# LIPOPROTEIN LIPASE GENE SEQUENCING AND PLASMA LIPID PROFILE

by

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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 ≥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≥ 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 [≥ 6.22mmol/L], arterial blood pressure≥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 discodial 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 *PON1),* 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 $10^{th}$ 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).

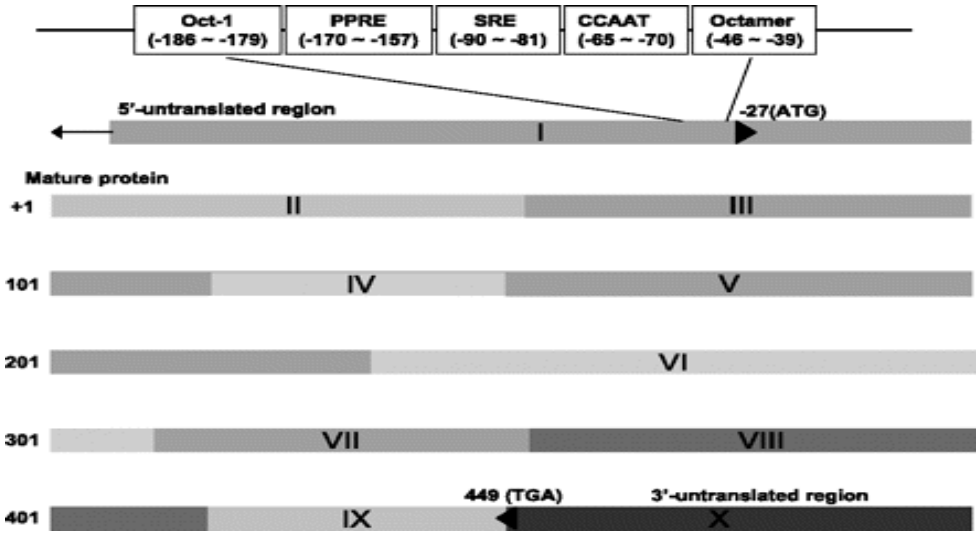


**Figure 1.** Lipoprotein lipase gene structure with promoter elements. (From Wang et al 2009, "Am Physiol Soc, used with permission")

### 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 steroidgenic 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-Olivecrone 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).

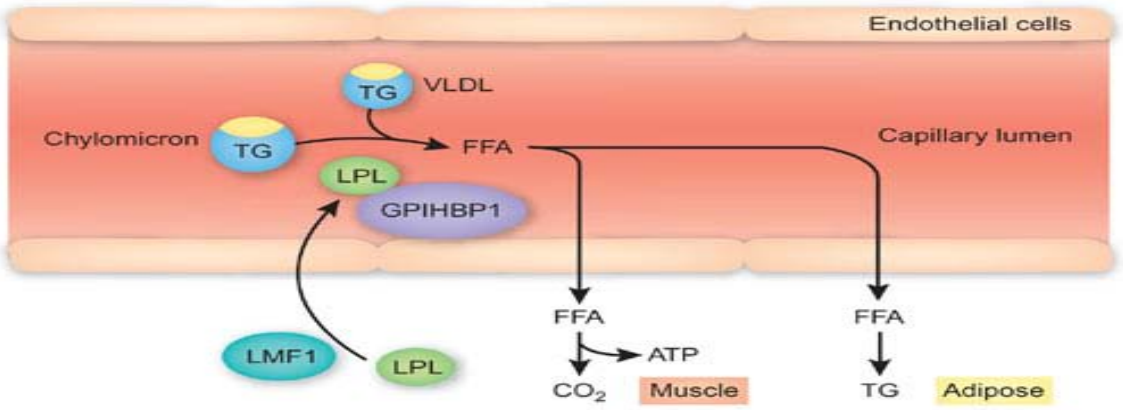


**Figure 2.** LPL activity in fatty acid transport to muscle and adipose tissues (Attie et al. 2010, used with permission).

### 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 dsylipidemia, 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

| *LPL* 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 showen 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; Mailly 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 *Hind*III (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 *Hind*III 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 (Wallacee 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) $5^{th}$ 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/m2) | 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 5$^{th}$ (n=47) and in the lower 5$^{th}$ (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/m2) | 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 Pimer 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.

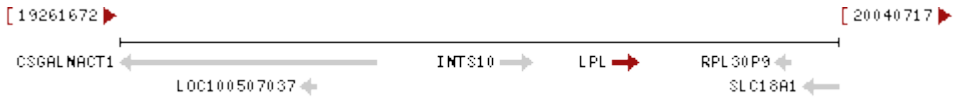


**Figure 3.** *LPL* and neighboring genes on chromosome 8.

**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'-TGTTTTCCAAGGAGGGAAAG-3' | |
| 2 | 722 | 5'-TGATCCATCTTGCCAATGTT-3' | 5'-AGGGCTTTGCTCTCCATCT-3' | |
| 3 | 851 | 5'-GAAAGCTGCCCACTTCTAGC-3' | 5'-GTACTTTCTCCACCCCGACA-3' | |
| 4 | 703 | 5'-TGACCTGCAGTCACCTCTCT-3' | 5'-GCTCTCTATGCTGCTGTTGC-3' | |
| 5 | 800 | 5'-GGGGCCAAATGAGAATGTC-3' | 5'-AGTTGGCTCCTACCATCTTC-3' | |
| 6 | 1177 | 5'-GGATCAGTTTGAAAACACTGGA -3' | 5'-CATTTTGATGGCTGGAACAT-3' | |
| 7 | 1112 | 5'-TGCCTTATGCCAGATTGTTC-3' | 5'-TTGAATGAAGGGCTGTTGAG-3' | |
| 8 | 1147 | 5'-ATACCATTCTGGCTTGGATT-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'-TCGAAAACACTTCAGAAACAAAA-3' | 5'-AGTAAATGGAGGCCCAGAGA-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'-AACCCGATTTTCTTGCCTTA-3' | 5'-TGAATGCCCCCAGAAAATA-3' | |
| 18 | 1084 | 5'-AGAGTTGGGTGCCAAAACTT-3' | 5'-GGGTATATATTTTCCCATTATTCC-3' | |
| 19 | 691 | 5'-AACCAGGTAATTGGAAGTAAAAA-3' | 5'-ACAGTTCTGCCAAAAATAAAACT-3' | |
| 20 | 1061 | 5'-TGTTTACGGAAAAGTGAAACAAA-3' | 5'-GGGGCTTCTGCATACTCAA-3' | |
| 21 * | 475 | 5'-GGCCAAATGTGTATATGAAAAC-3' | 5'-CCATGACTGTAGAATAGGAGC-3' | |
| 22 * | 1783 | 5'-AGAGGACTTGGAGGTAAATATT-3' | 5'-GACTCCTTGGTTTCCTTATTTA-3' | 5´-ATGTTACTGGAACAGAAGATG-3´<br>5´-CTGGTCCACATCTGGGTAAA-3´ |
| 23 * | 1229 | 5'-AGGCTGGAGACTGTTGTAAAT-3' | 5'-CTCAGGTTTCCATCTGGATTC-3' | 5´–CTATCAACTCTGTTATGGTGGC-3´ |
| 24 | 708 | 5'-CCCTCTATGTGCTCATGCAA-3' | 5'-TGGGGCCACTGTTCTTTAAT-3' | |
| 25 | 1169 | 5'-GGAATGGTCGGAAAATGAGA-3' | 5'-AAGGAAAGGCAGCAGGACTA-3' | |

**Table 4 Continued**

| | | | | |
|---|---|---|---|---|
| 26 | 915 | 5'-CCACGCCCAACTAATTTC-3' | 5'-CCTAGAAAATGCAGACCTTGAA-3' | |
| 27 | 1057 | 5'-TGTTTTGGCCTTCTGATTTG-3' | 5'-CATGGTGAGACCCTGTCG-3' | |
| 28 | 755 | 5'-AGTAAGAAGTCCATGACAAAGTGT -3' | 5'-TTTCCTGGGTTTCCTACAAT-3' | |
| 29* | 1881 | 5'-CATCAATTACAGTCGTACCTAT-3' | 5'-TCAGCTTTAGCCCAGAATGC-3' | 5´-GAGCAGTCTTATGTTACTGGGC-3´ |
| 30* | 794 | 5'-TCATTTGCAGAAAGGAAAGG-3' | 5'-AATTCAGAACAGGAGTAGTG-3' | |
| 31 | 874 | 5'-TGCCTCTTTCCTACCTGACC-3' | 5'-ATTTTTGTAAAGGACGAAAAACAT-3' | |
| 32 | 1072 | 5'-AAAAACATGCCTATTAGGAAAAG-3' | 5'-CGCATCTGAACATTCTCTGTC-3' | |
| 33 | 1078 | 5'-CGGCCCTAGATGCAGTTTTA-3' | 5'-AGATTCGCCCAGTTTCTGAG-3' | |
| 34 | 1049 | 5'-AGAAGTCATTTGGCCCAGTC-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 Corperation, 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 µL) | | PCR conditions |
|---|---|---|
| DNA | 3.0 µL | 1. 95º C for 5 minutes<br>2. 95º C for 45 seconds<br>3. 58-60º C for 45 seconds<br>4. 72º C for 1 minute<br>- Repeat steps 2-4 for 40 cycles<br>5. 72º C for 10 minutes<br>6. Cool to 4º C |
| dH2O | 12.25-13.75 µL | |
| 10x BufferGold | 2.5 µL | |
| MgCl2 (25 mM) | 1-3.5 µL | |
| dNTPs (1.25mM) | 3.8 µL | |
| Forward Primer (20mM) | 0.4 µL | |
| Forward Primer (20mM) | 0.4 µL | |
| AmpliTaqGold (5U/µL) | 0.15 µL | |

## 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 (MAF≥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≥ 4%, $r^2 \geq 0.7$)

| *Bin* | *Variants captured* |
|---|---|
| 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** | ***LPL* 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 μL) | | PCR Cycling Conditions |
|---|---|---|
| $dH_2O$ | 2.43 | 1. 95º C for 10 minutes<br>2. 95º C for 15 seconds<br>3. 60º C for 1 minute<br>-repeat steps 2-3 50x |
| TaqMan Master Mix | 2.50 μL | |
| TaqMan Assay Mix | 0.06 μL | |

## 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 $\chi 2$ 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 $\chi 2$ 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 ($0.05 \leq P \leq 0.01$).

# 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) $5^{th}$ 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 $\geq$0.05, 54 had a MAF 1-5% and 37 had MAF $\leq$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-synomous 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

| ***LPL* 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 (*Hind*III)** | | 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

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19,839,948  taatgtaaat cccttatatt taaaaagata tttaaaagta ttccaagcat
19,840,008  tttggcagaa aagcatagta tctaatgtta ttttttttctt attttatgtg
19,840,058  catgcctctt atccatttaa aaatagcttt actgacctat aatttacaca p.208/rs1470186-[T/C]
19,840,108  ctatataatt ctcccattga aagtgcataa ttctgttgct tttagtatat
19,840,158  ttacagagtt gtgcagcatc agcataatgt aatctagaac attgtcatca
19,840,208  actacccccca aatctctatt cttcccttcc cctattaatt acccagcccc p.351-[C/A]
19,840,258  aggcaagcac tgatctactt ttggtctcta tggatttgtc tatttgtgga p.428/rs73667465-[G/A]
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19,840,358  acttatcata actcaatacg gctttagatt atttgacctc gatgttctgc
19,840,408  ctctgaacat aaaatattat ccttgcattc cttgatgagt ttgaggattg p.549/rs17091742-[C/T]
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19,840,558  ttgtacaagc acaagctggg acgcaatgtg tgtccctcta tccctacatt
19,840,608  gactttgcgg gggtggggat ggggtgcggg gtgagtgagg gaggactgca
19,840,658  agtgacaaac aggattcgtc aaaagagagg tgtattaaag tgccgatcaa
19,840,708  atgtaattta acagctaaac tttccctcct tggaaaacag gtgattgttg
19,840,758  agtatttaac gtgaatcgat gtaaacctgt gtttggtgct tagacagggg
19,840,808  gcccccgggt agagtggaac cccttaagct aagcgaacag gagcctaaca p.958-[G/A]
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19,841,058  cccctcttcc tcctcctcaa gggaaagctg cccacttcta gctgccctgc Exon 1
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19,841,158  tctccagcct ccggctcagc cggctcatca gtcggtccgc gccttgcagc
19,841,208  tcctccagag ggacgcgccc cgagATGGAG AGCAAAGCCC TGCTCGTGCT
19,841,258  GACTCTGGCC GTGTGGCTCC AGAGTCTGAC CGCCTCCCGC GGAGGGGTGG
19,841,308  CCGCCGCCGA CCGTAAGTTT TGCGCGCAAA CTCCCCTCCA CCTGCAGACC Intron 1 rs11570895
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19,841,508  CCGATCGTGA AGCGGCGGCG CCCAGTTCCC GCTTTTTCTC TCTGCCGGGT
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19,841,858  GGTACGCTCG CCCTCGGCGG GGCCCCTCGC TCCGCTGTGG GAGTGGCAGT
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19,842,058  CCCGAAACCG TGGCGCAGCC ACCAGCAATC TGTGGTCGCC GACTCGGGGG
19,842,108  GTTGCCAGGT CTGCGTTTGG CCACCCTTTC TGTCCTGGGG GCTGAGGTCA
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19,842,308  GACCTGTCCG CAATGGAGGC AGCCTGCTTA ATTCGAACCT CGATTCAGTA
19,842,358  AACATGCAAC AGCAGCATAG AGAGCAGCTG AAGCCATTCA TAACACGGGA
19,842,408  CAACATTTCC TTTTTTCTTC CATGCTGGAA TTGCAATTAG GGCGGTGTCG
19,842,458  CTTGGATGTG CTCTCAGGCG GCACGTCCCC AGCGGTTCAA GTTATAATAG
19,842,508  AGTCTCTCCA TAGCTTTGAT GGCCGCTAAA CGTTTGTTTT ATTTTGGCAT
19,842,558  TAATTTGTGA AACATTTTTG TTAGGTTAAA AAACAAAAAG TTGGCAGGGA
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19,842,658  GACAGCTTGA GCCCAGAAGT TCGAAATCAG CCTGGGCAAC ATAGGGAGAC
19,842,708  ACCGTCTCTA TAAAAAAAAA ATACAAAAAT TAGCCGGGCG TGGTGGCCTG
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19,842,858  GGGTGACAGT GAGACCCTGT CTCATAAAAT TAAAACAAAA CAAAACAAAC
19,842,908  ACTAAGTATA GATTCCATCA AAGCAAAATT GGATAAGAAA AAAAGTATCT
19,842,958  TTTCTATTGG ATCAGTTTGA AAACACTGGA GAGTTGATGA GAAAGTCTTC
19,843,008  AACATCTTAG GTGGGGTATG TTTCGTATGT TTCCCTTCGT ACTGCTTAAT
19,843,058  GCTGACAAGA AGATGGTAGG AGCCAACTCC AAATTCTTAT TTCAGAAAGC
19,843,108  ACACCATAGA ATAACGTCAT TTTCATTGCA AAACAAGCAC CGAAATATGT
19,843,158  CATCACATTC AAGTTTTTCC TAGGCTCCCT TACAGGTTCA AGATCCTAAA
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19,843,608  TCATTCTGGA TTCTTTCTAA CAAGAATATG AGGACAAGAG GCACTGTCCT
19,843,658  CAGGCACTTC GTCCTGGGAG CCACCACCAT CTCTGCATGG CCCCAATTAG
19,843,708  GAAACGTGAA GAGCTAGGAG AGGGAGAGTA TGGTCAGTGC TTAGCAGCTG
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19,844,208  TTTTAAGGTC TGGATGAAGA GACCATTTGG ATTTACTAAT AAGGTAAATT
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19,845,808  TCTTCTACTAA AAATACAAAA ATTAGCCAGG CGTGGTGGCA GGCACCTGTA p.5949-[T/G]
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19,846,058  TGAACAGACC AATCAATTCA GTCTGATCAT GATATTGATG TTTTTCTTCA
19,846,108  CCAAATCGAC CACATCAGTA ATTCACTTTG TTCTTCGATA TCCTACAGAC
19,846,158  ACTGCCTGAG TCGATAACTA TGACTATCAG TCTCAGAGAG CAAATGAATT
19,846,208  ACTGAGGAAG CCCTGTAGGA GTGAGAGAAA AGGGGGGAGA GAGAGAGAAA p.6383-[G/T]
19,846,258  GGGGTGGGGG GATAACAGGA GAACAGAAAT TCCAAAGAGA ATTGCATTCT p.6435-[G/C]
19,846,308  CATTGAGTTC TTGTACCTCA TGTCATTGCA TTAAATGTTCA TCTTACTCAC p.6477-[T/C]
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19,846,408  GCTCAACACT TCCCTCTTTC TAGCAACAAG AATTACCACT CTTCCCCTCT p.6553/rs59254395-[C/T];p.6554/-
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 19,846,808  CCTGTACTCC TCTTCTTTCT CAATTCTAAG GTGGCCTTAA TTTCTAAGGG
 19,846,858  ACATGGCAAA AGACAGTCTA GTTGGATGAG TGCAGTCACT AATATTATTT
 19,846,908  CCATGTATGG AAAATAACTG TTTCCTTAGT AACAATTGCA TCAAATCAGT
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 19,847,108  GTTGCAAACA CAGGTAGTGT GAAAATTATC AGAACATCCA AGAAAAGGAA
 19,847,158  AGTTTGACTA AGTGCCGATA AGATTTATGA TGTCATGTCT GACATAGAAT p.7313/rs28645722-[G/A]
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 19,847,708  GAGCTATTTA AGGTGGAAAG GTGTGGGAGA GGGTGAAATT AGTTTTAAAT
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 19,858,308  TGTGACATGG GAGTTTGTTG TCCAGAATAT TCCATCACCC AGGTATTAAG p.18462-[T/G]
 19,858,358  CTTAGTACCC ATTAGTTACT TTTCCTGAAG CTCTCCCTCC TCCCACCCTC
 19,858,408  TGGGAGGCCC CAGTGTCTGT TGTTCCCCTC TATGTGCTCA TGCAAAGTTT
 19,858,458  TATTAGGACA CAGCCACACA CATTCATTAC CATATTGTCA AAGGCTGGTT p.18621-[C/T]
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 19,858,658  AGGCTGGAGG AGAGGGGCAT GATCTTGGCT CACCACAACC TCTGCCTCCC p.18822/rs277-[T/C]
 19,858,708  GGGTTCAAAT GATTCCCCTG CCTCAGCCTC CGGAGTAGCT GGGATAACAG
 19,858,758  GCATGCACCA TCATGCCCAG CTAATTTTTG TATTTTTAGT AGAGAGGGGG p.18942/rs278-[G/A]
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 19,858,858  GCCCTCCTTG GCCTCATCTG TCTTTTTAAA TGCAACTATT CCTGGAAGGC
 19,858,908  AAGAATATCT CACACCTTCT AAGATACTGC CATTTTGCCA GGAGTTTGTT
 19,858,958  TCACACTTGA ATTTCAAGCT TGGCCTCTTG TTTAGAGGCA GACCTAAAGG rs279
 19,859,008  AATGGTCGGA AAATGAGAGA GGAGGTCTTC GGATAAATCC GGTGAGAGGG
 19,859,058  ACCAACTTCA GGAAGGGTGG CTTTTGTGGA ATCCAGATGG AAACCTGAGG
 19,859,108  GAAGGGATGA TATTAAAGAA CAGTGGCCCC AGGTAAAACA TATGGCACCC
 19,859,158  ATGTGTAAGG TGATTCTTAG AATCTGTAGA GGTGTCTTTC GTGGTATAGA rs280
 19,859,208  GGTTGAGGCA CCTGTGCTTC AAGGAAACCT TAACTCTTCA AAATCAGGCA
 19,859,258  ATGCGTATGA GGTAAAGAGA GGACTGTGGG ACCATAATCT TGAAGACACA rs17091775;p.19442/rs281-
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 19,859,358  TCAGTGTCCC CTAGGGGCAC CTCACCACTC CCAGCTTCTT CAGCTCTGGC p.19517/rs283-[C/T];rs284
 19,859,408  CTGTCCTGCT GCCTGCAAGG GTTTTGCTTA ATTCTCAATT CAATGTCTCT
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 19,859,508  AGTATCTGAC TACTTTTTAA TTATAAAAAG AGATGTATCT AAACAAAATA p.19675/rs286-[A/T]
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 19,859,758  GAAAAAACAT TCCAAGAATT ATTTTATTTA TTTATTTATT TATTTATTTA
 19,859,808  TTTATTTATT TATTTATTTT TGAGACACAG TCTCGCTCAG TTACCCAGGC p.19975/rs287-[A/G]
 19,859,858  TGGAGTGCAG 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 CCCAACTAAT TTCTGTATTT TTCTTAGTAG AAACAGGGTT rs34951282
 19,860,008  TCACCATGTT GGCCAAGCTA GTCTCAAACT CCTGACCTCA GGTGATTCAC rs290
 19,860,058  CCACCAAGGC CTCCCAAAGT GCTGGGATTA CAGGCATGAG CCACCATGCC
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 19,860,208  TGGTAGAGAC CAGAGGAGGG GCAGGGAGGA GATATAGAAG TTCAACTACC p.20363-[A/T]
 19,860,258  TGCTTCCAGA GGCTGTCCCT AGTATAGAAT ACTTTAGGGG CTGGCTTTAC
 19,860,308  AAGGCAGTCC TTGTGGCCTC ACTGATGGCT CAATGAAATA AGTTCTTTTT rs292
 19,860,358  TAAAAAAAAT TTTATTTATT TCCATAGGTT ATTGGGGGAA CAGGTGGTGT p.20505_20506-[InsA];p.20544/rs294-
[T/C]
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 19,860,458  CCCATTACGG AATGGAAAAA TCAACGAAAT AAGTTCTATG ATGCACCTAC
 19,860,508  TAGACACCTA ATCTGCGCTA GATGGTGGGG GAATTAAGAG CATGGGCATG p.20657/rs295-[A/C];p.20663/rs296-
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 19,860,858  CAAGGTCTGC ATTTTCTAGG TATGAACACT GTGCATGATG AAGTCTTTCC
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 19,861,158  ATCCCATTCA CTCTGTGAGT AGCACAGGGG GGCGGTCATC ATGGCACCAG Intron 7 rs299 rs300
 19,861,208  TCCCTCTCCT GCCATAACCC TTGGTCTGAG CAGCAGAAGC AGAGAGCGAT p.21353/rs301-[T/C];rs302;rs34500595
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 19,861,458  GTTTGACATT AAAGAAAATG AGCAATGGTA ACAGGAAACC ACTCTATAGA
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 19,861,558  AGTGTTAGCT CTTTTTTTTT TTTTTTTTT TTTTTTTTT GAGATGGAGT rs303;rs13257355
 19,861,608  CTCTCTCTAT TGCCCAGGCT GGAGTGCAGT GATTCGATCT CAGCTCACTG p.21780/rs304-[T/G]
 19,861,658  CAACCTCTAC CTCCCGAGTT CAAACAATTC TTCTGTCTCA GCCTCCCGAG p.21820/rs305-[A/G]
 19,861,708  TAGCTGGGGC TGCAGGTGCC CACCACCATG CCCAGCTAAT TTTTGTATTT rs306;rs307; p.21895/rs308-[T/G]
 19,861,758  TTAGTAGCGA CAGGGTCTCA CCATGTTGGC CAAGCTGGTC TTGAATTCCT rs309
 19,861,808  GATCTCAGGT GATCCACCCG CCTCGGCCTC CCAAAGTGCT GGGATTACAG p.21965/rs310-[C/T]
 19,861,858  GTGTGAGCCA CCATGCCCAG CCTACCCTTT ACTACTAATC AAAGAAATAA p.22044_22047_[delTAAA]
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 19,862,008  GCATCATTCA CGGCTAGAAC CCTCCAGGTG CGGAAGGTAG TATTTTAATA
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 19,862,158  TACAGTCGTA CCTATACTAG CATAAATTAG AAACCCAGTA TCCAACATTG
 19,862,208  AGGCAGTGGG TAAATGAATC GTGGTTTATC AAGTCATTAA AATCAATCTA
 19,862,258  GCCTTTAAAA ACTATAATTG TAGGAAACCC AGGAAAACAT AGTAAAAAAT p.22416/rs312-[G/C];rs313
 19,862,308  GGAATATAAA ATCTGAAGAG AATAAAGAAT AGAGAATCGT ATGTGTGCTA p.22461/rs314-[G/A]
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 19,862,508  ATATAATTTA AAATATACTA AATGCCATCG ACCTTCATTT TGAGTTCTTT
 19,862,558  GTTGGACATT TTTGTGCATT TTTAAAATAT CCCCTAAATA ATAAAGCTAT
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 19,862,808  ACTGGTGGAG CAGTCCCGGC TTCGCCATTC AGAAGATCAG AGTAAAAGCA rs5934
 19,862,858  GGAGAGACTC AGAAAAAGTA ATTAAATGTA TTTTTCTTCC TTCACTTTAG Intron 8
 19,862,908  ACCCCCACCT GATGTCAGGA CCTAGGGGCT GTATTTCAGG GGCCTTCACA
 19,862,958  ATTCAGGGAG AGCTTTAGGA AACCTTGTAT TTATTACTGT ATGATGTAGA
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 19,863,158  GCTTCTCATT TGGCACTGTT TCTTGTAAGT ACAAAATAGT TAGGGAACAA
 19,863,208  ACCTCCGAGA TGCTACCTGG ATAATCAAAG ATTCAAACCA ACCTCTTCAA p.23388/rs318-[C/G];p23395/rs319-[A/C]
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 19,863,308  TGTGTACCCA TAAAATGAAT TACACAGAGA TCGCTATAGG ATTTAAAGCT p.23496/rs320-[T/G]
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 19,863,408  TAATTTAAAA AAACTCTAAG TTAGGATTGA CAAATTATTT CTCTTTAGTC p.23573-[T/C]
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19,863,658  ACTGCTCTAG GCTGTCTGCA TGCCTGTCTA TCTAAATTAA CTAGCTTGGT
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19,864,258  TTAGGTTTTA TTTTACTCCT TGAACTACCC CTGAATCTTC ACTTCTCCTT
19,864,308  TTTTCTCTAC TGCGTCTCTG CTGACTTTGC AGATGCCATC TGCAGAGCAT
19,864,358  GTAACACAAG TTTAGTAGTT GCCGTTCTGG CTGTGGGTGC AGCTCTTCCC p.24505/rs329-[A/G]
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19,864,658  GCTGCATCTT TCCAGAGCGT CAGTACTGAG AGGACACTAA GCATGTGACC p.24815/rs330-[G/A;p.24824/rs331-[G/A]
19,864,708  TTCACTACTC CTGTTCTGAA TTCCAGGAAT ATGCCCTTTT CAACCCTCCA p.24852/rs12679834-[T/C]
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19,865,158  CAGAGGTTGC AGTGAGATGA GATCGCGCCA TTATATTCCA GCCTGGGCAA p.25320-[C/T];p25335-[C/T]
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19,866,258  TATAGGATTA CTGACAGAAC AGTTATTTAG ACAGAGAATG TTCAGATGCG
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19,866,808  TGATACATTT TAGAATAAGA AATCCTCTAA ATTTAGAAGT CATTTGGCCC
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 19,868,808  tttattagct gtaaatacat gtgtggatgt gtaaatggag cttgtacata p.28982/rs1059611-[T/C]
 19,868,858  ttggaaaggt cattgtggct atctgcattt ataaatgtgt ggtgctaact rs17091815;p.29046_29047-[InsTT]
 19,868,908  gtatgtgtct ttatcagtga tggtctcaca gagccaactc actcttatga p.29086/rs15285-
[C/T];p.29088/rs3866471-[C/A]
 19,868,958  aatgggcttt aacaaaacaa gaaagaaacg tacttaactg tgtgaagaaa
 19,869,008  tggaatcagc ttttaataaa attgacaaca ttttattacc aca
 19,869,051  ctaagtcatt attttgtatc atttttaaag taaatttatt cttaggtcag rs71510671
 19,869,101  attcactcag catattttga ctaagtaacc actgtactta gtaaaccgaa p.29287/rs3916027-[G/A]
 19,869,151  gagcttctga gaattatagt gtacctatta gatatttttа acatttatat p.29315/rs9644636-[T/G]
 19,869,201  ttgtataaag ctaaagaaag ccttacatat cctttaaact gactatagaa
 19,869,251  gaaaatgata cagaattttg cctgcataaa gtacacagga ctattcttgc
 19,869,301  ctacaatatg ctttttcaca agcaaaatgt tagactaata taaggcatct p.29474-[C/T];p.29487/rs4921683-[T/A]
 19,869,351  ttggccattt tatagtgtac atcatctcta tttctgaggc ctcattgtta
 19,869,401  gctgtaacgc aagtagcatt tgtgcaataa aatgaactat ttgggatggg p.29547/rs4921684-[C/T];p.29557_29558-
[InsA]
 19,869,451  agggtacatt ttttagaact ttgctttggg ttgccttgat aattaatagc
 19,869,501  atatagtcca tttatgcagc taagtaggga ttgcttctta gtacagtcag
 19,869,551  gaagaattta gcccagaaaa caattattc aatggccact gacccaaact p.29716-[T/C]
 19,869,601  tccaggctga agagcaatgg cgtgatcatg gctcactgca cctccacctc
 19,869,651  ccaggctcaa gtgattctcc tgcctcagcc tcccaagtag atggtactac
 19,869,701  aagcacacgc cactgcaccc agctaatttt tgtatttttt gtagagatgg
 19,869,751  gggtttcacc atgttgccca ggctggtctt aaattcctgg cctcaagtgt
 19,869,801  ctgccccccc tggcctccca aagtgctgga attacaggca tgagccacca
 19,869,851  tgtccagcct tgacccaaac ttttattgtc agttagctat tggggcttct
 19,869,901  ggagtttggg tctcccctga caggaggggg ctccccagtt cacacttggc
 19,869,951  cactgcccat caattcctgt tgatatgatc aacaagatag acaattgcaa
 19,870,001  atgttgctga ggatgtggag 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 ≥0.05 and 9 1 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

| ***LPL* 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 (*Hind*III)** | 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

| ***LPL* 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

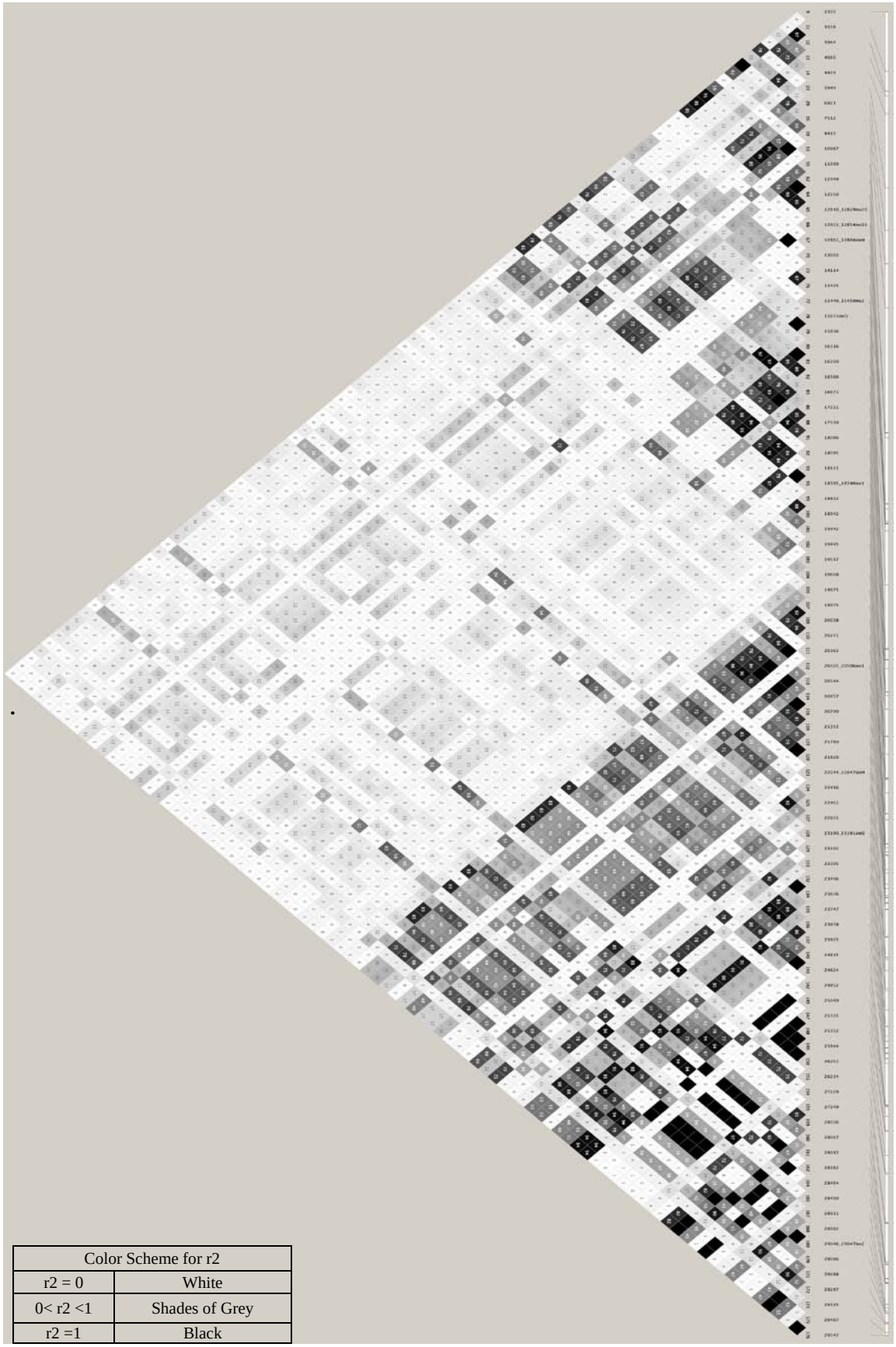
We used LD and Tagger analysis to identify tagSNPs for the common variants (MAF≥0.05) that we identified by resequencing. By using tagger analysis with an $r^2$ cutoff of 0.9, 43 tagSNP bins were identified. Table 12 shows the bins identified by Tagger analysis and Figure 5 demonstrates the LD plot. 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-value≤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, 28982 |
| 2 | 29547, 29487, 24824, 24815, 28036, 28382, 29088 |
| 3 | 28911, 29086, 23858, 29287, 28067 |
| 4 | 7512, 12810_12829ins20, 12550, 12449, 2335 |
| 5 | 16386, 18121, 17599, 18395_18396ins1 |
| 6 | 20790, 20505_20506ins1, 20657, 20271 |
| 7 | 16671, 15653del1, 15836 |
| 8 | 23955, 23636, 23496(*Hind*III) |
| 9 | 26234, 23395, 28490 |
| 10 | 22855, 22416, 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 | 15425 |
| 21 | 15449_15450ins2 |
| 22 | 25049 |
| 23 | 12861_12864del4 |
| 24 | 18095 |
| 25 | 14114 |
| 26 | 19442 |
| 27 | 19445 |
| 28 | 4424 |
| 29 | 23192 |
| 30 | 20544 |
| 31 | 19675 |
| 32 | 27229 |
| 33 | 20363 |
| 34 | 4060 |
| 35 | 18822 |
| 36 | 19517 |
| 37 | 10987 |
| 38 | 29315 |
| 39 | 22461 |
| 40 | 18942 |
| 41 | 22044_22047del2 |
| 42 | 23190_23191del2 |
| 43 | 5949 |



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

**Figure 5.** LD analysis of identified common variants in NHWs

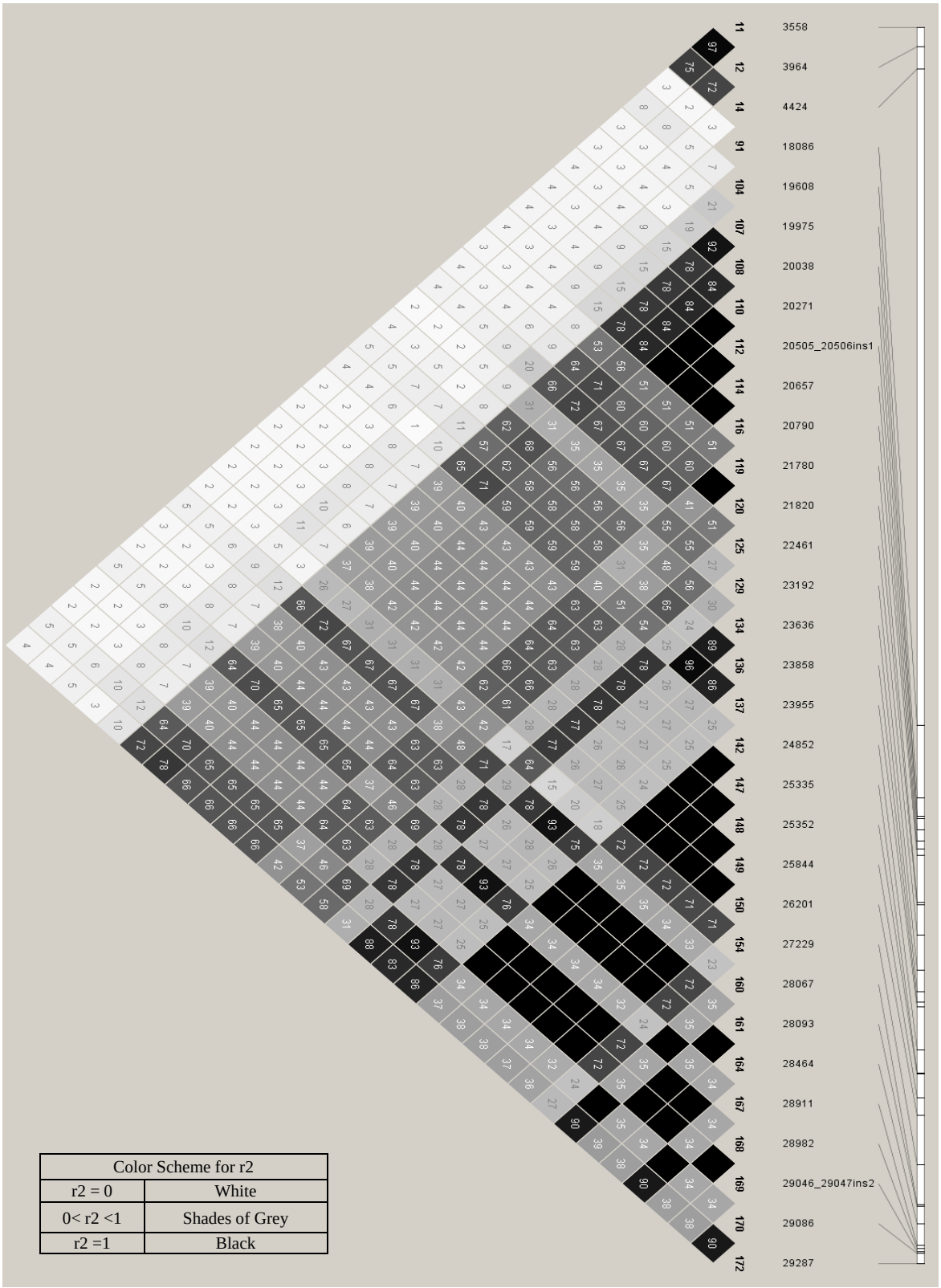



**Figure 6.** LD analysis of the 32 identified variants which had a statistically significant p-value <0.05 when comparing the allele frequencies between the high HDL-C and low HDL-C groups

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

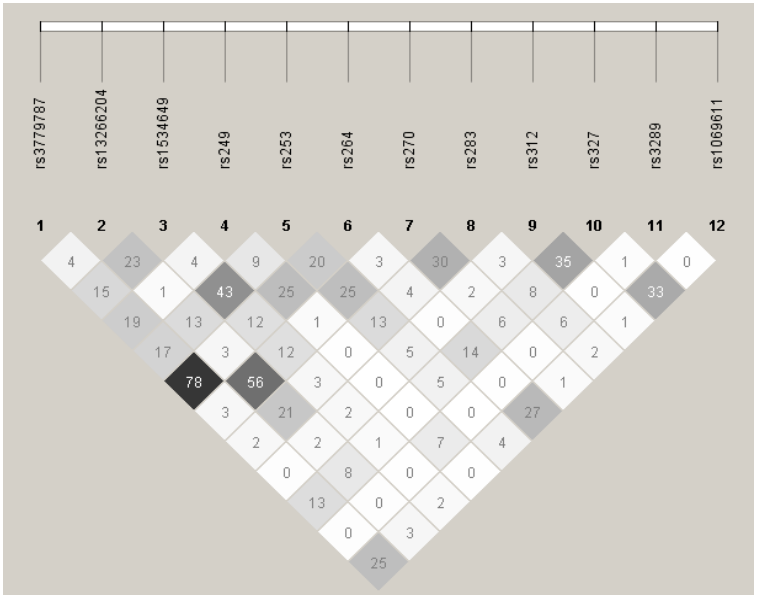
To date, we have genotyped 12 variants in our NHW sample (n=623) using pre-made TaqMan SNP Genoytping Assays. Genotyping results and features of these tagSNPs are shown in the Table 13.

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

| ***LPL*** **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 NHW 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** | |
|---|---|---|---|---|---|
| | AA[380] | GA[203] | GG[31] | AA[380] | GA/GG[234] |
| **Total Cholesterol Adjusted mean± SD** | 216.03±2.13 | 219.34±2.90 | 218.32±7.21 | 216.02±2.13 | 219.2±2.69 |
| **P-value** | P= 0.400 | | | P=0.342 | |
| | AA[383] | GA[203] | GG[31] | AA[383] | GA/GG[234] |
| **HDL-C Adjusted mean± SD** | 50.95±0.64 | 49.95±0.88 | 45.26±2.19 | 50.93±0.65 | 49.3±0.82 |
| **P-value** | **P=0.013** | | | **P=0.080** | |
| | AA[378] | GA[203] | GG[29] | AA[378] | GA/GG[232] |
| **LDL-C Adjusted mean± SD** | 137.40±1.97 | 141.56±2.68 | 143.13±6.89 | 137.41±1.97 | 141.76±2.50 |
| **P-value** | P=0.164 | | | P=0.160 | |
| | AA[380] | GA[203] | GG[31] | AA[380] | GA/GG[234] |
| **Triglycerides Adjusted mean± SD** | 140.70±3.52 | 139.13±4.79 | 180.90±11.92 | 140.9±3.54 | 144.89±4.49 |
| **P-value** | P=0.082 | | | P=0.329 | |
| | **rs312_Add** | | | **rs312_Dom** | |
| | GG[482 | GC[122] | CC[7] | GG[482] | GC/CC[129] |
| **Total Cholesterol Adjusted mean± SD** | 215.70±1.8 | 222.44±3.67 | 211.87±1.15 | 215.71±1.89 | 221.89±3.57 |
| **P-value** | P=0.174 | | | P=0.118 | |
| | GG[484] | GC[123] | CC[7] | GG[482] | GC/CC[129] |
| **HDL-C Adjusted mean± SD** | 50.21±0.58 | 51.06±1.12 | 57.26±4.66 | 50.21±0.58 | 51.39±1.1 |
| **P-value** | P=0.118 | | | P=0.201 | |
| | GG[480] | GC[120] | CC[7] | GG[480] | GC/CC[127] |
| **LDL-C Adjusted mean± SD** | 137.47±1.75 | 144.04±3.42 | 138.97±13.99 | 137.47±1.75 | 143.77±3.33 |
| **P-value** | P=0.114 | | | **P=0.087** | |
| | GG[482] | GC[122] | CC[7] | GG[482] | GC/CC[129] |
| **Triglycerides Adjusted mean± SD** | 144.13±3.1 | 136.49±6.14 82 | 85.52±25.36 | 144.19±3.16 | 133.65±6.00 |
| **P-value** | **P=0.009** | | | **P=0.050** | |
| | **rs1059611_Add** | | | **rs1059611_Dom** | |
| | TT[490] | TC[116] | CC[7] | TT[490] | TC/CC[123] |
| **Total Cholesterol Adjusted mean± SD** | 216.74±1.8 | 219.38±3.73 | 210.19±15.10 | 216.76±1.89 | 218.88±3.63 |
| **P-value** | P=0.713 | | | P=0.599 | |
| | TT[491] | TC[117] | CC[7] | TT[491] | TC/CC[124] |
| **HDL-C Adjusted mean± SD** | 49.71±0.58 | 52.78±1.13 | 49.36±4.61 | 49.72±0.58 | 52.6±1.1 |
| **P-value** | **P=0.036** | | | **P=0.018** | |
| | TT[486] | TC[117] | CC[7] | TT[486] | TC/CC[123] |
| **LDL-C Adjusted mean± SD** | 139.10±1.75 | 138.78±3.45 | 138.41±13.96 | 139.10±1.75 | 138.76±3.36 |
| **P-value** | P=0.925 | | | P=0.928 | |
| | TT[490] | TC[115] | CC[7] | TT[490] | TC/CC[122] |
| **Triglycerides Adjusted mean± SD** | 143.66±3.117 | 139.91±6.29 | 113.17±25.35 | 143.72±3.17 | 138.44±6.13 |
| **P-value** | P=0.346 | | | P=0.481 | |

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** | |
| **LDL-C Adjusted mean± SD** | TT[335] | TG[227] | GG[47] | TT[335] | TG/GG[274] |
| **P-value** | 139.14±2.07 | 137.65±2.50 | 144.17±5.40 | 139.14±2.07 | 138.76±2.29 |
| | 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 | |
|---|---|---|---|---|---|
| | AA[394] | AT[189] | TT[29] | AA[394] | AT/TT[218] |
| **Total Cholesterol Adjusted mean± SD** | 217.60±2.10 | 216.97±2.99 | 214.60±7.52 | 217.59±2.1 | 216.66±2.79 |
| **P-value** | P=0.717 | | | P=0.782 | |
| | AA[397] | AT[189] | TT[29] | AA[397] | AT/TT[218] |
| **HDL-C Adjusted mean± SD** | 50.68±0.64 | 50.09±0.91 | 48.17±2.29 | 50.68±064 | 49.83±0.85 |
| **P-value** | P=0.350 | | | P=0.402 | |
| | AA[392] | AT[187] | TT[29] | AA[392] | AT/TT[216] |
| **LDL-C Adjusted mean± SD** | 139.80±1.95 | 137.57±2.78 | 138.53±6.96 | 139.80±1.95 | 137.70±2.59 |
| **P-value** | P=0.560 | | | P=0.504 | |
| | AA[394] | AT[189] | TT[29] | AA[394] | AT/TT[218] |
| **Triglycerides Adjusted mean± SD** | 138.20±3.48 | 151.36±4.96 | 138.14±12.49 | 138.17±3.48 | 149.59±4.63 |
| **P-value** | **P=0.068** | | | **P=0.024** | |
| | **rs3289_Add** | | | **rs3289_Dom** | |
| | TT[584] | TC[32] | CC[0] | TT[584] | TC/CC[32] |
| **Total Cholesterol Adjusted mean± SD** | 217.66±1.75 | 209.97±7.09 | | 217.66±1.75 | 209.97±7.09 |
| **P-value** | P=0.289 | | | P=0.289 | |
| | TT[587] | TC[32] | CC[0] | TT[587] | TC/CC[32] |
| **HDL-C Adjusted mean± SD** | 50.44±0.53 | 50.42±2.17 | | 50.44±0.53 | 50.42±2.17 |
| **P-value** | P=0.876 | | | P=0.876 | |
| | TT[580] | TC[32] | CC[0] | TT[580] | TC/CC[32] |
| **LDL-C Adjusted mean± SD** | 139.62±1.62 | 129.13±6.55 | | 139.62±1.62 | 129.13±6.55 |
| **P-value** | P=0.118 | | | P=0.118 | |
| | TT[584] | TC[32] | CC[0] | TT[584] | TC/CC[32] |
| **Triglycerides Adjusted mean± SD** | 141.28±2.91 | 152.51±11.81 | | 141/28±2.91 | 152.51±11.81 |
| **P-value** | P=0.378 | | | P=0.378 | |
| | **rs3779787_Add** | | | **rs3779787_Dom** | |
| | GG[436] | GT[166] | TT[11] | GG[436] | GT/TT[177] |
| **Total Cholesterol Adjusted mean± SD** | 217.06±1.98 | 216.70±3.19 | 234±12.15 | 217.04±1.98 | 217.74±3.1 |
| **P-value** | P=0.582 | | | P=0.844 | |
| | GG[436] | GT[167] | TT[11] | GG[438] | GT/TT[178] |
| **HDL-C Adjusted mean± SD** | 49.73±0.60 | 52.06±0.97 | 48.94±3.69 | 49.74±0.6 | 51.88±0.94 |
| **P-value** | **P=0.033** | | | **P=0.018** | |
| | GG[433] | GT[165] | TT[11] | GG[433] | GT/TT[176] |
| **LDL-C Adjusted mean± SD** | 139.35 ±1.84 | 136.99±2.95 | 159.09±11.22 | 139.31±1.84 | 138.33±2.88 |
| **P-value** | P=0.830 | | | P=0.770 | |
| | GG[438] | GT[164] | TT[11] | GG[438] | GT/TT[175] |
| **Triglycerides Adjusted mean± SD** | 144.66±3.29 | 137.02±5.33 | 129.24±20.22 | 144.68±3.28 | 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; Reymer 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; Wallacee 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≥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 ≥0.05) observed in this study 32 showed significantly different distribution (P ≤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 ($P=1.4\times10^{-7}$) 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, *Hind*III (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 *Hind*III/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 *Hind*III/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 *Hind*III 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 *Hind*III site binds to a transcription factor and affects LPL expression and

thus, this is a functional variant by itself. Although we identified the *Hind*III 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 *Pvu*II (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 ($P=5.5\times10^{-18}$) (Aulchenko et al. 2009), rs10503669 ($P =4.1\times10^{-19}$) (Willer et al. 2008), rs331 ($P=9.1\times10^{-7}$) (Chasman et al. 2008), rs328 ($P =9\times10^{-23}$) (Kathiresan et al. 2008b), rs17482753 ($P =2.71\times10^{-5}$) (Heid et al. 2008), rs17411031 ($P =1.28\times10^{-10}$) (Wallacee et al. 2008), and rs326 ($P =1.4\times10^{-7}$) (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 ($5^{th}$ percentile) and high ($95^{th}$ 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 (HDLCQ11) 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 activty. –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 $5^{th}$ percentile of HDL-C in a NHW sample. We identified 179 variants, of which 88 had MAF≥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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