcct	ccggctcagc	cggtctcatc	gtcggctccg	gccttgcagc
19,841,208	ttctccagag	ggacgcgccc	cgagATGGAG	AGCAAAGCCC	TGCTCGTCT
19,841,258	GACTCTGGCC	GTGTGGCTCC	AGAGTCTGAC	CGCCTCCCGC	GGAGGGGTGG
19,841,308	CCGCCGCGA	CCGTAAGTTT	TGCGCGAAA	CTCCCTCCA	CCTGCAGACC
19,841,358	CGGCGGGTGG	CCACTGCCAC	CCGAAGTGG	GATGAGAAGA	AGGAAGTTGG
19,841,408	AAGGGGCGGT	GGATGCGCCC	AGGGACTCTC	CCAGCCTGGG	CTCTAGCCCC
19,841,458	GAAACGGTCC	CCGGAGTGGG	ATCCAGGAGG	GGCCGGGAGG	GAATCTCCTC
19,841,508	CCGATCGTGA	AGCGGCGGCG	CCCAGTTCCC	GCTTTTCTC	TCTGCCGGGT
19,841,558	TCCCGGCGTA	TCCCTTCCAC	TCTGGCTGGG	ACCGGCTTCC	CGGGCTCGCA
19,841,608	GGCTCCGCGG	GGGAGTTC	GGGGTGTGGG	GGCCGGGACG	GCGGAGGCGG
19,841,658	GGAGTAAGGG	CCCGGCTGGC	GGTGACCTGC	AGTCACTCT	CTGCCGGAGG
19,841,708	GGCCCTGGAA	TGAAAGCGCG	GCGGGCCAAG	GTGACCTCGC	CTTGTGTGGC
19,841,758	ACTGCGGCTC	AGCCCCGCC	CGGGACTCG	CGGGCCGACT	GTGGCCCTT
19,841,808	CTGGGGAAGC	CGGGGCGCGG	GGAGGCGTTC	CGGGCATCTC	AGCCGCACGG
19,841,858	GGTACGCTCG	CCCTCGGCGG	GGCCCCCGC	TCCGCTGTGG	GAGTGGCAGT
19,841,908	GGGTGTGGG	GTGGAGAAAG	TACGCGTGGC	CCGGAGTCTC	rs7839976
19,841,958	CGTCCACACC	GCTCTGGGGA	GCCCCGACT	CTCTCCAGCT	TCCAGGCTCG
19,842,008	CATGCCCTCT	TTTTCTTAGT	GCCCTGAGAA	CCCAGCGAGG	GGCTGACCCT
19,842,058	CCGAAACCG	TGGCGCAGCC	ACCAGCAATC	TGTGGTCCGC	GACTCGGGGG
19,842,108	GTTGCCAGGT	CTGCGTTTGG	CCACCCCTTC	TGTCCTGGGG	GCTGAGGTCA
19,842,158	GCTCCGGGGC	CCCGGCCCGG	CCGCGCGGCT	GCGAGCACGT	GGGGTTGACG
19,842,208	GGCGCCGCGT	GAGGGCAGCG	AGCAAAACGG	TGGTCACCGC	CGCCAGGGAA
19,842,258	CCGCCCGCTC	GCTGGGGTCC	AGGCGTTCGG	GGCAAAATGA	GAATGTCTCA
19,842,308	GACCTGTCCG	CAATGGAGGC	AGCCTGCTTA	ATTGAAACCT	CGATTTCAGTA
19,842,358	AACATGCAAC	AGCAGCATAG	AGAGCAGCTG	AAGCCATTCA	TAACACGGGA
19,842,408	CAACATTTCC	TTTTTTCTTC	CATGCTGAAA	TTGCAATTAG	GGCGGTGTCT
19,842,458	CTTGGATGTG	CTCTCAGGCG	GCACGTCCCC	AGCGGTTCAA	GTTATAATAG
19,842,508	AGTCTCTCCA	TAGCTTTGAT	GGCCGCTAAA	CGTTTGTTTT	ATTTTGGCAT
19,842,558	TAATTTGTGA	AACATTTTGG	TTAGGTTAAA	AAACAAAAG	TTGGCAGGGA
19,842,608	GCAGTGGCTC	ACCCATGTAG	TCCAGCACT	TCGGGAGGCC	GAGGCCGGAA
19,842,658	GACAGCTTGA	GCCAGAAAGT	TCGAAATCAG	CCTGGGCAAC	ATAGGGAGAC
19,842,708	ACCGTCTCTA	TAAAAAAAAT	ATACAAAAT	TACCGGGCGG	TGGTGGCCTG
19,842,758	TGCCTGTGGT	CCCAGT T AGT	CAGCAGGCTG	AGAGATCACT	TGAGCCAGGG
19,842,808	AGTTTCAGTA	GACTGCAGTG	AGCTGTGATT	GCCCCACTGC	ACTTCATCCT
19,842,858	GGGTGACAGT	GAGACCCTGT	CTCATAAAAT	TAAACAAAA	CAAAACAAAC
19,842,908	ACTAAGTATA	GATTCATCA	AAGCAAAAT	GGATAAGAAA	AAAAGTATCT
19,842,958	TTTCTATTGG	ATCAGTTTGA	AAACACTGGA	GAGTTGATGA	GAAAGTCTTC
19,843,008	AACATCTTAG	GTGGGGTATG	TTTTCGTATG	TTCCCTTCGT	ACTGCTTAAT
19,843,058	GCTGACAAGA	AGATGGTAGG	AGCCAACTCC	AAATCTTAT	TTCAGAAAGC
19,843,108	ACACCATAGA	ATAACGTCAT	TTTCATTGCA	AAACAAGCAC	CGAAATATGT
19,843,158	CATCACATTC	AAGTTTTTCC	TAGGCTCCCT	TACAGGTCA	AGATCCTAAA

p.208/rs1470186-[T/C]

p.351-[C/A]

p.428/rs73667465-[G/A]

p.549/rs17091742-[C/T]

p.958-[G/A]

p.1088-[G/T];p.1090/rs1800590-[T/G]

p.1130-[G/C]

Exon 1

Intron 1 rs11570895

rs2898492

rs7839976

p.2335/rs3779787-[G/T]

p.2913-[T/C]

19,843,208 TTCTTGGGAG TCAGTGTGAC CTCTCTGGGT TTAGGTTCTT CAACTCTGCA
19,843,258 ATGAGTTTGG ATGAGGCCAA TGTTCCTCTGA GCCTGGTGTA ACTCTTGCCT
19,843,308 CTTTAAAGTGG ACACCTTATGT GATTAATTAG TTTAATTGAG TTGTAGCCAA
19,843,358 CACATGCCTTT TCCTAGCTGT AAAATATATTA AGGAAGGATT ATTTCCAAGT
19,843,408 AGACTGGAAA **G**ATGCCCCTC CCATCCCCTC CACTTTCAC TACTCACC **p.3558/rs34309063-[G/A]**
19,843,458 AATATATCAT GCCTCTCCCA TCACAGCAAC TTTCTCCCTC TTTCTCCTCC
19,843,508 AGATGCATTC ATCTAGGAAG GTAAGAATTT CAGGGAGAGA AAGATGTGAC
19,843,558 CGTGGTAGAA AGACAGGGAT CACCTCCTCT GGGCTCTTGA GTTTACTTAT
19,843,608 TCATTTCTGGA TTCTTTCTAA CAAGAATATG AGGACAAGAG GCACTGTCTT
19,843,658 CAGGCACTTC GTCCCTGGGAG CCACCACCAT CTCTGCATGG CCCCAATTAG
19,843,708 GAAACGTGAA GAGCTAGGAG AGGGAGAGTA TGGTCAGTGC TTAGCAGCTG
19,843,758 AAGTTCACAT TGCCTGGCCA TCGTGAATTT CCAGGCTGTC TTCTGAGTTG
19,843,808 AACATGATGG CAAAGGA**G**AG CAAAATAGCA GATGTCAC TGGAGAGCT **p.3964/rs17410577-[G/C]**
19,843,858 CAGCGAGGGA GTGATTGATT AATAGCTGTA TTGAAAGGTG GGAGTCAGGT
19,843,908 ACGGGGGAAG AG**C**GCGATG GAAAATTTTC GCTTCTTTC AGCAGCTTAT **p.4060/rs1534649-[G/T]**
19,843,958 TTTTAACTCA GCTTTCTGTT CTTGCTTTAT TATGGAGGAA AAATTGGGCC
19,844,008 ATAGAGTTTA CTGCCTTATG CCAGATTGTT CAAGAAAATG CCTTGCAACT
19,844,058 TACAAATATT TGCAGCTAGT TTCTTCC**G**TG ACCACCACAA AGACTGCATT **rs73667466**
19,844,108 GACTTAAATA TGAAGATGTT CCAGCCATCA AAATGATGGT TGGTGATGAT
19,844,158 TTTGGATCAC AAAGTGTAAG GAAAGTATTC AAGACATGAG TATCATGATT
19,844,208 TTTTAAAGTTC TGGATGAAGA GACCATTGG ATTTACTAAT AAGTAAATT
19,844,258 CCAACTTTTA TGGCAATAAA AACAA**C**AAA ACACCTATCA GTGTAAAGCT **p.4424/rs13266204-[A/G]**
19,844,308 TTTGGATCAT CTATCCATTA AATGAGTTTG TCACCACAGT GAACTAAATA
19,844,358 CCTTTTATCA ACAGAGACTT TCTAACCTGG GAGTAAATTT CTTGTACAG
19,844,408 TGCTTTTGTG ACATTTCTGTC TTTGCAAAAG TTGAAGGCTC CAATAGTTTC
19,844,458 TGAAGGACTA ATAGGATAGG GTT**C**CAACTT ACTCAGAGGC TAAGAGTTTG **p.4621-[C/G]**
19,844,508 AAATTTACTC TGAACGATGT CTGTTCACTA **G**ACTGCGTGA CTGCAGTTTG **rs58877654**
19,844,558 CTGTCTTGTG GCCATTTTAA GATTGTCTGT GTGCACTGAC ACCATTTGCG
19,844,608 TACTCAACAA CAG**G**TATCTA CTAGGAAGGA AGGAATGGAT TATCTTAGGT **rs10503668**
19,844,658 GCTATATATA TATGTAAGTT CTGCCGGAAG TGAGCCTATT AAACCTGTGC
19,844,708 CAATTCATTC CTTTGTCTCT TCCAGTCTG TGCTCTCAGA ATGATCAAAT
19,844,758 GCTATCTAAG TAGTGTGGTG ATTTTGACTG TTTGAATGAA TAAACGAAAA
19,844,808 **C**TCTCCAC CATTATTTGA CAGTAGAATA GAACAAAAGAT GACCCAGTTG **p.4948/rs6997330-[C/G]**
19,844,858 GCGTGGCCAC ACTGTGTCTA ATCCAGCCAC CTTGCTCACA GCACCAACC
19,844,908 CACTTTGGTG TGTGCGCTCA TCTGTATTTT CGTAAATGTT GAAGTCT**C**TT **p.5094-[C/G]**
19,844,958 TCTATGCATC **C**AGGTAGGGG **T**AATACCATT CTGCTTGGGA TTAATTTGAG **p.5107-[C/T]; p.5118-[A/T]**
19,845,008 TGTAGTACAA AGTTTAGAGA AGCTTTTAAAG TAGCATGAAA AGTCAGATGC
19,845,058 TTT**C**SGAGGG ATGGTGGTGA ATGTAGGTAA ATGGATCTGC ACTTAGAGAT **p.5200-[C/T]**
19,845,108 CCTCAACAGC CCTTCATTCA AGATACAGCT ATCATGACAT CAATAATGTA
19,845,158 CCTTTGAAAA AAAATTGGCT GCAGAAATTA TTACGGTGA AAACAGTATA
19,845,208 GGAGTTAGCA CTTACATTTT AACTAAAAAG AATAGCGTCC CCATGTTTAT
19,845,258 TCAGCCCTCC CTCCAATAAA ACAATTGTTG GCAAAGTAAAT CATGACTTTT
19,845,308 CATTGTGTTA GTTTGAGACA ACTGGATGTT TCCATTTGCC ATCCTCAGCA
19,845,358 ACAAGAAGAA AGTAGTCTCA GATTAACAGG GTAA**G**TATTT TGTAATTTCC **p.5531/rs1031045-[G/A]**
19,845,408 TTAATAAATC TTAGCTGAAA TATGCCTATG TGATGACACT CAACCAAAAT
19,845,458 ATTTTCAATA CCAGTCCACA AGATCTCCTT AAATAATGAT GGCTTATTCA
19,845,508 CACTTGATGG TCTCATTCAG TGGGGCAATT TTAATACACA TCTCTGAACC
19,845,558 TATTTTTTAA CCCCTCTTTT TCAGTAGTGT GGAAGGTTAG CCCTAATATT
19,845,608 GGAGAAAAAT CAGGGTAAAA TTCAG**A**TGAT TCATACAGGA TTTATTTTTT **p.5772/rs60633545-[A/G]**
19,845,658 CTATTCATT AATAAAAAAA CTTTATATAA AATAAAAAAGT AGGCTGGCAC
19,845,708 AGTGGCTCAC TCCTGTAATC CCAGCATTTT GGGAGGCCGA GGCGGGTGA
19,845,758 TCATGAGGTC GGGAGTTCAA GACCAGCGTG GCCAGGATGG TGAAACCCCA
19,845,808 **T**CTACTATA AAATACAAAA ATTAGCCAGG CGTGGTGGCA GGCACCTGTA **p.5949-[T/G]**
19,845,858 ATCCCGGCTA CTCGGGAGGC TGAGGCAGAG AATGGCTTGA ACCCAGGAGG
19,845,908 CATAGTTTGC AGTGAGTCAC GATCGTGCCA CTGCACTCCA GCCTGGGTGA
19,845,958 CAGAGCAAGA CTCCGTTTCA AAAAATAATA ATAAAAATA ATGAAATAAA
19,846,008 GTAAAGCTGC ATGTTAGAGA AGTCAAGAGC ATTACTTACG TTAGAATATC
19,846,058 TGAACAGACC AATCAATTCA GTCTGATCAT GATATTGATG TTTTCTTCA
19,846,108 CCAAATCGAC CACATCAGTA ATTCACCTTG TTCTTCGATA TCCTACAGAC
19,846,158 ACTGCCTGAG TCGATAACTA TGACTATCAG TCTCAGAGAG CAAATGAATT
19,846,208 ACTGAGGAAG CCCTGTAGGA GTGAGAGAAA AGGGGG**G**AGA GAGAGAGAAA **p.6383-[G/T]**
19,846,258 GGGTGGGGG GATAACAGGA GAACAGAAAT TCCAAAG**A**G ATTGCATCT **p.6435-[G/C]**
19,846,308 CATTGAGTTC TTGTACCTCA TGTCAATTGCA **T**AAATGTTCA TCTTACTCAC **p.6477-[T/C]**
19,846,358 GTGATGACTT TGATCTGCCT TTAAGCACCC ATCTGCTGCT TTCTCTGGGAT
19,846,408 GCTCAA**C**ACT TCCCTCTTTC TAGCAACAAG AATTACCACT CTTCCCTCT **p.6553/rs59254395-[C/T]; p.6554/-**
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19,846,508 CTGTGAGTTA TTATCTATGT TAGAATAAAT TCTTTGTCTT TGTTTACACA
19,846,558 CTCAGATTTG TAGTTATTTA TTTAGGAATT TAGGAATAAA GATTCACATG
19,846,608 TCAGGAAAGG CACAATTTAT AACTTGCCTG TTACCCAAAA CTCTCCCTTA **rs61274012**

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19,846,758 CTTCCCTTTC CCCTTCTTCC TTCTTCTCCT CCTTCCCTCC ATCCCTCCCT
19,846,808 CCTGTACTCC TCTTCTTCTT CAATTCTAAG GTGGCCTTAA TTTCTAAGGG
19,846,858 ACATGGCAAA AGACAGTCTA GTTGGATGAG TGCAGTCACT AATATTATTT
19,846,908 CCATGTATGG AAAATAACTG TTCTCTTAGT AACAAATGCA TCAAATCAGT
19,846,958 TCACCTGCTG CCCAATAGCA ATCACAGGAT GCA~~T~~GGGAC AAATAAATAT p.7130/rs28615996-[T/C];p.7131-[T/G]
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19,847,108 GTTGCAACA CAGGTAGTGT GAAAATTATC AGAACATCCA AGAAAAGGAA
19,847,158 AGTTTGAATA AGTGCCGATA AGATTTATGA TGTCATGTCT GACATAGAAT p.7313/rs28645722-[G/A]
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19,848,058 CAAAACCTCT TTTCTCATAA TTAG~~A~~ATCAT TAAGAAATTT CTCCTACATT p.8221/rs7000460-[A/C]
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19,857,458 CGCTACACG CAAGAATATC TCCTGAAATG GGGCCTACAA TCATAAATGC p.17599/rs264-[G/A]
19,857,508 ACAGGACTAT ATCCTTGGGT GATTCTACTC TAACACCACA TCTCACCTAT rs265
19,857,558 TTTAGACATG CCAAATGAAA CACTCTTTGT GAATTTCTGC CGAGATACAA rs266;rs267
19,857,608 TCTTGGTGTG TCTTTTTTAC CCAGATGTGG ACCAGCTAGT GAAGTGTCTC Exon 6
19,857,658 AAGGAGCCGT CCATTCATCT CTTCACTGAC TCTCTGTTGA ATGAAGAAAA rs28934893;rs35414700
19,857,708 TCCAAGTAAG CCCTACAGGT GCAGTTCCAA GGAAGCCTTT GAGAAAGGGC rs1800011
19,857,758 TCTGCTTGAG TTGTAGAAAG AACCGCTGCA ACAATCTGGG CTATGAGATC
19,857,808 AATAAAGTCA GAGCCAAAAG AAGCAGCAAA ATGTACCTGA AGACTCGTTC p.17948/rs268-[A/G]
19,857,858 TCAGATGCC TACAAGGTA GGCTGGAGTA TGTTGTAAT AAGGAAACCA Intron 6 rs59184895
19,857,908 AGGAGTCCTA TTTCATCATG CTCACTGCAT CACATGTACT GATTCTGTCT p.18065-[T/G];p.18086/rs269-[T/G];
rs2075651; p.18095/rs270-[C/A]
19,857,958 ATTGGAACAG AGATGATGAC TGGTGTACT AAACCTGAG CCCTGGTGTG p.18121/rs271-[G/A]
19,858,008 TCTGTTGATA GGGGGTTGCA TTGATCCATT TGTCTGAGGC TTCTAATTC
19,858,058 CATTGTGAGC AAGGTCCAG TGCTCAGTGT GGGATTGCA GCCTTGCTCG
19,858,108 CTGCCCTCCC CTGTAATATGT GGCCATTAGC ATGGGCTAGG CTATCAGCAC
19,858,158 AGAGCTCAGA GCTCATTTGG AACCATCCAC CTCGGGTCAA CAAACTATAA p.18297-[A/C]
19,858,208 CCCTTGTGCC AAATCCAGCC TACTTCTCTG TTTTGTAAT AGTTTTTTA rs272;p.18395_18396-[InsT]
19,858,258 AAACCTTTAA GTTCAGGGGT ACGTATGTAG GTTTGCTAAA AAGGTAAACT
19,858,308 TGTGACATGG GAGTTTGTG TCCAGAATAT TCCATCACCC AGGTATTAAG p.18462-[T/G]
19,858,358 CTTAGTACCC ATTAGTACT TTTCTGTGAG CTCTCCCTCC TCCCACCCTC
19,858,408 TGGGAGGCC CAGTGTCTGT TGTCCCCCTC TATGTGCTCA TGCAAAGTTT
19,858,458 TATTAGGACA CAGCCACACA CATTCATTAC CATATTGTCA AAGGCTGGTT p.18621-[C/T]
19,858,508 TCATGCCACC ATAACAGAGT TGATAGCCCA CAGAGCCTAA AATATTTACT rs275
19,858,558 CCCTGGCCCT TTACAGAATG TTCACAACCT ACATAAAGGC AAGGACCATC p.18708/rs276-[T/C]
19,858,608 TGTCTATTT ATTTATTTAT TTAATTTGAG ATGAAGTCTA GCTTTCTCCT
19,858,658 AGGCTGGAGG AGAGGGGCAT GATCTTGGCT CACCACAACC TCTGCCCTCC p.18822/rs277-[T/C]
19,858,708 GGGTTCAAAT GATTCCCCTG CCTCAGCCTC CGGAGTAGCT GGATAACAG
19,858,758 CATGCAACCA TCATGCCAG CTAATTTTTG TATTTTTAGT AGAGAGGGGG p.18942/rs278-[G/A]
19,858,808 TTTACCCTGG TTGACCAGGC TGGTCTCGAA CTGCTGACCT CAGGTGATCT
19,858,858 GCCCTCCTTG GCCTCATCTG TCTTTTTAAA TGCAACTATF CCTGGAAGGC
19,858,908 AAGAATATCT CACACCTTCT AAGATACTGC CATTTTGCCA GGAGTTTGT
19,858,958 TCACACTTGA ATTTCAAGCT TGGCCTCTTG TTTAGAGGCA GACCTAAAGG rs279
19,859,008 AATGGTCGGA AAATGAGAGA GGAGGTCTTC GGATAAATCC GGTGAGAGGG
19,859,058 ACCAACTTCA GGAAGGGTGG CTTTGTGTTG ATCCAGATGG AAACCTGAGG
19,859,108 GAAGGGATGA TATTAAGAA CAGTGGCCCC AGGTAAAACA TATGGCACCC
19,859,158 ATGTGTAAGG TGATTCTTAG AATCTGTAGA GGTGTCTTTC GTGGTATAGA rs280
19,859,208 GGTGAGGCA CCTGTGCTTC AAGGAAACCT TAACCTTCA AAATCAGGCA
19,859,258 ATCGTATGA GGTAAAGAGA GGACTGTGG ACCATAATCT TGAAGCACA rs17091775;p.19442/rs281-
[A/T];p.19445/rs282-[C/G]
19,859,308 GACAGGCCTC ACTCATCCCT GCCTCCTGCA CCAGTGGGTT CAAGGCTCTG
19,859,358 TCAGTGTCCC CTAGGGGCAC CTCACCACTC CCAGCTTCTT CAGCTCTGGC p.19517/rs283-[C/T];rs284
19,859,408 CTGTCCTGCT GCCTGCAAGG GTTTTGCTTA ATTCTCAATT CAATGTCTCT
19,859,458 TCATCTTTTA CCAGCTGTGG GGTTTTGTG TTGTTCTTCT GTTTTGTCTT p.19608/rs285-[C/T]
19,859,508 AGTATCTGAC TACTTTTTAA TTATAAAAAG AGATGTATCT AAACAAAATA p.19675/rs286-[A/T]
19,859,558 GAGATTGTTA TCAGAAGTTC ACAACATTTA TTAATAATTT TTTACCTGG
19,859,608 ACAAGAGTCT AAAGCAGCAT AAAAAATATG TCTGCTATAT TCTAAACCAT
19,859,658 CAGTCTTAAG AGATCTGTG CTCAGCTTAA GAGAAAATAC ATTTAATAGA p.19815-[G/A]
19,859,708 CAGTAACACA AATAAGAAAA AAATCTGACC AAGGATAGTG GGATATAGAA
19,859,758 GAAAAACAT TCCAAGAATT ATTTTATTTA TTTATTTATT TATTTATTTA
19,859,808 TTTATTTATT TATTTATTTT TGAGACACAG TCTCGCTCAG TTACCCAGGC p.19975/rs287-[A/G]
19,859,858 TGGAGTGCAC CGGCGCAATC TTAGCTCACT GCAACCTCTG CTTTCCGGTT rs288;rs2583659;p.20038/rs289-[T/C]
19,859,908 CAAGCGATTC TCCTGCCTCA GCCTCCTGAG TAACTGGGAT TACAGGCACC p.20080-[C/T]
19,859,958 CGCCACCACG CCCACTAAT TCTGTATTT TTCTTAGTAG AAACAGGGTT rs34951282
19,860,008 TCACCATGTT GGCCAAGCTA GTCTCAAACCT CCTGACCTCA GGTGATTAC rs290
19,860,058 CCACCAAGGC CTCCCAAAGT GCTGGGATTA CAGGCATGAG CCACCATGCC

19,860,108 TGGCTCCAA **G**AACTCTTTT TTCC**T**CCATC ATCATGGTTC TATTTTAGTC rs2698206;p.20271/rs291-[T/C]
19,860,158 CTGCTGCCTT TCCTTTTAAAC CTC**T**CCCCAG GCCCATTGTC TCAGGGTTTT
19,860,208 TGGTAGAGAC CAGAG**A**GGG GCAGGGAGGA GATATAGAAG TTCAACTACC p.20363-[A/T]
19,860,258 TGCTTCCAGA GGCTGTCCCT AGTATAGAAT ACTTTAGGGG CTGGCTTTAC
19,860,308 AAGGCAGTCC TTGTGGCCTC ACTGAT**G**CT CAATGAAATA AGTTCTTTTT rs292
19,860,358 TAAAAAA**A**TT TTTATTATT TCCATAGGTT ATTGGGGGAA CAGGTGG**T**GT p.20505_20506-[InsA];p.20544/rs294-
[T/C]
19,860,408 TTGGTTACAT GAGTAAGTTC TTTAGTAGTG ATTTGTGAGA TTTTGGTGTG
19,860,458 CCCATTACGG AATGGAAAAA TCAACGAAAT AAGTTCTATG ATGCACCTAC
19,860,508 TAGACACCTA **A**TCTGC**G**CTA GATGGTGGGG GAATTAAGAG CATGGGCATG p.20657/rs295-[A/C];p.20663/rs296-
[G/A]
19,860,558 ATCCTGTGAC CGGAAGCCCG CTTACAGTCA GGTGGGAGGA CAGACCTACT
19,860,608 CATGAAACAA ACACAGTGAC ATATAGTGAC ACAGAAGCAA ATG**T**CAAATA p.20790/rs297-[T/C]
19,860,658 TGCTTGCTCC AGATGCTAAG GCACAAGATG GCCAAGGATG GCGGAGTTCA
19,860,708 TGGAGAAAGC ATCATGAGTG TTTTGGCCTT CTGATTGAT CTCCCTAGCA
19,860,758 CCCCTCAAAG ATGGCTACTT CCTAATGCTG CTGGCAATT CAGACACATT
19,860,808 TGGGTTTTTC CTATGCATAT AACCACACTT TTCTGAAAGG GAGTAGAATT
19,860,858 CAAGGTCTGC ATTTTCTAGG TATGAACACT GTGCATGATG AAGTCTTTCC
19,860,908 AAGCCACACC AGTGGTTCCA TGTGTGTGCA CTTCCGGTTT GAGTGCTAGT
19,860,958 GAGATACTTC TGTGGTCTG AATTGCCT**GA** **CA**ATTTGGGG TTGTGATATT p.21125_21128-[delGACT];rs73601683
19,861,008 TTCATAAAGA TTGATCAACA TGTTCGAATT TCCTCCCCAA CAGTCTTCCA Exon 7
19,861,058 TTACCAAGTA AAGATTCAAT TTTCTGGGCA TGAGAGTGAA ACCCATACCA
19,861,108 ATCAGGCCTT TGAGATTCT CTGTATGGCA CCGTGCCGA GAGTGAGAAC rs298
19,861,158 ATCCCATCA CTCTGTGAGT AGCACAGGGG GCGGGTCAATC ATGGCACCAG Intron 7 rs299 rs300
19,861,208 TCCCTC**T**CCT GCCATAACCC TTGGTCTGAG CAGCAGAAGC AGAGAGCGAT p.21353/rs301-[T/C];rs302;rs34500595
19,861,258 GCCTAGAAAA CAAGTCTTTA GTTAAAAAAA TCAGAAT**T**TC AAAATTTGAGG
19,861,308 TCTTCTCTT ATTTGATATT GAGAAAAAAA TGCTTCAAAT TGGCCATTTT
19,861,358 ATTTTCACTT ACTAGTTATA TTTTTTTATT TATCATCTTA TATCTGTTTA
19,861,408 TTTCTTTTAT AAAGCTGCTG TTAACAATA TAATTAAGT ATCTCAAAG
19,861,458 GTTTGACATT AAAGAAAATG AGCAATGGTA ACAGGAAACC ACTCTATAGA
19,861,508 TGTACATATA ATATGTACAG AAAATATAAG TAGTAAGAAG TCCATGACAA
19,861,558 AGTGTAGCT **C**TTTTTTTTT TTTTTTTTTT TTTTTTTTTT GAGATGGAGT rs303;rs13257355
19,861,608 CTCTCTCTAT TGCCAGGCT GGAGTGCAGT GAT**T**CGATCT CAGCTCAGTG p.21780/rs304-[T/G]
19,861,658 CAACCTCTAC CTCCCGAGT CAA**A**CAATTC TTCTGTCTCA GCCTCCCGAG p.21820/rs305-[A/G]
19,861,708 TAGCTGGGGC TGCAG**T**GGCC CACCACCA**T**G CCCAGCTAAT TTTTGTAT**T** rs306;rs307; p.21895/rs308-[T/G]
19,861,758 TTAGTAGCGA CAGGGTCTCA CCATGTTGGC CAAGCTGGTC TTGAAT**T**CCT rs309
19,861,808 GATCTCAGGT GATCCACC**C**G CCTCGGCCTC CCAAAGTCT GGGATTACAG p.21965/rs310-[C/T]
19,861,858 GTGTGAGCCA CCATGCCAG CCTACCCTTT ACTACTAATC AAAGAAA**TAA** p.22044_22047_[delTAAA]
19,861,908 **A**AGTAAGCA ACTTGATACT TTTACAATA CTAGATGAAC AAATCTTTAA
19,861,958 AAATAGCCAG TGCAGACAAG GTGGTGAAGC AGAACATGCG AACCTACCAT
19,862,008 GCATCATTCA CGGCTAGAAC CCTCCAGGTG CGGAAGGTAG TATTTTAAATA
19,862,058 ACTTTCCATA GCTACAAAAT ATTATTACAT AGAAGGGAGT GATTTTTTTC
19,862,108 TAATATTTAT CCTAAAAGAAA TAGTCAACAA ACATTTTTAA AAACATCAAT
19,862,158 TACAGTCGTA CCTATACTAG CATAAATTAG AAACCCAGTA TCCAACATTG
19,862,208 AGGCAGTGGG TAAATGAATC GTGGTTTATC AAGTCATTA AATCAATCTA
19,862,258 GCCTTTAAA ACTATAATT**G** TAGGAAACCC AGGAAAACAT AGTAAAA**AT** p.22416/rs312-[G/C];rs313
19,862,308 GGAATATAAA ATCT**G**AAGAG AATAAAGAAT AGAGAATCGT ATGTGTGCTA p.22461/rs314-[G/A]
19,862,358 TGATTGTAGC TAAATA**T**GT TCAAGTATCA ACACAAATTG AAAAGGAATA p.22514/rs315-[T/C]
19,862,408 CATGAAAAAT AAAATTATAT TTCTGAATGA TTGACTTCAG GATTTTCTTT
19,862,458 TAGAATTGTA TTAATAGTT CATGTCATTA GGATAAATGC TGGAAATGTTG
19,862,508 ATATAATTTA AAATATACTA AATGCCATCG ACCTTCATTT TGAGTTCTTT
19,862,558 GTTGGACATT TTTGTGCATT TTTAAAATAT CCCTAAATA ATAAAGCTAT
19,862,608 TTATATTTGG AGAGGAGAAA AAAAAGTGGG GGGCAGGGAG AGCTGATCTC
19,862,658 TATAACTAAC CAAATTTATT GCTTTTTTGT TTAGGCCTGA AGTTTCCACA Exon 8
19,862,708 AATAAGAC**C**T ACTCCTTCCT AATTTACACA GAGGTAGATA TTGGAGA**ACT** p.22855/rs316-[C/A]
19,862,758 ACTCATGTTG AAGCTCAAAT GGAAGAGTGA TTCATACTTT AGCTGGTCAG
19,862,808 ACTGGTGGAG CAGTCCCGGC TTC**G**CCATTC AGAAGATCAG AGTAAAAGCA rs5934
19,862,858 GGAGAGACTC AGAAAAAGTA ATTAATGTA TTTTCTTCC TTCACTTTAG Intron 8
19,862,908 ACCCCACCT GATGTCAGGA CCTAGGGGCT GTATTTCAGG GGCCCTCACA
19,862,958 ATTCAGGGAG AGCTTTAGGA AACCTTGAT TTATTACTGT ATGATGTAGA
19,863,008 TTTTCTTTAG GAGTCTTCTT TTAITTTCTT ATTTTGGGG GGC**AG**GGGG p.23190_23191-[delAG];p23192-[G/T]
19,863,058 GGAAGTGAC AGTATTTTGG TATTTCATGT AAGGAAAACA TAAGCCCTGA
19,863,108 ATCGCTCACA GTTATTCACT GAGAGCTGGG ATTAGAAGTC AGGAATCTCA
19,863,158 GCTTCTCATT TGGCACTGTT TCTTGTAAGT ACAAATAGT TAGGGAACAA
19,863,208 ACCTCCGAGA TGCTACCTGG ATAATCAAAG ATTCAAACCA **A**CTCTTCA**A** p.23388/rs318-[C/G];p23395/rs319-[A/C]
19,863,258 GAAGGGTGAG ATTCCAAGAT AATCTCAACC TGTCTCCGCA GCCCCACCA
19,863,308 TGTGTACCCA TAAAATGAAT TACACAGAGA TCGCTATAGG ATTTAAAG**C**T p.23496/rs320-[T/G]
19,863,358 TTTATACTAA ATGTGCTGGG ATTTTGCAA CTATAGTGTG CTGTTATTGT
19,863,408 TAATTTAAAA AAACCTAAG TTAGGA**T**TGA CAAATATTTT CTCTTTAGTC p.23573-[T/C]
19,863,458 ATTTGCTT**G**T ATCACCAAAG AAGCAAACAA ACAAACAAA **A**AAAAAAGAA rs321;p.23636/rs322-[A/C];rs323

19,863,508 AAAGATCTTG GGGATGGAAA TGTTATAAAG AATCTTTTTT AACTAGACAA
19,863,558 TGTCTAGCTG AAGGCAGATG CCCTAATTCC TTAATGCAGA TGCTAAGAGA
19,863,608 **T**GGCAGAGTT GATCTTTTAT CATCTCTTGG TGAAAGCCCA GTAACATAAG p.23747/rs325-[T/C]
19,863,658 ACTGCTCTAG GCTGTCTGCA TGCCTGTCTA TCTAAATTA CTAGCTTGGT
19,863,708 TGCTGAACAC **A**AGGTTAGGC TCTCAAATTA CCCTCTGATT CTGATGTGGC p.23858/rs326-[A/G];rs7005541
19,863,758 CTGAGTGTGA CAGTTAATTA TTGGGAATAT CAAAACAATT ACCCAGCATG
19,863,808 ATCATGTAT **T** ATTTAAACAG TCCTGACAGA ACTGTACCTT TGTGAACAGT p.23955/rs327-[T/G]
19,863,808 GCTTTTGGATT **G**TTCTACATG GCATATTCAC ATCCATTTTC TTCCACAGGG Exon 9
19,863,908 TGATCTTCTG TTCTAGGGAG AAAGTGTCTC ATTTGCAGAA AGGAAAGGCA
19,863,958 CCTGCGGTAT TTGTGAAATG CCATGACAAG TCTCTGAATA AGAAGT**C**AGG p.24143/rs328-[C/G]
19,864,008 AGAATGAGCA TTCTGGGCTA AAGCTGACTG GGCATCCTGA GCTTGCACCC Intron 9
19,864,058 TAAGGGAGGC AGCTTCATGC ATTCTCTTCC ACCCCATCAC CAGCAGCTTG
19,864,108 CCCTGACTCA TGTGATCAAA GCATTCAATC AGTCTTTCTT AGTCTTCTG
19,864,158 CATATGTATC AAATGGGTCT GTTGTCTTAT GCAATACTTC CTCTTTTTTT
19,864,208 CTTTCTCCTC TTGTTTCTCC CAGCCCGGAC CTCAACCCA GGCACACATT
19,864,258 TTAGGTTTAA TTTTACTCCT TGAACACCCT CTGAATCTTC ACTTCTCCTT
19,864,308 TTTTCTCTAC TGGCTCTCTG CTGACTTTTG AGATGCCATT TGCAGAGCAT
19,864,358 GTAACACA**A**G TTTAGTAGTT GCCGTCTCTG CTGTGGGTGC AGTCTTCCCC p.24505/rs329-[A/G]
19,864,408 AGGATGTATT CAGGGAAGTA AAAAGA**T**CTC ACTGCATCAC CTGCAGCCAC p.24573-[T/C]
19,864,458 ATAGTTCTTG ATTCTCCAAG TGCCAGCATA CTCCGGGACA CACAGCCAAC
19,864,508 AGGGCTGCCC CAAGCACCCA TCTCAAAACC **C**TCAAAGCTG CCAAGCAAAAC rs12544438
19,864,558 AGAATGAGCA TTATAGGAAA CTGTTCTCTC TTCTATCTCC AAACAACCTCT
19,864,608 GTGCCTCTTT CCTACCTGAC CTTTAGGGCT AATCCATGTG GCAGCTGTTA
19,864,658 GCTGCATCTT TCCAGAGG**G**T CAGTACT**G**AG AGGCACATA GCATGTGACC p.24815/rs330-[G/A;p.24824/rs331-[G/A]
19,864,708 TTPCA**T**ACTC CTGTTCTGAA TTCCAGGATT ATGCCCTTTT CAACCTCCCA p.24852/rs12679834-[T/C]
19,864,758 CA**C**ATCCCCT GCCAGACAGC AAGTGCTAAT GGGTTACAGG AACAAAGGGG p.24899-[C/T]
19,864,808 AGAAATATTA GATCATGTCA TACAAGCCAG TGACACAAGA AATGAAGGGA
19,864,858 AAGGCTAG**A**C ACAGTGTCTC CTGGAACAG GAAAAGCAAT TGCTTTTGGT p.25005-[A/G]
19,864,908 TT**G**TTCTTTT CTAGTTTGC ATTTGGGACA AAT**G**TATAGA ATAAGAATTG p.25049-[G/A];rs28681081
19,864,958 CCTTCATGCC TGCAATCCCA GCACTTTGGG AGGCTGAGGC AGGTGGATCA
19,865,008 CCTGAGGTCA GGAGTTTGGG ACCAGCCTGG CCAACCTGGC GAAACCACCT
19,865,058 CTCTACTAAA AATATAAAAA TTAGCTGGGT GTGGCGGCAC ATGCCTGTAA
19,865,108 TCCCAGTAA TCGCAGGCT GAGCGGGAG AATTGCTTGA ACCCGGGAGG
19,865,158 CAGAGGTTGC AGTGAAGTGA GAT**C**GGGCCA TTATATTC**C**A GCCTGGGCAA p.25320-[C/T];p25335-[C/T]
19,865,208 CAGAG**A**AAGA CTCATCTCA AAAAAAAAAA AAACATGCCT ATT**A**GGAAAA p.25352-[A/C];rs28578146
19,865,258 GTATATTA**A**A GACCCTA**T**GT GTAACATCTT TAATGTTTTT AAATCTACT rs28599962
19,865,308 TTATAATGA TTTTATACAT GTTTACTATA AATAGATTAG GAAAAATAAG rs13261181
19,865,358 CAAAAATAAA ATAAAATCAC TGTGACCATA TCACTCAGAG ACAACCCCAA
19,865,408 TTAACGTTTT TATTTATATT CTTTCGGACT TTATATATAC ATAATATTTA
19,865,458 TATGTTTTTT GTCCTTTACA AAAATAGAAAT TATGGTGTAT ATACTCTGAA
19,865,508 TGACT**A**GATG AGAACATCT**G** GATCAAAAGC ATTAATGTAA GAGCATTACAG rs17116619 rs28439839
19,865,558 GATAAACTCA AAATGGAGAA TAGTTAGTGG TATTGAGCCA GGCAAAATAA
19,865,608 CGCAATCTCT ATCTAACTGG AGACTTTTCT TCTAAGAGTT TATTACGTTG
19,865,658 TTTTCTCT**C**A TCACAAATCT GAGGCAATAT CATACTTTCT TCAGTT**C**T**T**A rs28424158;p25844-[T/G]
19,865,708 GAAAGAGACT TTTAGATGAA GTTTTTTTTG TTTGTTT**G**G TTTTTTTTTT rs28716400
19,865,758 CTTGAGATGG AGTTTTGCTC TTGCTGCCCA GGCTGGAGTG TAGTGGCTCG
19,865,808 ATCTCAGCTC ACTGCAACCT CCACCTCCTG GGTCAAGCA ATTCCTCTGC
19,865,858 CTCAGCCTCC CAAGTAGCTG GGATTACACG TGTCCGCCAC CACACCTGGC
19,865,908 TAATTT**C**GT**A** TTTT**T**AGTAG AGAAAGGGTT TCACCATGTT GGT**C**AGGCTG rs4416836
19,865,958 GTCTTGA**A**CT CCTAACCTCA GGTATCCAC CTGCCTCGGC CTCCAAAGT
19,866,008 GCTGGGATTA TAGGTGTGAG TCACCACACC CGGCCCTAGA TGCAGTTTTA
19,866,058 TACA**T**GCATT TGTATTACAC ATAAATAGCA TGCATAT**T**CT p.26201-[T/G];p.26234/rs10099160-[T/G]
19,866,108 CTACA**A**CTTT AAATCTACAT GTGAATGTGA AAATAAAACC TCATTA**A**ATT
19,866,158 AGTAAATAAC TCTAGCTGCT TGTAAGCAC GTCCAGTCGT ATTTTTTATA
19,866,208 TGTTACAAGA CTTTATCTGA GAAAGCCTAA TGAAGCATT CTTGTCTGAT
19,866,258 TATAGGATTA CTGACAGAAC AGTTATTTAG ACAGAGAATG TTCAGATGCG
19,866,308 TTTTATTTTT ATTTTTTACT TTTATTTATT TTTGAGACAG TCTCGTCTG
19,866,358 TTGCCAGGC TAGAATGTGG TGGCGTGATC TCGGCTCAAT GCAACTCTGC
19,866,408 CTCC**C**GGGTT CAAGTGATTC TTGTGCCTCA GCCTGACAAG TAGCTGGGAT rs58844409
19,866,458 TATAGGTGCC CGTTACCATG CCCAGCTAAT TTTTCTGTTT TTAGTAGAGA
19,866,508 CGGAGTTTCA CCATATTGGC CAGGCTGGTC ATTGA**A**CTCC TAACCTCAGG
19,866,558 TGATGTGCCT GTCTCAGCCT CCCAATGTGC TGGGATTACA GGCATGAGCC
19,866,608 ACAGCACCCA GCCAGATGCA TTTTTAAAAA CGTACCTGAA CTTTATCTAG
19,866,658 GAGGTAATTA TAAATTAGAC TAATAATCTT CTACAGTTTC TTTCTCTCTG
19,866,708 GATTAAAAATC AATCAAAATC AAGAT**T**CTCT TTCTCACACC TTCTGCTAAC
19,866,758 TCCTCAGAAA CCTCATATCA CAAGAAATGA AATGGAACAG GCCTTCTGTT
19,866,808 TGATACATTT TAGAATAAGA AATCCTCTAA ATTTAGAAGT CATTTGGCCC
19,866,858 AGT**C**CTCCAA AAATGATGCA CCTTATTGGG ACGGGGCTAA ATAGTTGCTC p.27000-[C/T]
19,866,908 CAGTGTCTTC CATTCCTACA AACCTGCCAT TCTCTGATCC ATTATACACA
19,866,958 TCTCCCTG**G** GTTTAT**T**CTC ACAACCTTTG TTCTGAAATT CCATTTGAAG rs10283151

19,867,008 GCTTTTTCCA TCC**T**AAAAACC AGTGGGGGAC AGGCGGGAAT TGTA AACACAC p.27160-[T/A]
19,867,058 TCAGAAGATA ATAAATTGCC CTTTTTCTCG TG**C**TTTTTCT CAGAAactgg Exon 10 p.27229/rs11570891-[C/T]
19,867,108 gcg**g**aactctac agaacaaaga acg**g**catgtg aattctgtga agaatgaagt p.27249/rs4922115-[G/A];rs7818177
19,867,158 ggaggaagta acttttaca aacataccca gtgtttggg tgtttcaaaa
19,867,208 gtggatttct ctgaatatta atcccagccc tacccttgtt agttatttta
19,867,258 ggagacagtc tcaagcacta aaaagtggct aattcaattt atggggtata
19,867,308 gtggccaaat agcacatcct ccaacgttaa aagacagtgg atcatgaaaa
19,867,358 gtgctgtttt gtcctttgag aaagaataaa ttgtttgagc gcagagtaaa
19,867,408 ataaggctcc ttcattgtggc gtattggggc atagcctata attggttaga
19,867,458 acctoctatt ttaa**t**tgga tctctggatct ttcggactga ggcttctca p.27611/rs3289-[T/C]
19,867,508 aactttactc taagtctcca agaatacaga aaatgctttt **c**gcggcagc p.27688-[C/T]
19,867,558 aatcacagc atctacacag cagtatgaat gatgttttag aatgattccc
19,867,608 tcttgctatt ggaatgtggt ccagacgtca accagga**a**ca tghtaactgg p.27783-[A/T]
19,867,658 agagggacga agaaagggtc tgataaacac agaggtttta aacagtcctc rs11542064
19,867,708 accattggcc tgcattcatga caaagttaca aattcaagga gatataaaat
19,867,758 ctgatcaat taattcttaa taggctttat cgtttattg ttaatccctc
19,867,808 tctccccctt cttttttgtc tcaagattat attataataa tgttctctgg
19,867,858 gtagggtgtg aaaatgagcc tgtaatcctc agctgacac**a** taatttgaat p.28036/rs11570892-[A/G]
19,867,908 ggtgcagaaa aaaaaaaga **a**accgtaatt ttattattag attctc**c**aaa rs1059497;p.28067/rs3208305-[A/T];
rs11570896;p.28093/rs1803924-[C/T]
19,867,958 tgattttcat caatttaaaa tcaattcaata tctgacagtt actcttcagt
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19,868,058 tgttctctggc tttgacatga aaagataggt ttgagttcaa attttgcatt
19,868,108 gtgtgagctt ctacagatct tagacaagga ccgtttttac taagtaaaag
19,868,158 ggtggagagg ttcctggggt ggattcctaa gcagtgtctg taaacctatg
19,868,208 cgtgcaatga gccagatgga gtaccatgag ggttg**c**tatt tgttgttttt p.28382/rs1059507-[C/T]
19,868,258 aacaactaat **c**aagagtgag tgaacaacta tttataaact agatctccta p.28407-[C/A]
19,868,308 tttttcagaa tgcctt**c**ta cgtataaata tgaatgata aag**a**tgta p.28464/rs3735964-
[C/A];p.28490/rs3200218-[A/G]
19,868,358 atatctcaga ggctatagct gggaaacc**g**ga ctgtgaaagt atgtgatatc p.28524-[C/T]
19,868,408 tgaacacata ctagaaagct ctgcatgtgt gttgtcctc agcataattc
19,868,458 ggaagggaaa acagtcgatc aagggatgta **t**tggaaacatg tcggagtaga rs1803923
19,868,508 aattgttcc**t** gatgtgccag aacttcgacc ctttctctga gagagatgat rs58998793
19,868,558 cgtgcctata aatagtagga ccaatgttgt gattaacatc atcaggcttg
19,868,608 gaatgaattc tctctaaaa taaaatgatg tatgatttgt tgttggcatc
19,868,658 ccctttatta attcattaaa tttctggatt tgggttgtga cccagggtgc
19,868,708 attaacttaa aagattcact aaagcagcac atagcactgg gaactctggc
19,868,758 tccgaaaaac tttg**t**atat atatacaagga tgttctggct ttacatttta p.28911/rs13702-[T/C]
19,868,808 tttattagct gtaaatacat gtgtggatgt gtaaa**g**ggc cttgtacata p.28982/rs1059611-[T/C]
19,868,858 ttggaaaggt cattgtggct atctgc**a**ttt ataaatgtgt ggtgctaact rs17091815;p.29046_29047-[InsTT]
19,868,908 **g**tatgtgtct ttatcagtga tggctcaca gagccaact**c** **a**ctcttatga p.29086/rs15285-
[C/T];p.29088/rs3866471-[C/A]
19,868,958 aatgggcttt aacaaaacaa gaaagaacg tacttaactg tgtgaagaaa
19,869,008 tggaaatcagc ttttaataaa attgacaaca ttttattacc aca
19,869,051 **c**taagtcatt attttgtatc atttt**ta**aag taaatattatt cttaggtoag rs71510671
19,869,101 attcactcag catattttga ctaagt**aa**cc actgtactta gtaaac**g**aa p.29287/rs3916027-[G/A]
19,869,151 gagcttctga gaattatagt gtacc**t**tata gatattttta acattttat p.29315/rs9644636-[T/G]
19,869,201 ttgtataaag ctaaagaaag ccttacaat cctttaaact gactatagaa
19,869,251 gaaaatgata cagaattttg cctgcataaa gtacacagga ctattcttgc
19,869,301 ctacaatatg ctttttcaca agcaaatgt taga**c**taata taaggca**t**ct p.29474-[C/T];p.29487/rs4921683-[T/A]
19,869,351 ttggccattt tatagtgtac atcatctcta tttctgaggc ctcatgttta
19,869,401 gctgta**g**cgc aagtagc**at**t ttgtcaataa aatgaactat ttgggatggg p.29547/rs4921684-[C/T];p.29557_29558-
[InsA]
19,869,451 agggtagact ttttagaact ttgctttggg ttgccttgat aattaatagc
19,869,501 atatagtcca tttatgcagc taagtggga ttgcttcta gtacagt**ca**g
19,869,551 gaagaattta gccagaaaa caatta**t**ttc aatggcact gacccaaact p.29716-[T/C]
19,869,601 tccaggctga agagcaatgg cgtgatcatg gctcaactga cctccactc
19,869,651 ccaggctcaa gtgattctcc tgcctcagcc tcccagtag atggtagctac
19,869,701 aagcacacgc cactgcaccc agctaatttt tgtatttttt gtagagatgg
19,869,751 gggtttcacc atggtgcccc ggctggtctt aaattcttgg cctcaagtgt
19,869,801 ctgccccctt tggcctcccc aagtgcctga attacaggca tgagccacca
19,869,851 tgtccagcct tgacccaaac ttttattgtc agtttagctt tggggcttct
19,869,901 gggattttggg tctcccctga caggaggggg ctcccagtt cacacttggc
19,869,951 cactgcccatt caattctctg tgatatgatc aacaagatag acaattgcaa
19,870,001 atgttgctga ggaatgtggag aagtgtgaac ctgtgtaagt ggctgatggg

Figure 4. LPL Annotated Sequence

3.2 DISTRIBUTION OF *LPL* VARIANTS IN HIGH AND LOW HDL-C GROUPS

Of the 178 identified variants (excluding microsatellite), 88 had $MAF \geq 0.05$ and 91 had a $MAF < 0.05$. The MAF range for the 74 variants which have not been previously identified in the dbSNP build 130 was 0.005-0.500%. Among common variants, 32 had a statistically significant difference (P -value <0.05) when comparing the allele frequencies between the high HDL-C and low HDL-C groups; the P -value range was 0.048-0.002. Figure 6 shows the LD pattern of these significant variants in [Section 3.3](#). In addition to 32 variants, 5 of the common variants have a P -value of between 0.05 and 0.10.

Of the 91 relatively uncommon or rare variants ($MAF < 0.05$), 21 were present only in the low HDL-C group and 25 were present only in the high HDL-C group and the remaining were present in both groups. Forty of 47 (85.1%) individuals with high HDL-C had minimum one rare variant versus 35 out of 48 (72.9%) individuals with low HDL-C; 23 of 47 (72.9%) individuals high HDL-C had minimum two rare variants versus 16 out of 48 (33.3%) individuals with low HDL-C; 17 of 47 (36.1%) individuals with high HDL-C had minimum three rare variants versus 8 out of 48 (16.6%) individuals with low HDL-C. There were some individuals that had low genotyping call rates so it is worth mentioning that they may be underestimated in the analysis of rare variants.

Among 23 identified exonic variants, 10 were relatively uncommon or rare variants ($MAF < 0.05$). Of these 10 relatively uncommon or rare exonic variants, two were found only in the high HDL-C group (24143C>G, Ser447X) and (27783A>T, $P=0.142$) which are located in exon 9 and untranslated region of the exon 10, respectively. On the other hand, two were found only in low HDL-C group (27688C>T, $P=0.326$ and 28524C>T, $P=0.321$), however, they are located in the

untranslated region of exon 10. There was a one base insertion in the 3' flanking region which was found only in the low HDL-C group (29557_29558InsA, P=0.321) but the remaining 19 exonic variants were found in both the high and low HDL-groups. Table 9 summarizes the distribution of common *LPL* variants in high and low HDL-C groups. The relatively uncommon or rare *LPL* variants in high and low HDL-C groups are listed in Table 10 and the variants which were found only in high or low HDL-C groups are specifically highlighted; the 25 variants that were found in only high HDL-C group are highlighted in yellow and the 21 variants that were identified in only low HDL-C group are highlighted in blue.

Table 10. Distribution of common *LPL* variants in high and low HDL-C groups in NHWs

<i>LPL</i> variant (Nucleotide #)	Alleles	Location	refSNP ID	All MAF (n=95)	Assoc. Allele	%MAF of High HDL-C	%MAF of Low HDL-C	P value
2335	G>T	Intron 1	rs3779787	0.122	T	0.163	0.083	0.096
3558	G>A	Intron 1	rs34309063	0.247	A	0.160	0.333	0.006
3964	G>C	Intron 1	rs17410577	0.239	C	0.160	0.320	0.010
4060	G>T	Intron 1	rs1534649	0.430	T	0.383	0.478	0.189
4424	A>G	Intron 1	rs13266204	0.263	G	0.200	0.330	0.038
5949	T>G	Intron 1		0.137	G	0.160	0.115	0.367
6821	C>T	Intron 1	rs10104051	0.426	T	0.404	0.447	0.555
7512	C>T	Intron 1	rs3779788	0.121	T	0.160	0.083	0.107
8415	T>A	Intron 1	rs56321069	0.183	A	0.228	0.138	0.113
10987	C>A	Intron 2		0.122	A	0.140	0.106	0.498
11090	C>G	Intron 2	rs8176337	0.190	G	0.239	0.146	0.109
12449	G>A	Intron 2		0.117	A	0.152	0.083	0.142
12550	G>A	Intron 2		0.118	A	0.156	0.083	0.128
12810_12829	dup20	Intron 2		0.126	G	0.163	0.089	0.132
12853_12854	Ins16	Intron 2		0.055	C	0.054	0.056	0.972
12861_12864	del4	Intron 2		0.060	C	0.054	0.933	0.727
13003	G>T	Intron 2		0.053	T	0.053	0.052	0.973
14114	T>C	Intron 3	rs73667472	0.080	C	0.065	0.094	0.471
15425	T>C	Intron 4	rs249	0.095	C	0.085	0.104	0.654
15449_15450	Ins2	Intron 4		0.063	C	0.096	0.031	0.068
15653	delA	Intron 4		0.410	C	0.394	0.426	0.656
15836	C>T	Intron 4	rs253	0.410	C	0.394	0.426	0.656
16316	C>G	Intron 5	rs254	0.074	G	0.060	0.913	0.487
16320	T>C	Intron 5	rs255	0.105	C	0.106	0.104	0.960
16386	C>T	Intron 5	rs256	0.118	T	0.133	0.104	0.538
16671	G>C	Intron 5	rs258	0.409	C	0.380	0.436	0.440
17231	C>T	Intron 5	rs263	0.135	T	0.148	0.122	0.618
17599	G>A	Intron 5	rs264	0.116	A	0.128	0.104	0.613
18086	T>G	Intron 6	rs269	0.119	G	0.144	0.093	0.293
18095	C>A	Intron 6	rs270	0.201	A	0.140	0.261	0.045
18121	G>A	Intron 6	rs271	0.109	A	0.130	0.087	0.343
18395_18396	insT	Intron 6		0.121	G	0.138	0.104	0.471
18822	T>C	Intron 6	rs277	0.232	C	0.181	0.281	0.101
18942	G>A	Intron 6	rs278	0.211	A	0.170	0.250	0.177
19442	A>T	Intron 6	rs281	0.242	T	0.207	0.278	0.262
19445	C>G	Intron 6	rs282	0.129	G	0.133	0.125	0.866
19517	C>T	Intron 6	rs283	0.161	T	0.167	0.156	0.847
19608	C>T	Intron 6	rs285 (PvuII)	0.407	T	0.444	0.370	0.304
19675	A>T	Intron 6	rs286	0.060	T	0.111	0.011	0.004
19975	A>G	Intron 6	rs287	0.172	G	0.244	0.104	0.011
20038	T>C	Intron 6	rs289	0.159	C	0.221	0.104	0.032
20271	T>C	Intron 6	rs291	0.163	C	0.234	0.094	0.009
20363	A>T	Intron 6		0.053	T	0.043	0.062	0.538

Table 10 (Continued)

20505_20506	insA	Intron 6		0.163	G	0.234	0.094	0.009
20544	T>C	Intron 6	rs294	0.080	C	0.087	0.073	0.723
20657	A>C	Intron 6	rs295	0.163	C	0.234	0.094	0.009
20790	T>C	Intron 6	rs297	0.163	C	0.234	0.094	0.009
21353	T>C	Intron 7	rs301	0.172	C	0.223	0.120	0.061
21780	T>G	Intron 7	rs304	0.103	G	0.174	0.034	0.002
21820	A>G	Intron 7	rs305	0.108	G	0.179	0.037	0.003
22044_22047	del4	Intron 7		0.075	A	0.087	0.064	0.550
22416	G>C	Intron 7	rs312	0.072	C	0.080	0.065	0.711
22461	G>A	Intron 7	rs314	0.180	A	0.244	0.120	0.031
22855	C>A	Exon 8	rs316(T361T)	0.079	A	0.074	0.083	0.821
23190_23191	del2	Intron 8		0.500	A	0.456	0.453	0.228
23192	G>T	Intron 8		0.068	T	0.122	0.012	0.004
23395	A>C	Intron 8	rs319	0.293	C	0.282	0.302	0.776
23636	A>C	Intron 8	rs322	0.170	C	0.227	0.117	0.048
23747	T>C	Intron 8	rs325	0.067	C	0.102	0.033	0.067
23858	A>G	Intron 8	rs326	0.206	G	0.278	0.133	0.017
23955	T>G	Intron 8	rs327	0.192	G	0.261	0.122	0.018
23496	T>G	Intron 8	rs320 (HindIII)	0.185	G	0.244	0.128	0.053
24815	G>A	Intron 9	rs330	0.111	A	0.128	0.094	0.456
24824	G>A	Intron 9	rs331	0.122	A	0.154	0.096	0.247
24852	T>C	Intron 9	rs12679834	0.080	C	0.138	0.021	0.003
25049	G>A	Intron 9		0.063	A	0.043	0.083	0.248
25335	C>T	Intron 9		0.079	T	0.138	0.021	0.003
25352	A>C	Intron 9		0.079	C	0.138	0.021	0.003
25844	T>G	Intron 9		0.074	G	0.130	0.021	0.004
26201	T>G	Intron 9		0.077	G	0.136	0.021	0.004
26234	T>G	Intron 9	rs10099160	0.282	G	0.250	0.312	0.341
27229	C>T	Intron 9	rs11570891	0.082	T	0.144	0.021	0.002
27249	G>A	Exon 10-3' UTR	rs4922115	0.085	A	0.087	0.083	0.929
28036	A>G	Exon 10-3' UTR	rs11570892	0.101	G	0.117	0.085	0.468
28067	A>T	Exon 10-3' UTR	rs3208305	0.203	T	0.278	0.130	0.014
28093	C>T	Exon 10-3' UTR	rs1803924	0.082	T	0.141	0.022	0.004
28382	C>T	Exon 10-3' UTR	rs1059507	0.110	T	0.120	0.100	0.673
28464	C>A	Exon 10-3' UTR	rs3735964	0.079	A	0.138	0.021	0.003
28490	A>G	Exon 10-3' UTR	rs3200218	0.279	G	0.255	0.302	0.472
28911	T>C	Exon 10-3' UTR	rs13702	0.200	C	0.277	0.125	0.009
28982	T>C	Exon 10-3' UTR	rs1059611	0.079	C	0.138	0.021	0.003
29046_29047	Ins2	Exon 10-3' UTR		0.079	G	0.138	0.021	0.003
29086	C>T	Exon 10-3' UTR	rs15285	0.200	T	0.277	0.125	0.009

Table 10 (Continued)

29088	C>A	Exon 10- 3' UTR	rs3866471	0.105	A	0.117	0.094	0.601
29287	G>A	3' flanking	rs3916027	0.184	A	0.255	0.115	0.012
29315	T>G	3' flanking	rs9644636	0.339	G	0.348	0.330	0.795
29487	T>A	3' flanking	rs4921683	0.105	A	0.117	0.094	0.601
29547	C>T	3' flanking	rs4921684	0.105	T	0.117	0.094	0.601

Table 11. Distribution of relatively uncommon or rare *LPL* variants in high and low HDL-C groups in NHWs

<i>LPL</i> variant (Nucleotide #)	Alleles	Location	refSNP ID	All MAF (n=95)	MAF(%) assoc. allele High HDL-C	MAF(%) assoc. allele Low HDL-C
208	T>C	5' flanking	rs1470186	0.016	0.021	0.011
351	C>A	5' flanking		0.005	0.011	0.000
428	G>A	5' flanking	rs73667465	0.016	0.021	0.011
549	C>T	5' flanking	rs17091742	0.016	0.021	0.011
958	G>A	5' flanking		0.005	0.000	0.011
1088	G>T	5' flanking		0.005	0.011	0.000
1090	T>G	5' flanking	rs1800590 (-T93G)	0.011	0.011	0.010
1130	G>C	5' flanking		0.005	0.000	0.010
2913	T>C	Intron 1		0.005	0.000	0.011
4621	C>G	Intron 1		0.021	0.021	0.021
4948	C>G	Intron 1	rs6997330	0.022	0.033	0.010
5094	C>G	Intron 1		0.005	0.000	0.011
5107	C>T	Intron 1		0.005	0.011	0.000
5118	A>T	Intron 1		0.022	0.043	0.000
5200	C>T	Intron 1		0.006	0.000	0.011
5531	G>A	Intron 1	rs1031045	0.016	0.022	0.011
5772	A>G	Intron 1	rs60633545	0.016	0.022	0.010
6383	G>T	Intron 1		0.005	0.011	0.000
6435	G>C	Intron 1		0.005	0.011	0.000
6477	T>C	Intron 1		0.005	0.011	0.000
6553	C>T	Intron 1	rs59254395	0.016	0.021	0.010
6554	A>G	Intron 1	rs56043715	0.016	0.021	0.010
7130	T>C	Intron 1	rs28615996	0.021	0.029	0.014
7131	T>G	Intron 1		0.007	0.014	0.000
7313	G>A	Intron 1	rs28645722	0.016	0.022	0.010
7388	C>G	Intron 1	rs28575919	0.016	0.021	0.010
7503	T>C	Intron 1	rs6999612	0.016	0.021	0.010
7556	T>C	Intron 1	rs59811201	0.016	0.021	0.010
8221	A>C	Intron 1	rs7000460	0.018	0.026	0.011
8250	G>A	Intron 1	rs59630933	0.019	0.026	0.012
8467	C>T	Intron 1		0.005	0.000	0.010
8516	delG	Intron 1		0.011	0.011	0.010
9015	A>G	Intron 1	rs28445964	0.021	0.022	0.020
9024	T>C	Intron 1		0.008	0.000	0.014
9130	T>A	Intron 1	rs13252357	0.005	0.011	0.000
9411	A>C	Intron 1	rs28689946	0.016	0.021	0.011

Table 11 (Continued)

9418	G>A	Intron 1	rs28582042	0.016	0.021	0.011
9589	C>T	Intron 1		0.016	0.021	0.011
9696	G>T	Intron 1	rs73667468	0.016	0.022	0.010
9914	T>G	Intron 1	rs73667469	0.016	0.022	0.011
10127	G>A	Exon 2	rs1801177 (D9N)	0.016	0.021	0.010
10632	C>T	Intron 2		0.005	0.000	0.001
10912	A>G	Intron 2		0.022	0.000	0.043
11050	T>C	Intron 2	rs7016529	0.016	0.023	0.010
11228	T>C	Intron 2		0.005	0.011	0.000
11574	G>A	Intron 2	rs34123038	0.049	0.056	0.043
11600	G>C	Intron 2		0.011	0.000	0.021
11760	A>C	Intron 2	rs73667470	0.016	0.021	0.010
11888_11889	insA	Intron 2		0.011	0.021	0.000
12224_12920	del697	Intron 2		0.005	0.011	0.000
12484	C>A	Intron 2		0.048	0.044	0.052
12878_12889	del12	Intron 2		0.005	0.011	0.000
12884_12887	del4	Intron 2		0.016	0.033	0.000
13639	G>A	Intron 2		0.011	0.021	0.000
13854	G>A	Exon 3	rs1121923 (V108V)	0.016	0.021	0.010
15206	C>A	Intron 3	rs343	0.042	0.064	0.021
15245	G>A	Exon 4	rs248 (E118E)	0.047	0.053	0.042
16442	G>C	Intron 5		0.005	0.000	0.010
16563	T>A	Intron 5		0.016	0.011	0.021
17476	A>C	Intron 5		0.005	0.000	0.010
17948	A>G	Exon 6	rs268 (N291S)	0.033	0.022	0.043
18065	T>G	Intron 6		0.009	0.000	0.017
18297	A>C	Intron 6		0.005	0.011	0.000
18462	T>G	Intron 6		0.005	0.000	0.010
18621	C>T	Intron 6		0.005	0.011	0.000
18708	T>C	Intron 6	rs276	0.021	0.021	0.021
19815	G>A	Intron 6		0.005	0.011	0.000
20080	C>T	Intron 6		0.005	0.011	0.000
20663	G>A	Intron 6	rs296	0.005	0.000	0.010
21125_21128	del4	Intron 6		0.005	0.000	0.011
21895	T>G	Intron 7	rs308	0.012	0.013	0.011
21965	C>T	Intron 7	rs310	0.006	0.013	0.000
22514	T>C	Intron 7	rs315	0.011	0.000	0.022

Table 11 (Continued)

23388	C>G	Intron 8	rs318	0.018	0.024	0.012
23573	T>C	Intron 8		0.017	0.024	0.011
24143	C>G	Exon 9	rs328 (Ser447X)	0.005	0.011	0.000
24505	A>G	Intron 9	rs329	0.016	0.021	0.011
24573	T>C	Intron 9		0.005	0.000	0.011
24899	C>T	Intron 9		0.016	0.021	0.010
25005	A>G	Intron 9		0.005	0.011	0.000
25320	C>T	Intron 9		0.005	0.011	0.000
27000	C>T	Intron 9		0.029	0.044	0.012
27160	T>A	Intron 9		0.027	0.058	0.000
27611	T>C	Exon 10-3' UTR	rs3289	0.016	0.022	0.010
27688	C>T	Exon 10-3' UTR		0.005	0.000	0.010
27783	A>T	Exon 10-3' UTR		0.011	0.022	0.000
28407	C>A	Exon 10-3' UTR		0.026	0.021	0.031
28524	C>T	Exon 10-3' UTR		0.005	0.000	0.010
29474	C>T	3' flanking		0.026	0.043	0.010
29557_29558	InsA	3' flanking		0.005	0.000	0.010
29716	T>C	3' flanking		0.026	0.043	0.010

3.3 LINKAGE DISEQUILIBRIUM (LD) AND TAGGER ANALYSES OF *LPL* VARIANTS

We used LD and Tagger analysis to identify tagSNPs for the common variants ($MAF \geq 0.05$) that we identified by resequencing. By using tagger analysis with an r^2 cutoff of 0.9, 43 tagSNP bins were identified. Table 12 shows the bins identified by Tagger analysis and Figure 5 demonstrates the LD plot. Twelve variants that were genotyped in our entire NHW sample are underlined in Table 12. We also used LD analysis in Haploview to see the LD pattern of the 32 common variants that had $p\text{-value} \leq 0.05$ when comparing the allele frequencies between the high HDL-C and low HDL-C groups (Figure 6).

Table 12. Tagger results using Haploview of *LPL* common variants in NHWs

Bin	Variants captured
1	28093, 23747, 26201, 25352, 25844, 25335, 24852, 29046_29047ins2, 28464, <u>28982</u>
2	29547, 29487, 24824, 24815, 28036, 28382, 29088
3	28911, 29086, 23858, 29287, 28067
4	7512, 12810_12829ins20, 12550, 12449, <u>2335</u>
5	16386, 18121, <u>17599</u> , 18395_18396ins1
6	20790, 20505_20506ins1, 20657, 20271
7	16671, 15653del1, <u>15836</u>
8	<u>23955</u> , 23636, 23496(<i>HindIII</i>)
9	26234, 23395, 28490
10	22855, <u>22416</u> , 27249
11	20038, 19975, 21353
12	3558, 3964
13	16320, 16316
14	21780, 21820
15	8415, 11090
16	13003, 12853_12854ins16
17	18086, 1723
18	6821

Table 12 (Continued)	
19	19608
20	<u>15425</u>
21	15449_15450ins2
22	25049
23	12861_12864del4
24	<u>18095</u>
25	14114
26	19442
27	19445
28	<u>4424</u>
29	23192
30	20544
31	19675
32	27229
33	20363
34	<u>4060</u>
35	18822
36	<u>19517</u>
37	10987
38	29315
39	22461
40	18942
41	22044_22047del2
42	23190_23191del2
43	5949

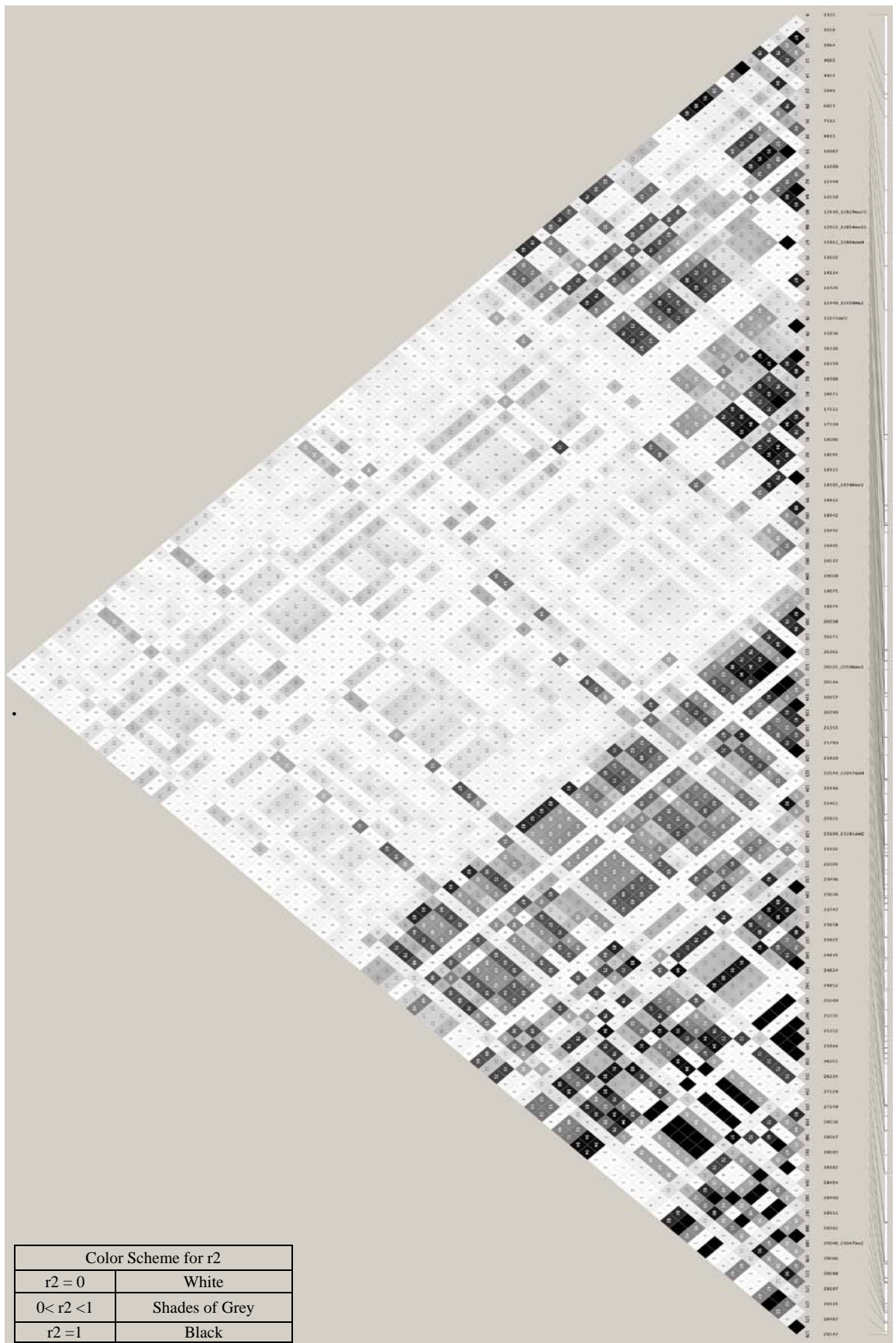


Figure 5. LD analysis of identified common variants in NHWs

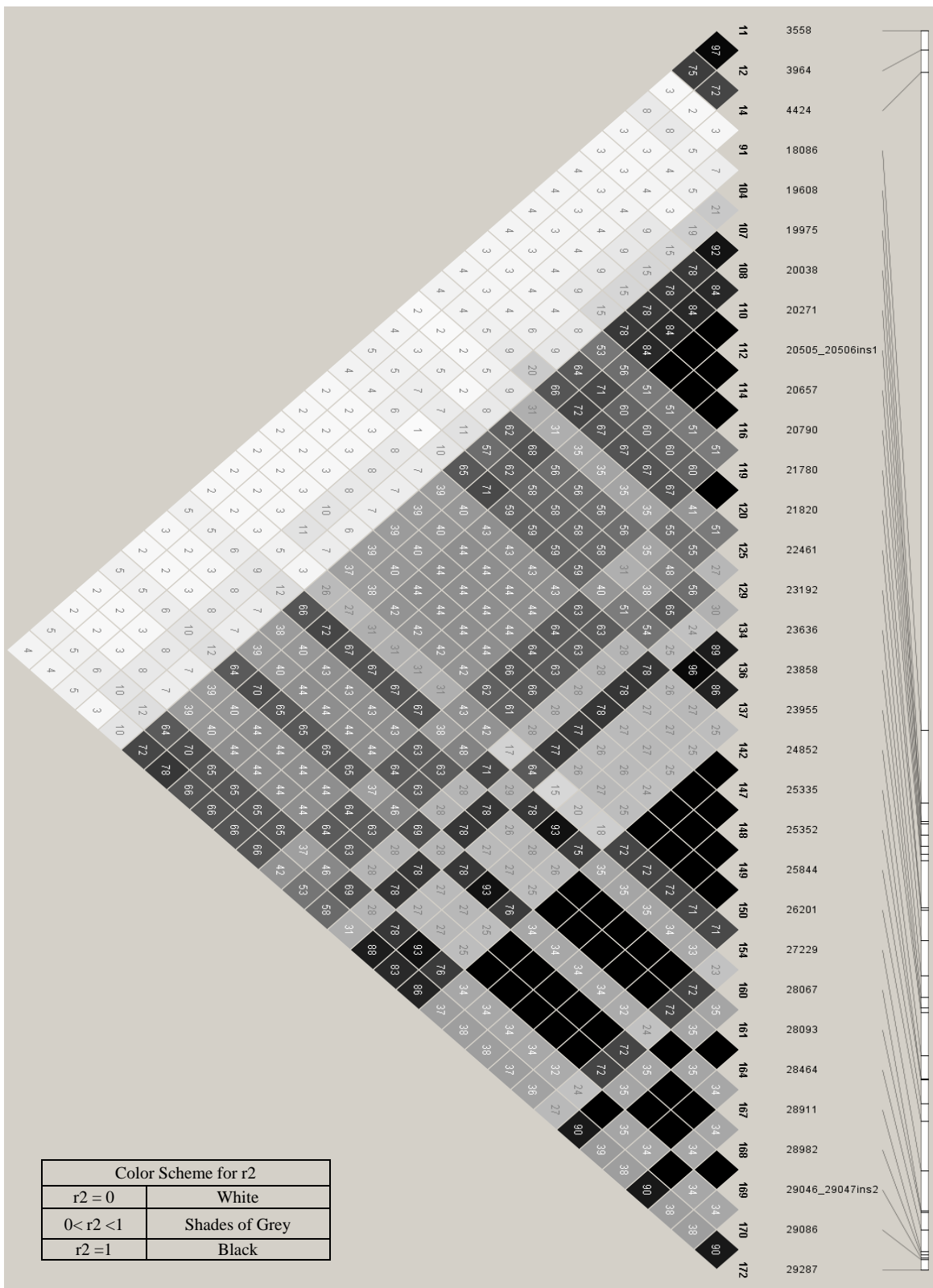


Figure 6. LD analysis of the 32 identified variants which had a statistically significant p-value <0.05 when comparing the allele frequencies between the high HDL-C and low HDL-C groups

3.4 GENOTYPING OF IDENTIFIED VARIANTS IN THE ENTIRE NHW SAMPLE AND THEIR ASSOCIATION ANALYSIS WITH PLASMA LIPOPROTEIN LEVELS

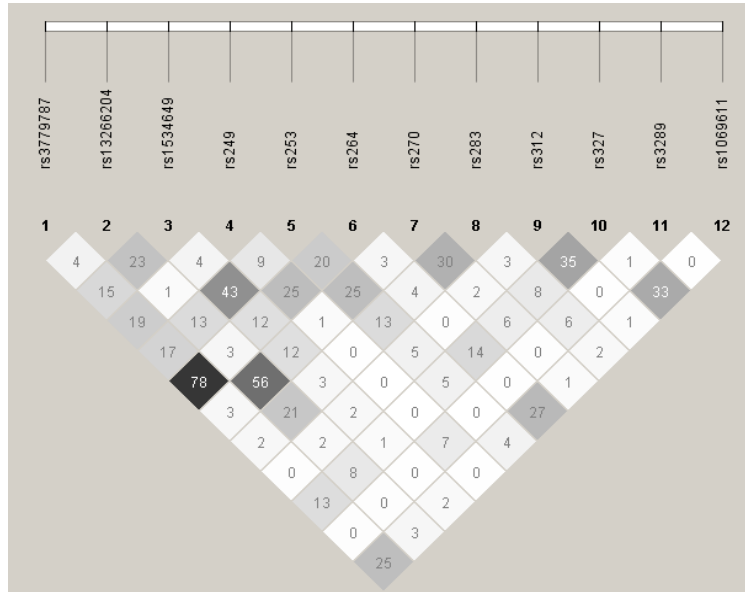
To date, we have genotyped 12 variants in our NHW sample (n=623) using pre-made TaqMan SNP Genotyping Assays. Genotyping results and features of these tagSNPs are shown in the Table 13.

Table 13. Genotyping results of 12 tagSNPs screened in the entire NHW sample

<i>LPL</i> refSNP ID	Location	Position (Nucleotide #)	Number of individuals genotyped in NHWs	MAF % in *CEU (HAPMAP)	MAF % in NHWs	Call rates (%)	P-value
rs3779787	Intron 1	2335	619	0.175	0.153	99.40	0.376
rs13266204	Intron 1	4424	620	0.259	0.215	99.50	0.616
rs1534649	Intron 1	4060	618	0.472	0.453	99.20	0.599
rs249	Intron 4	15425	614	0.075	0.074	98.60	0.211
rs253	Intron 4	15836	623	0.450	0.451	100.00	0.505
rs264	Intron 5	17599	605	0.142	0.140	97.10	0.428
rs270	Intron 6	18095	618	0.208	0.172	99.20	0.896
rs283	Intron 6	19517	618	0.188	0.201	99.20	0.352
rs312	Intron 7	22416	617	0.092	0.112	99.00	0.976
rs327	Intron 8	23955	619	0.250	0.264	99.40	0.478
rs3289	Exon 10 3'UTR	27611	622	0.042	0.026	99.80	1.000
rs1059611	Exon 10 3'UTR	28982	618	0.133	0.106	99.20	1.000

* CEU: U.S. residents with northern and western European

None of the variants showed deviation from HWE ($P > 0.05$) in the total sample. Figure 7 shows the LD analysis that was repeated for the variants screened using TaqMan assays. The LD patterns between the 12 variants were similar to those observed in the subset (high and low HDL groups) of our sample that we used for sequencing.



Color Scheme for r2	
r2 = 0	White
0 < r2 < 1	Shades of Grey
r2 = 1	Black

Figure 7. LD analysis of the variants screened in the entire NWH sample

These 12 variants screened in total NWH sample were analyzed by using both additive and dominant models for their relation to plasma total cholesterol, HDL-C, LDL-C and TG levels. Table 14 shows the genotype counts, covariate adjusted mean for each lipid level and adjusted p-values for additive and dominant models, respectively. The remaining common tagSNPs that are not included in these analyzes are currently being genotyped by Sequenome IPLEX genotyping platform so they are not included in this study.

Of the 12 variants, 4 (rs1059611, rs270, rs3779787, rs327) had statistically significant p-values associated with HDL-C level in both additive and dominant models, but rs13266204 had a significant P-value (0.013) only in additive model and had a marginally significant p-value (0.080) in the dominant model.

Two of the 12 variants (rs327, rs270) showed statistically significant association with triglycerides levels in both additive and dominant models. rs312 showed statistically significant

association with triglyceride levels in additive model ($P=0.0091$) and it showed marginally significant association in the dominant ($P=0.050$) model. On the other hand, rs283 had a statistically significant P-value (0.024) associated with TG levels only in dominant model but it had a marginally significant P-value (0.068) in additive model.

There were two variants (rs270 and rs312) that had marginally statistically significant P-values associated with LDL-C levels; rs270 showed marginally significant association in only additive model ($P=0.056$), however, rs312 showed marginally significant association in only dominant model ($P=0.087$).

Table 14. Genotype distributions and adjusted p-values for 12 *LPL* variants in NHWs

	rs13266204_Add			rs13266204_Dom	
Total Cholesterol Adjusted mean± SD	AA[380] 216.03±2.13 P= 0.400	GA[203] 219.34±2.90	GG[31] 218.32±7.21	AA[380] 216.02±2.13 P=0.342	GA/GG[234] 219.2±2.69
HDL-C Adjusted mean± SD	AA[383] 50.95±0.64 P=0.013	GA[203] 49.95±0.88	GG[31] 45.26±2.19	AA[383] 50.93±0.65 P=0.080	GA/GG[234] 49.3±0.82
LDL-C Adjusted mean± SD	AA[378] 137.40±1.97 P=0.164	GA[203] 141.56±2.68	GG[29] 143.13±6.89	AA[378] 137.41±1.97 P=0.160	GA/GG[232] 141.76±2.50
Triglycerides Adjusted mean± SD	AA[380] 140.70±3.52 P=0.082	GA[203] 139.13±4.79	GG[31] 180.90±11.92	AA[380] 140.9±3.54 P=0.329	GA/GG[234] 144.89±4.49
	rs312_Add			rs312_Dom	
Total Cholesterol Adjusted mean± SD	GG[482] 215.70±1.8 P=0.174	GC[122] 222.44±3.67	CC[7] 211.87±1.15	GG[482] 215.71±1.89 P=0.118	GC/CC[129] 221.89±3.57
HDL-C Adjusted mean± SD	GG[484] 50.21±0.58 P=0.118	GC[123] 51.06±1.12	CC[7] 57.26±4.66	GG[482] 50.21±0.58 P=0.201	GC/CC[129] 51.39±1.1
LDL-C Adjusted mean± SD	GG[480] 137.47±1.75 P=0.114	GC[120] 144.04±3.42	CC[7] 138.97±13.99	GG[480] 137.47±1.75 P=0.087	GC/CC[127] 143.77±3.33
Triglycerides Adjusted mean± SD	GG[482] 144.13±3.1 P=0.009	GC[122] 136.49±6.14	CC[7] 85.52±25.36	GG[482] 144.19±3.16 P=0.050	GC/CC[129] 133.65±6.00
	rs1059611_Add			rs1059611_Dom	
Total Cholesterol Adjusted mean± SD	TT[490] 216.74±1.8 P=0.713	TC[116] 219.38±3.73	CC[7] 210.19±15.10	TT[490] 216.76±1.89 P=0.599	TC/CC[123] 218.88±3.63
HDL-C Adjusted mean± SD	TT[491] 49.71±0.58 P=0.036	TC[117] 52.78±1.13	CC[7] 49.36±4.61	TT[491] 49.72±0.58 P=0.018	TC/CC[124] 52.6±1.1
LDL-C Adjusted mean± SD	TT[486] 139.10±1.75 P=0.925	TC[117] 138.78±3.45	CC[7] 138.41±13.96	TT[486] 139.10±1.75 P=0.928	TC/CC[123] 138.76±3.36
Triglycerides Adjusted mean± SD	TT[490] 143.66±3.117 P=0.346	TC[115] 139.91±6.29	CC[7] 113.17±25.35	TT[490] 143.72±3.17 P=0.481	TC/CC[122] 138.44±6.13

Mean and *p*-values adjusted for BMI, age, smoking, and sex
 Natural log transformed data were used for association analysis of triglycerides and HDL-C levels

Table 14 (Continued)

	rs253_Add			rs253_Dom	
	CC[181]	TC[315]	TT[121]	CC[181]	TC/TT[436]
Total Cholesterol Adjusted mean± SD	213.91±3.01	218.61±2.32	218.93±3.71	213.82±3	218.69±2
P-value	P=0.231			P=0.168	
	CC[182]	TC[316]	TT[122]	CC[182]	TC/TT[438]
HDL-C Adjusted mean± SD	51.19±0.92	49.94±0.71	50.38±1.13	51.19±0.92	50.06±0.61
P-value	P=0.595			P=-0.290	
	CC[181]	TC[312]	TT[120]	CC[181]	TC/TT[432]
LDL-C Adjusted mean± SD	136.34±2.78	140.15±2.16	140.34±3.45	136.34±2.78	140.20±1.86
P-value	P=0.309			P=0.239	
	CC[182]	TC[314]	TT[121]	CC[182]	TC/TT[435]
Triglycerides Adjusted mean± SD	139.61±5.01	143.13±3.87	143.24±6.20	139.61±5.00	143.16±3.35
P-value	P=0.686			P=0.683	
	rs1534649_Add			rs1534649_Dom	
	GG[177]	GT[314]	TT [122]	GG[177]	GT/TT [436]
Total Cholesterol Adjusted mean± SD	216.50±3.022	217.63±2.32	215.99±3.65	216.51±3.02	217.17±2
P-value	P=0.956			P=0.850	
	GG[179]	GT[314]	TT [123]	GG[179]	GT/TT [437]
HDL-C Adjusted mean± SD	50.47±0/92	50.52±0.71	49.82±1.12	50.48±0.92	50.32±0.61
P-value	P=0.730			P=0.815	
	GG[177]	GT[311]	TT [121]	GG[177]	GT/TT [432]
LDL-C Adjusted mean± SD	138.43	138.81±2.16	139.61±3.39	138.43±2.78	140.20±1.86
P-value	P=0.792			P=0.855	
	GG[179]	GT[312]	TT [122]	GG[179]	GT/TT [434]
Triglycerides Adjusted mean± SD	145.73±5.04	140.98±3.91	138.33±6.13	145.74±5.04	140.24±3.36
P-value	P=0.175			P=0.219	
	rs327_Add			rs327_Dom	
	TT[337]	TG[229]	GG[47]	TT[337]	TG/GG[276]
Total Cholesterol Adjusted mean± SD	217.07±2.23	216.02±2.68	222.89±5.84	217.07±2.23	217.18±2.47
P-value	P=0.645			P=0.971	
	TT[338]	TG[231]	GG[47]	TT[338]	TG/GG[278]
HDL-C Adjusted mean± SD	49.64±0.68	50.97±0.82	52.52±1.80	49.64±0.68	51.23±0.76
P-value	P=0.030			P=0.038	
	TT[335]	TG[227]	GG[47]	TT[335]	TG/GG[274]
LDL-C Adjusted mean± SD	139.14±2.07	137.65±2.50	144.17±5.40	139.14±2.07	138.76±2.29
P-value	P=0.746			P=0.900	
	TT[337]	TG[229]	GG[47]	TT[337]	TG/GG[276]
Triglycerides Adjusted mean± SD	146.94±3.71	136.95±4.48	132.08±9.72	146.94±3.70	136.12±4.10
P-value	P=0.014			P=0.015	

Mean and *p*-values adjusted for BMI, age, smoking, and sex
 Natural log transformed data were used for association analysis of triglycerides and HDL-C levels

Table 14 (Continued)

	rs264_Add			rs264_Dom	
	GG[440]	GA[150]	AA[9]	GG[440]	GA/AA[159]
Total Cholesterol Adjusted mean± SD	216.77±1.96	217.53±3.31	222.74±13.31	216.76±1.96	217.81±3.22
P-value	P=0.709			P=0.774	
	GG[442]	GA[151]	AA[9]	GA[442]	GA/AA[160]
HDL-C Adjusted mean± SD	50.20±0.60	51.03±1.02	50.24±4.12	50.2±0.6	50.99±0.99
P-value	P=0.380			P=0.370	
	GG[438]	GA[148]	AA[9]	G[438]	GA/AA[157]
LDL-C Adjusted mean± SD	138.77±1.82	138.18±3.08	147.98±12.31	138.75±1.82	138.72±3.00
P-value	P=0.850			P=0.993	
	GG[442]	GA[148]	AA[9]	G[442]	GA/AA[157]
Triglycerides Adjusted mean± SD	143.18±3.30	141.75±5.62	124.00±22.50	143.21±3.30	140.77±5.47
P-value	P=0.660			P=0.783	
	rs270_Add			rs270_Dom	
	CC[419]	CA[174]	AA[19]	CC[419]	CA/AA[193]
Total Cholesterol Adjusted mean± SD	215.90±2.02	219.26±3.04	231.19±9.08	215.91±2.02	220.45±2.89
P-value	P=0.100			P=0.187	
	CC[422]	CA[174]	AA[19]	CC[422]	CA/AA[193]
HDL-C Adjusted mean± SD	51.22±0.62	48.58±0.94	49.91±2.80	51.23±0.62	48.71±0.89
P-value	P=0.020			P=0.008	
	CC[417]	CA[172]	AA[19]	CC[417]	CA/AA[191]
LDL-C Adjusted mean± SD	137.53±1.87	141.17±2.83	153.95±8.49	137.54±1.87	142.26±2.69
P-value	P=0.056			P=0.123	
	CC[419]	CA[174]	AA[19]	CC[419]	CA/AA[193]
Triglycerides Adjusted mean± SD	137.93±3.39	153.27±5.13	136.79±15.31	137.93±3.39	151.62±4.87
P-value	P=0.031			P=0.012	
	rs249_Add			rs249_Dom	
	TT[523]	TC[79]	CC[6]	TT[523]	TC/CC[85]
Total Cholesterol Adjusted mean± SD	217.39±1.83	214.25±4.55	247.51±16.36	217.34±1.83	216.54±4.41
P-value	P=0.745			P=0.864	
	TT[526]	TC[79]	CC[6]	TT[526]	TC/CC[85]
HDL-C Adjusted mean± SD	50.62±0.56	49.41±1.39	46.93±5.00	50.63±0.45	49.24±1.34
P-value	P=0.343			P=0.355	
	TT[521]	TC[77]	CC[6]	TT[521]	TC/CC[83]
LDL-C Adjusted mean± SD	139.39±1.70	134.85±4.26	170.56±15.15	139.34±1.70	137.36±4.13
P-value	P=0.890			P=0.653	
	TT[524]	TC[78]	CC[6]	TT[524]	TC/CC[84]
Triglycerides Adjusted mean± SD	140.82±3.03	147.94±7.61	149.88±27.24	140.82±3.03	148.08±7.35
P-value	P=0.380			P=0.4213	

Mean and *p*-values adjusted for BMI, age, smoking, and sex

Natural log transformed data were used for association analysis of triglycerides and HDL-C levels

Table 14 (Continued)

	rs283_Add			rs283_Dom	
Total Cholesterol Adjusted mean± SD	AA[394] 217.60±2.10	AT[189] 216.97±2.99	TT[29] 214.60±7.52	AA[394] 217.59±2.1	AT/TT[218] 216.66±2.79
P-value	P=0.717			P=0.782	
HDL-C Adjusted mean± SD	AA[397] 50.68±0.64	AT[189] 50.09±0.91	TT[29] 48.17±2.29	AA[397] 50.68±0.64	AT/TT[218] 49.83±0.85
P-value	P=0.350			P=0.402	
LDL-C Adjusted mean± SD	AA[392] 139.80±1.95	AT[187] 137.57±2.78	TT[29] 138.53±6.96	AA[392] 139.80±1.95	AT/TT[216] 137.70±2.59
P-value	P=0.560			P=0.504	
Triglycerides Adjusted mean± SD	AA[394] 138.20±3.48	AT[189] 151.36±4.96	TT[29] 138.14±12.49	AA[394] 138.17±3.48	AT/TT[218] 149.59±4.63
P-value	P=0.068			P=0.024	
	rs3289_Add			rs3289_Dom	
Total Cholesterol Adjusted mean± SD	TT[584] 217.66±1.75	TC[32] 209.97±7.09	CC[0]	TT[584] 217.66±1.75	TC/CC[32] 209.97±7.09
P-value	P=0.289			P=0.289	
HDL-C Adjusted mean± SD	TT[587] 50.44±0.53	TC[32] 50.42±2.17	CC[0]	TT[587] 50.44±0.53	TC/CC[32] 50.42±2.17
P-value	P=0.876			P=0.876	
LDL-C Adjusted mean± SD	TT[580] 139.62±1.62	TC[32] 129.13±6.55	CC[0]	TT[580] 139.62±1.62	TC/CC[32] 129.13±6.55
P-value	P=0.118			P=0.118	
Triglycerides Adjusted mean± SD	TT[584] 141.28±2.91	TC[32] 152.51±11.81	CC[0]	TT[584] 141.28±2.91	TC/CC[32] 152.51±11.81
P-value	P=0.378			P=0.378	
	rs3779787_Add			rs3779787_Dom	
Total Cholesterol Adjusted mean± SD	GG[436] 217.06±1.98	GT[166] 216.70±3.19	TT[11] 234±12.15	GG[436] 217.04±1.98	GT/TT[177] 217.74±3.1
P-value	P=0.582			P=0.844	
HDL-C Adjusted mean± SD	GG[436] 49.73±0.60	GT[167] 52.06±0.97	TT[11] 48.94±3.69	GG[438] 49.74±0.6	GT/TT[178] 51.88±0.94
P-value	P=0.033			P=0.018	
LDL-C Adjusted mean± SD	GG[433] 139.35 ±1.84	GT[165] 136.99±2.95	TT[11] 159.09±11.22	GG[433] 139.31±1.84	GT/TT[176] 138.33±2.88
P-value	P=0.830			P=0.770	
Triglycerides Adjusted mean± SD	GG[438] 144.66±3.29	GT[164] 137.02±5.33	TT[11] 129.24±20.22	GG[438] 144.68±3.28	GT/TT[175] 136.55±5.18
P-value	P=0.225			P=0.222	

Mean and *p*-values adjusted for BMI, age, smoking, and sex

Natural log transformed data were used for association analysis of triglycerides and HDL-C levels

4.0 DISCUSSION

Lipoprotein lipase (*LPL*) is a biological candidate gene for cardiovascular disease due to its major role in the catabolism of TG rich lipoproteins. Clinically abnormal lipid levels have been associated with CHD, atherosclerosis and obesity in individuals who have functional DNA sequence variation in their *LPL* gene (Murthy et al. 1996; Reymers et al. 1995; Brunzell et al. 1995; Wiebusch et al. 1992). The sequence variation of *LPL* is also associated with Mendelian disorders. *LPL* deficiency, known as Type 1 hyperlipoproteinemia or familial chylomicronemia (MIM 238600), is a rare autosomal recessive disorder (1/1,000,000) caused by defects in the *LPL* gene and it is correlated with severe hypertriglyceridemia due to chylomicronemia and VLDL accumulation with very low levels of LDL-C and HDL-C levels (<20mg/dL).

Several candidate gene studies and genome wide association studies (GWAS) have identified many SNPs in the *LPL* gene associated with lipid levels (Aulchenko et al. 2009; Kooner et al. 2008; Klos et al. 2006; Chasman et al. 2008; Ahn et al. 1993; Razzaghi et al. 2000; Willer et al. 2008; Chamberlein et al. 1999; Wallace et al. 2008; Humphries et al. 1998). In this study, we resequenced the entire *LPL* gene (~30kb) in 95 healthy NHW individuals having extreme HDL-C to evaluate the impact of *LPL* genetic variation on HDL-C and correlated lipid levels. Our comprehensive resequencing effort of this gene, the first to our knowledge, revealed a total of 179 variants; 162 single nucleotide substitutions, 17 indels and a microsatellite (tetranucleotide repeat marker). Of 178 variants, 88 had a $MAF \geq 0.05$ and 91 had a $MAF < 0.05$.

4.1 COMPARISON OF OUR RESEQUENCING RESULTS WITH TWO PUBLISHED STUDIES

We compared our sequencing results with two previous published sequencing reports (Nickerson et al. 1998; Wright et al. 2008). Nickerson et al. (1998) examined a portion of the *LPL* gene (9.7kb), from 3' end of intron 3 to 5' end of intron 9, among 71 individuals from three racial groups including European-Americans from Rochester, Minnesota (n=23), African-Americans from Jackson, Mississippi (n=24) and Europeans from North Karelia, Finland (n=24). They identified 88 variants, of which 79 were single nucleotide substitutions, 8 were indels and one was a microsatellite. Of 88 variant, 56 and 59 were identified in their white populations, North Karelia, Finland and Rochester, Minnesota, respectively. We compared our sequencing results with the variants they identified in Rochester samples. Fifty three of the 59 reported variants were founded in our NHW sample in this 9.7 kb region, including the reported microsatellite (ATTT)_n. Of the 6 reported variants, which we did not find in this study, 5 had $MAF \leq 0.05$ and one was 8538delA with a MAF of 0.20 which was observed as a sequencing artifact in our sample. On the other hand, we identified 17 variants ($0.005 \leq MAF \leq 0.053$) that were not reported in their study. This inconsistency might be due to the sample size differences or due to the different software that were used in identifying variants (Nickerson et al. used PolyPhred Program, but we used Variant Reporter and Sequencher).

Wright et al. (2008) sequenced the 10 exons and intron-exon boundaries of the *LPL* gene, plus 1kb in the promoter region, and about 300 bases in the 3' flanking region in 19 Northern Irish individuals with extreme hypertriglyceridemia (HTG). They identified a total of 42 variants and found rs268 (N291S) to be a major predisposing factor for HTG. We identified 39 of the reported

42 variants, including N291S (discussed later on). The three variants they identified (590G>A, 1018G>A and 345A>C) but we did not, had MAF <0.05 in their Northern Irish sample. Two of these 3 variants were exonic (590(G>A) in exon 5 and 1018G>A in exon 6). Interestingly, these two variants were not identified by Nickerson et al. (1998), either. Since Wright et al. (2008) used a selected sample with HTG, it is not surprising that these variants were not identified in ours and Nickerson et al.'s population-based samples. On the other hand, although we identified 23 exonic variants in our sample, they identified only 15 exonic variants including the 2 we did not identify. Of 11 exonic variants that they did not identify, ten were located in the UTR of exon 10, and one was [rs328 (Ser447X)] located in exon 9.

4.2 DISTRIBUTION OF *LPL* VARIANTS AMONG HIGH AND LOW HDL-C GROUPS

4.2.1 Distribution of common *LPL* variants among high and low HDL-C groups in NHWs

Of the 88 common variants (MAF \geq 0.05) observed in this study 32 showed significantly different distribution ($P \leq$ 0.05) between high and low HDL-C groups. Of these 32 variants, 25 have been previously reported in Chip Bioinformatics, and remaining 7 are the new variants.

Two of the common variants, rs326 (23858) and rs13702 (28911), that revealed significant difference between the high and low HDL-C groups in our study, have also been reported previously to be associated with HDL-C levels (Klos et al. 2006; Kooner et al. 2008). Klos et al. (2006) genotyped 3,993 individuals (1,132 black females, 807 black males, 1,101 white females and 953 white males) for rs326 and rs13702 variants and found significant association with HDL-C in black males ($P=0.013$) and black females ($P=0.004$). The association

between HDL-C levels and rs326 was also confirmed in a GWAS ($P=1.4\times 10^{-7}$) comprising 5,968 individuals (Kooner et al. 2008). In both studies, the rs326/G allele was found to be associated with high HDL-C levels than the other allele. Likewise, in our study the frequency of the G allele for rs326 was higher in the high HDL-C group than the low HDL-group (0.278 vs. 0.133; $P=0.017$). As reported previously, the rs326 and rs13702 variants were in strong LD in our sample ($r^2=0.933$). Our tagger analysis showed that rs13702 and rs326 were in the same bin along with rs15285 (29086C>T) and rs3916027 (29287G>A), which also demonstrated significant difference between the high and low HDL-C groups ($P=0.009$ and $P=0.012$, respectively).

A common *LPL* variant in intron 8, *HindIII* (23496T>G, rs320), has been reported to be associated with plasma TG and HDL-C in several, but not all studies (Ahn et al. 1993; Razzaghi et al. 2000; Chamberlain et al. 1989) As expected, the frequency of the *HindIII*/G allele in the high HDL-C group was almost twice to that observed in the low HDL-C group (0.244 vs 0.128; $P=0.053$) in our sequencing sample, The *HindIII*/G allele is in strong LD with the 447X allele of the functional Ser447X polymorphism (rs328) in exon 9 (Razzaghi et al. 2000; Humphries et al. 1998;). The S447X variant, which has been reported as a gain-of-function mutation, leads to a premature stop codon, resulting a truncated protein which is two amino acid shorter than the full-length product that increases LPL protein expression without changing specific activity (Merkel et al. 2002; Rip et al. 2006). Wittrup et al. (1999) found that the carriers of Ser447X had reduced TGs, increased HDL-C and 0.8-fold reduced risk of ischemic heart disease. Since these two variants are in strong LD, there has been a debate in the literature whether *HindIII* is a genetic marker for the functional Ser447X variant or this is functional by itself (Razzaghi et al. 2000; Humphries et al. 1998; Merkel et al. 2002). However, Chen et al (2008) have recently demonstrated that the *HindIII* site binds to a transcription factor and affects LPL expression and

thus, this is a functional variant by itself. Although we identified the *HindIII* polymorphism in our sample, we found only one example of S447X (24143C>G) in the high HDL group using Variant Reporter. We further looked for this variant in Sequencher. However, we did not see any clear peaks as an evidence of variation at this site in Sequencher.

Another common variant *PvuII* (rs285) that we identified in our sequencing sample has been previously reported to be associated with TG levels and hypertriglyceridemia in some but not all studies (Chamberlein et al. 1999; Ahn et al. 1993). However, in our sequencing sample we found a comparable frequency of the T allele between the high and low HDL-C groups (0.444 vs 0.370; P =0.304).

To our knowledge, seven GWA studies have found significant association signals within the *LPL* gene and flanking regions associated with HDL-C levels (Boes et al. 2010). The reported SNPs with the lowest p-values are: rs2083637 (P=5.5×10⁻¹⁸) (Aulchenko et al. 2009), rs10503669 (P =4.1×10⁻¹⁹) (Willer et al. 2008), rs331 (P=9.1×10⁻⁷) (Chasman et al. 2008), rs328 (P =9×10⁻²³) (Kathiresan et al. 2008b), rs17482753 (P =2.71×10⁻⁵) (Heid et al. 2008), rs17411031 (P =1.28×10⁻¹⁰) (Wallace et al. 2008), and rs326 (P =1.4×10⁻⁷) (Kooner et al. 2008). Of these 7 SNPs, we identified rs326 and rs331 SNPs in our study. As mentioned above, our result for rs326 was consistent with reported GWAS, but we did not find a significant difference between high and low HDL-C groups for rs331 (0.154 vs. 0.096; P =0.247), although the frequency of the A allele was higher in high HDL-C group which is consistent with the previous reports. The other 5 GWAS significant SNPs are located in the distant 5' flanking region of the *LPL* that was outside the region we sequenced.

4.2.2 Distribution of relatively uncommon or rare *LPL* variants among high and low HDL-C groups in NHWs

In addition to the 88 common variants identified in sequencing, we identified 91 uncommon or rare variants (MAF < 0.05). Of the 91 relatively uncommon or rare variants, 21 were present only in the low HDL-C group and 25 were present only in the high HDL-C group; 45 exist in both groups. Since the P-values for the uncommon or rare variants (MAF < 0.05) between the two HDL groups were unreliable due to the small resequencing sample size (47 in high HDL and 48 in low HDL), we performed a preliminary analysis for 91 variants to determine the accumulation of rare variants in the high and low HDL-C group. Forty one of 47 (87.2%) individuals with high HDL-C had minimum one rare variant versus 35 of 48 (72.91%) individuals with low HDL-C; 24 of 47 (51.1%) individuals high HDL-C had minimum two rare variants versus 16 of 48 (33.3%) individuals with low HDL-C; 18 of 47 (38.2%) individuals with high HDL-C had minimum three rare variants versus 8 out of 48 (16.6%) individuals with low HDL-C. Overall, the prevalence of uncommon or rare variants was higher in the high HDL-C group than the low HDL-C group, which contradicts observation made by Cohen et al. (2004) by sequencing three other lipid genes. Cohen et al (2004) resequenced the *ABCA1*, *APOA1* and *LCAT* genes in individuals having extremely low (5th percentile) and high (95th percentile) HDL-C levels from a population-based sample to test whether the accumulation of rare variants collectively contribute to variation in HDL-C levels. They observed accumulation of rare sequence variants in subjects with low HDL-C levels and concluded that multiple rare variants were significant contributor to low HDL-C levels.

To date, in Online Mendelian Inheritance in Man database (OMIM), (<http://www.ncbi.nlm.nih.gov/omim>) a list of 41 causative mutations or rare variants have been reported in *LPL*; 35 mutations were reported causing LPL deficiency; 4 were reported causing Combined familial hyperlipidemia (CFH); one single nucleotide polymorphism (SNP) (Ser447X) reported as contributing a defect in lipid interface recognition in a Type 1 hyperlipidemia patient and another SNP (rs326), a HDL-C level quantitative trait locus 11 (HDLQ11) was reported for being associated with high HDL-C levels. Not surprisingly, we did not identify any example of the reported 35 rare mutation associated with LPL deficiency because our sample was population-based from apparently healthy individuals. However, of the 4 reported variants associated with CFH, three were identified in our resequencing sample; D9N (rs1801177), -T93G (rs1800590) and N291S (rs268). Both coding variants, D9N and N291S, are associated with reduced enzymatic activity. -T93G is a functional promoter variant located in the 5' flanking region of the *LPL* gene and is in LD with D9N among Caucasians (Zhang et al. 1996; Mailly et al. 1995; Merkel et al. 2002). The minor allele frequency of these variants in our resequencing sample was ≤ 0.05 (Table 15) and so there were not enough individuals in the high and low HDL groups to make a conclusive statement about their role in affecting HDL-C levels. However, these variants will be genotyped in our entire sample in addition to other rare variants that we identified in the current study to evaluate their association with lipid levels.

Table 15. Distribution of identified functional variants in high and low HDL-C groups

Nucleotide #	Alleles	Location	refSNP ID	All MAF (n=95)	MAF(%) assoc. allele High HDL-C	MAF(%) assoc.allele Low HDL-C
1090	T>G	5' flanking	rs1800590 (-T93G)	0.011	0.011	0.010
10127	G>A	Exon 2	rs1801177 (D9N)	0.016	0.021	0.010
17948	A>G	Exon 6	rs268 (N291S)	0.033	0.022	0.043
24143	C>G	Exon 9	rs328(Ser447X)	0.005	0.011	0.000

4.3 DISTRIBUTION OF SELECTED COMMON VARIANTS IN THE TOTAL NHW SAMPLE

To date, we have screened a total of 12 common variants in the entire NHW sample (n=623) by using TaqMan Genotyping assays (Table 6 and Table 7 in [Section 2.3](#)). Of the 12 variants, rs1059611, rs270, rs3779787 and rs327 showed statistically significant association with HDL-C level ($P < 0.05$) in both additive and dominant models, but rs13266204 had a significant P-value (0.013) only in the additive model, but showed a marginally significant association ($P = 0.080$) in the dominant model. To our knowledge, none of these SNPs were previously reported as being significantly associated with HDL-C levels. In our resequencing samples (n=95), we also observed significantly different distribution of rs1059611 (28982T>C), rs270 (18095C>A), rs327 (23955T>G) and rs13266204 (4424A>G) between the high and low HDL-C groups ($P = 0.003$, 0.045, 0.018, and 0.038, respectively). Although we observed significant association of rs3779787 in the total sample ($P_{\text{additive model}} = 0.033$ and $P_{\text{dominant model}} = 0.018$) the difference of minor allele frequencies between the high and low HDL-C groups was marginally significant

($P=0.096$). This can be explained by the sample size differences in genotyping and resequencing. For rs3779787, T allele was associated with high HDL-C levels in our genotyping results ($P_{\text{additive model}}=0.033$ and $P_{\text{dominant model}}=0.018$) and T allele frequency was also higher in high HDL-C group than low HDL-C group, although it did not achieve statistical significance at 5% level ($P=0.096$). Noteworthy, although we identify only one example of the Ser447X variant in our resequencing sample, it was in LD with rs1059611 which was genotyped in our entire NHW sample and showed significant association with HDL-C levels, as discussed above.

Two of the 12 variants, rs327 (T>G), rs270 (C>A), showed statistically significant association with TGs levels in both additive ($P=0.014$ and $P=0.031$, respectively) and dominant models ($P=0.015$ and $P=0.012$, respectively). rs312 (G>C) also showed statistically significant association with TG levels in additive model ($P=0.009$), but it showed marginally significant association in dominant model ($P=0.050$). The rs327/T and rs270/A alleles were associated with high TG levels as compared to other corresponding alleles of these two SNPs. Both of these variants have also demonstrated significant differences between the high and low HDL-C groups in the subset sequencing sample, as discussed above. Recently, the association between TG levels and rs327 was also reported by Smith et al. (2010), but they did not observe significant association between rs270 and TG levels in any of the statistical models that they used. In their study, rs327 was shown to affect regulation of *LPL* in vitro and they reported this as a new potential functional variant of *LPL* (Smith et al. 2010). We also observed association of rs283 with TG levels ($P=0.024$); however this association was not identified by Smith et al. (2010).

There was one variant, rs270 (C>A), that showed statistically significant association ($P=0.020$) with LDL-C levels; rs270/A allele was associated with high LDL-C levels. The same allele was also associated with low HDL-C ($P=0.020$ and 0.008 , for additive and dominant

models, respectively) and high TG levels (P=0.031 and 0.012 for additive and dominant models, respectively). Additionally, rs312 (G>C) showed marginally significant association with TG levels in only dominant model (P=0.087); the G allele was associated with low LDL-C levels for this variant.

Previously, rs3289 located in UTR of exon 10, has shown to affect LPL regulation in vitro and also revealed significant association with TG levels (Smith et al. 2010). However, in our study we found no such association with TG or other lipid traits. This might be due to different sample sizes and sample selection criteria that were used in genotyping.

5.0 CONCLUSIONS

LPL plays a vital role in lipid metabolism and genetic variation in this gene affects monogenic lipid traits and also HDL-C and TG variation in the general population. To further investigate the role of the rare and common variation in the *LPL* gene, we resequenced the entire gene in selected individuals falling in the upper and lower 5th percentile of HDL-C in a NHW sample. We identified 179 variants, of which 88 had $MAF \geq 0.05$ and 91 had $MAF < 0.05$. Of the 88 common variants, 32 demonstrated significant association with HDL-C levels. Overall, the prevalence of uncommon or rare variants was higher in the high HDL-C group than the low HDL-C group. Our comprehensive resequencing study reconfirms the functional significance of the *LPL* gene in lipid metabolism.

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