Lipoproteins and Hyperlipoproteinemia: Links to Gout

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Abstract

Gout and cardiovascular disease are both complex and multi-factorial with genetic predisposition and environmental triggers. Not only do both have high co-morbidity with other metabolic conditions, gout patients have high risk for cardiovascular events. Māori and Pacific populations have high risk for both gout and cardiovascular disease. The general aim of this study was to elucidate whether cardiovascular risk factors contribute to gout.

New Zealand Caucasian and Māori/Pacific gout case and control sample sets were used. The serum lipid profile was investigated, including serum lipoprotein(a) (Lp(a)), measured with an enzyme-linked immunosorbent assay. Size exclusion chromatography was used on Māori and Pacific samples for detailed lipoprotein, apolipoprotein (apo) and lipid analyses. Increased prevalence for type III hyperlipoproteinemia (TIIIH; increased remnant lipoproteins which may result from the apo E isoform apo E2) and type IV hyperlipoproteinemia (TIVH; increased triglyceride (TG) due to increased very low density lipoprotein (VLDL) TG) was investigated. Single nucleotide polymorphisms (SNPs) in \textit{APOE} determining isoforms \textit{APOE2} (rs7412) and \textit{APOE4} (rs429358) were genotyped. SNPs associated with an increased risk for cardiovascular disease were also genotyped, including \textit{rs3798220} and \textit{rs10455872} of \textit{LPA} which also associate with Lp(a) levels, and \textit{rs1333049} of \textit{CDKN2BAS}.

Gout cases had increased total TG levels ($p < 0.001$) compared to gout controls in both Caucasian and Māori/Pacific sample sets. Māori and Pacific cases also had higher total and low density lipoprotein cholesterol, and lower high density lipoprotein cholesterol, than controls ($p < 0.01$). Chromatography analysis revealed a higher prevalence for TIVH in cases than controls ($p < 0.001$, odds ratio [95% confidence interval] = 7.7 [2.3 - 25.9]), due to an increased concentration of TG from an increased number of VLDL particles. No differences were found between VLDL cholesterol in cases and controls, indicating TIIIH prevalence was not increased in gout. Supporting this, \textit{APOE} isoforms did not associate with gout. Lp(a) levels did not differ between Caucasian and Māori/Pacific subjects, nor by gout affliction. No associations were found between the two \textit{LPA} SNPs and gout, or between the \textit{CDKN2BAS} SNP and gout.

The current study implicates that hypertriglyceridemia, inherent of the metabolic syndrome, is a linking factor between gout and cardiovascular disease. Future research exploring the relationship between the two diseases should be directed in this area, including testing genetic variants associated with hypertriglyceridemia for association with gout.
Acknowledgements

This project would not have been possible without the help of a large number of people.

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http://asia.ensembl.org/index.html
List of Abbreviations

Apo     Apolipoprotein
Apo(a)  Apolipoprotein(a)
ARIC    Atherosclerosis Risk in Communities
CAD     Coronary artery disease
CDKN2BAS Cyclin dependant kinase inhibitor 2B anti-sense RNA 1
CI      Confidence interval
CM      Chylomicron
EDTA    Ethylenediaminetetraacetic acid
ELISA   Enzyme-linked immunosorbant assay
EP      Eastern Polynesian
FHS     Framingham Heart Study
FPLC    Fast protein liquid chromatography
GWAS    Genome-wide association study
HDL     High density lipoprotein
hrp     Horseradish peroxidase
HSPG    Heparin sulfate proteoglycan
LD      Linkage disequilibrium
LDL     Low density lipoprotein
Lp(a)   Lipoprotein(a)
LPL     Lipoprotein lipase
MI      Myocardial infarction
MW      Mann-Whitney
NZCMP   New Zealand Caucasian, Māori and Pacific
OR      Odds ratio
rcf     Relative centrifugal force
SCL     Southern Community Laboratories
SD      Standard deviation
SNP     Single nucleotide polymorphism
SST     Serum separator tube
TG      Triglyceride
TIIDM   Type II diabetes mellitus
TIIIH   Type III hyperlipoproteinemia
TIVH    Type IV hyperlipoproteinemia
UA      Uric acid
VLDL    Very low density lipoprotein
WP      Western Polynesian

Note: gene names are italicized.
1. Introduction

1.1. Lipoproteins

The blood contains many types of lipoproteins (Table 1) which are molecules that provide solubility to lipids in the bloodstream and aid in the utilization of these lipids by various tissues (Voet et al., 2008). Lipoproteins are made of a single phospholipid layer studded with cholesterol and proteins collectively called apolipoproteins (abbreviated as apo when naming apolipoprotein types, for example, apolipoprotein B = apo B). These encase triglycerides (TG) and cholesterol esters derived from the diet or endogenously synthesized (Voet et al., 2008). Different lipoproteins are distinguished primarily by density, determined by the composition of lipids and proteins in the particle (Voet et al., 2008).

Table 1. Classes and properties of lipoproteins.

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Diameter (Å)</th>
<th>Density (g/mL)</th>
<th>Core lipid composition (%)</th>
<th>Electrophoretic mobility position</th>
<th>Apolipoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td>800-5000</td>
<td>0.930</td>
<td>86  3</td>
<td>α-2</td>
<td>AI, B48, C, E</td>
</tr>
<tr>
<td>VLDL</td>
<td>300-800</td>
<td>0.950-1.006</td>
<td>55  12</td>
<td>Pre-β</td>
<td>B100, CI, CII, CIII, E</td>
</tr>
<tr>
<td>VLDL remnants</td>
<td>250-350</td>
<td>1.006-1.019</td>
<td>23  29</td>
<td>Slow pre-β</td>
<td>B100, E</td>
</tr>
<tr>
<td>LDL</td>
<td>216</td>
<td>1.019-1.063</td>
<td>6   42</td>
<td>β</td>
<td>B100</td>
</tr>
<tr>
<td>HDL</td>
<td>75-100</td>
<td>1.063-1.210</td>
<td>4   15</td>
<td>α-1</td>
<td>AI, AII, AIV, C, D, E</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>300</td>
<td>1.055-1.085</td>
<td>3   33</td>
<td>Slow pre-β</td>
<td>(a)*, B100</td>
</tr>
</tbody>
</table>

*highly homologous to plasminogen. TG = triglyceride. CE = cholesterol ester. CM = chylomicron. VLDL = very low density lipoprotein. LDL = low density lipoprotein. HDL = high density lipoprotein. Table adapted from Pownall and Gotto Jr (1999).

1.1.1. Apolipoprotein B-containing lipoproteins

Chylomicrons (CM) and very low density lipoproteins (VLDL) transport and deliver TG to peripheral tissues (Voet et al., 2008). They obtain their core lipids from the intestines (diet) and liver (biosynthesis), respectively (Voet et al., 2008; Figure 1). Only one apo B molecule is present per apo B-containing lipoprotein (Elovson et al., 1988; Phillips et al., 1997). VLDL
contains apo B100 (Pownall and Gotto Jr, 1999), the full length version of the apo B protein. CM contains apo B48, a truncated version of apo B100 with 48% of the amino acid length due to the presence of a stop codon for apo B proteins produced by the intestines (Chen et al., 1987). The enzyme lipoprotein lipase (LPL) on capillary endothelial cells, and cofactor apo CII (Pownall and Gotto Jr, 1999) hydrolys the TG in the core of CM and VLDL to yield free fatty acids for peripheral tissue use (Figure 1). The resultant lipoproteins are collectively known as remnant lipoproteins.

Remnant lipoproteins are cleared from the circulation relatively quickly via two mechanisms. Approximately half of remnants derived from VLDL are converted into low density lipoprotein (LDL) molecules (Mahley and Ji, 1999) via the action of hepatic lipase (Figure 1), which is synthesized and expressed in the liver (Perret et al., 2002). The presence of apo E increases hepatic lipase activity, allowing for more efficient lipoprotein metabolism via this enzyme (Medh et al., 2000). LDL retains the apo B100 from VLDL particles, which acts as the ligand for binding by the LDL receptor (Shireman et al., 1977). Remnant lipoproteins not converted to LDL are taken up by hepatocytes for further processing (Figure 1). Remnant lipoproteins first enter the space of Disse, that is, the space between hepatocytes and sinusoids (liver blood capillaries) where they interact with the LDL receptor, heparin sulfate proteoglycans (HSPG) and apo E particles found within the space of Disse (Ji et al., 1994a; Kita et al., 1982). HSPG and apo E may cooperate with the LDL receptor-like protein for the uptake of remnants into hepatocytes (Ji et al., 1994a; Kowal et al., 1989).

Figure 1. Metabolism of apolipoprotein B-containing lipoproteins. Chylomicrons (CM) derived from the diet and biosynthesized very low density lipoproteins (VLDL) are converted to remnant lipoproteins via the action of lipoprotein lipase (LPL) and apolipoprotein (apo) CII, which hydrolyse triglycerides in the lipoproteins to yield free fatty acids. Some remnant VLDL are converted to low density lipoproteins (LDL) by the action of hepatic lipase (HL) and apo E. The liver uptakes remaining remnant lipoproteins via interactions with the LDL receptor (LDLr), heparin sulfate proteoglycans (HSPG), apo E and/or the LDLr-like protein (LRP).
1.1.2. High density lipoprotein

High density lipoproteins (HDL) are a heterogeneous class of lipoproteins with a large range of densities, protein types and sizes (Table 1). The biosynthesis of HDL involves the integration of apo AI with phospholipids and cholesterol by the action of ubiquitously expressed ATP-binding cassette A1 (Tsompanidi et al., 2010). These HDL precursors are discoidal in shape, but eventually become circular when lipid content, namely TG and cholesterol esters, increases (Tsompanidi et al., 2010). Lipid content may increase by the action of lecithin cholesterol acyltransferase, which interacts with apo AI and esterifies cholesterol on the molecule (Glomset, 1968; Scott et al., 2001). Further metabolism involves the exchange of cholesterol esters from HDL with TG from apo B-containing lipoproteins by the action of cholesterol ester transfer protein (Tall, 1993). The last stage of metabolism involves the recognition of HDL on hepatocytes via scavenger receptor class B type I, and consequential uptake of HDL cholesterol into the liver (Burgess et al., 2006).

HDL possesses many mechanisms that decreases and reverses atherosclerotic progression, acquiring the nickname of “good cholesterol.” A major function of HDL involves the removal of cholesterol from macrophages at the atherosclerotic lesion (see section 1.2), a process named reverse cholesterol transport (Burgess et al., 2006). The apo AI on HDL interacts with ATP-binding cassette A1 on macrophages for the delivery of cholesterol from the lesion onto HDL molecules (Oram and Vaughan, 2000). Other well-documented anti-atherosclerotic effects of HDL include anti-oxidant, anti-inflammatory and anti-thrombogenic properties (Mineo et al., 2006; Tsompanidi et al., 2010).

1.1.3. Lipoprotein(a)

Apolipoprotein(a) (apo(a)) is a recently evolved protein highly homologous to plasminogen (McLean et al., 1987), a regulatory protease in the blood coagulation system (Ponting et al., 1992). In humans, apo(a) is found covalently bound to LDL, forming lipoprotein(a) (Lp(a); Figure 2). Lp(a) has been reported to have plasma levels that range over 1000 fold between individuals (Nordestgaard et al., 2010). However, the distribution of Lp(a) is hugely skewed towards lower levels in most populations (Figure 3). Lp(a) levels differ by ethnicity, especially for individuals of African descent. People of African descent do not show as pronounced skewing of Lp(a) levels when compared with other ethnicities, and have higher
median values (27 mg/dl to 39 mg/dl compared to 12 mg/dl or less in Caucasians, 19 mg/dl in Hispanics and 13 mg/dl or less in Asians; Matthews et al., 2005; Trommsdorff et al., 1995).

Apo(a) consists of ten types of kringle IV motifs, denoted as kringle IV type 1 up to kringle IV type 10 (Figure 2). The kringle IV type 2 of apo(a) has variable repeat numbers ranging from two to over 40 between individuals (Nordestgaard et al., 2010), giving rise to a protein size polymorphism which inversely correlates with Lp(a) levels (Amemiya et al., 1996; Boerwinkle et al., 1992; Utermann et al., 1987). Apo(a) further consists of one kringle V motif and one inactive plasminogen protease-like domain (Figure 2). Apo(a) is linked to the apo B moiety of LDL via a single disulfide bond between the Cys4326 in the C-terminal of apo B100 (Callow and Rubin, 1995; McCormick et al., 1995) and the Cys4057 in kringle IV type 9 of apo(a) (Brunner et al., 1993; Koschinsky et al., 1993). Lysine residues in the N-terminal of apo B promote this bond formation by non-covalent interactions with kringle IV types 6 to 8 of apo(a) (Becker et al., 2001; Gabel and Koschinsky, 1998). Other important residues also contribute to a favourable conformation for Lp(a) formation, such as a highly basic lysine-rich α-helical region in the C-terminal of apo B100 (Liu et al., 2004).

Apo(a) particles arise from the liver (Kraft et al., 1989), where it assembles in the extracellular space with the lipoprotein portion (White and Lanford, 1995) to form Lp(a). Cell culture studies have shown that VLDL receptors recognize apo(a) to control the internalization and degradation of Lp(a) (Argraves et al., 1997). Evidence also suggests that apo(a) is fragmented by proteases and excreted in the urine, whereas the remaining lipoprotein portion is recognized by receptors on hepatocytes, and cleared (Kostner et al., 1997).

Figure 2. The lipoprotein(a) molecule. The lipoprotein portion is nearly identical to low density lipoprotein, with similar compositions of core lipids (triglycerides and cholesterol esters), and one single apolipoprotein B100 (apoB) particle. ApoB is attached via a disulfide bond to apolipoprotein(a). Apolipoprotein(a) consists of ten types of kringle IV domains (4_1 - 4_10), one kringle V domain (5), and one inactive plasminogen protease-like domain (P). Figure taken from Koschinsky and Marcovina (2004).
1.1.4. Hyperlipoproteinemia

There are six major forms of hyperlipoproteinemia, all characterized by the abnormal accumulation of particular lipoproteins (Table 2). Paper electrophoresis of plasma samples was the original method used for distinguishing different types of hyperlipoproteinemia (Levy and Fredrickson, 1968). Four main bands were visible with paper electrophoresis, namely at the positions termed α (representing HDL), β (representing LDL), pre-β (representing VLDL) and the origin (representing CM). Briefly, type I and type V hyperlipoproteinemia are characterized by an increase of blood TG levels resulting from increased CM, and both CM and VLDL, respectively (Levy and Fredrickson, 1968). Type IIa hyperlipoproteinemia is characterized by increased LDL cholesterol, and if increased TG is additionally present, the phenotype becomes type IIb hyperlipoproteinemia (Levy and Fredrickson, 1968).

1.1.4.1. Type III hyperlipoproteinemia and apolipoprotein E

Type III hyperlipoproteinemia (TIIIH) is a late onset disorder that predisposes to cardiovascular diseases such as coronary artery disease (CAD), peripheral vascular disease, stenosis, ischemic heart disease and cerebrovascular disease (Brewer et al., 1983; Feussner et al., 1996; Feussner et al., 1993; Hakim et al., 2002; Morganroth et al., 1975). The main characteristic for all TIIIH cases is the increased level of remnant lipoproteins, characterized by a broad β-band (appears as a smear between the β and pre-β positions) on gel electrophoresis (Levy and Fredrickson, 1968). One common diagnostic measure for TIIIH is an increased plasma VLDL cholesterol to total TG ratio (Fredrickson et al., 1975). It has been suggested that
Table 2. Types and properties of hyperlipoproteinemia.

<table>
<thead>
<tr>
<th>Hyperlipoproteinemia</th>
<th>Increased lipoprotein</th>
<th>Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>CM</td>
<td>Decreased LPL, altered apolipoprotein CII</td>
</tr>
<tr>
<td>Type IIa</td>
<td>LDL</td>
<td>LDL receptor deficiency</td>
</tr>
<tr>
<td>Type IIb</td>
<td>VLDL and LDL</td>
<td>Decreased LDL receptor, increased apolipoprotein B</td>
</tr>
<tr>
<td>Type III</td>
<td>Remnant lipoproteins</td>
<td>Defective apolipoprotein E</td>
</tr>
<tr>
<td>Type IV</td>
<td>VLDL</td>
<td>Increased VLDL production, decreased VLDL elimination</td>
</tr>
<tr>
<td>Type V</td>
<td>CM and VLDL</td>
<td>Increased VLDL production, decreased LPL</td>
</tr>
</tbody>
</table>

NB: CM = chylomicron. LPL = lipoprotein lipase. LDL = low density lipoprotein. VLDL = very low density lipoprotein.

A ratio of greater than 0.30 is indicative of TIIIH (Fredrickson et al., 1975). Physical characteristics include xanthoma (lipid deposits under the surface of the skin) development, namely tuberoeruptive xanthomatous lesions on elbows and knees, and xanthomas on palm and finger creases (Brewer et al., 1983; Burnside et al., 2005; Feussner et al., 1993; Feussner et al., 1996; Hakim et al., 2002; Morganroth et al., 1975).

Apo E is found in many lipoprotein classes (Table 1). Two specific domains are present: the N-terminal domain (amino acids one to 191) joined by a relatively unstructured sequence to the C-terminal domain (amino acids 216 to 299; Wetterau et al., 1988). Most cases of TIIIH are influenced by the single nucleotide polymorphism (SNP) rs7412 in the apo E gene (APOE), which results in the amino acid change Arg158Cys (Mahley and Rall Jr, 2000). The resulting gene isoform APOE2 (encoding protein isoform apo E2) has an allelic frequency of up to 13% in several ethnic groups, including Caucasians, Asians and American Indians (Davignon et al., 1988).

The large majority of TIIIH patients are homozygous for the APOE2 polymorphism (Mahley and Rall Jr, 2000). However, this is only a predisposition, emphasized by the statistic that greater than 90% of APOE2 homozygote individuals do not have hyperlipoproteinemia (Mahley and Rall Jr, 2000). Additional factors and other genetic causes contribute to the affect of TIIIH. Hormonal influences, such as increased estrogen (Falko et al., 1979; Kushwaha et al., 1977) and hypothyroidism (Brewer et al., 1983) diminish and aggravate TIIIH characteristics, respectively. Several other infrequent genetic defects in APOE may also produce phenotypes
which induce the onset of TIIIH, including Arg136Ser (Wardell et al., 1987), Lys146Gln (Smit et al., 1990), Gly127Asp and a frameshift G deletion at position 2919 (Feussner et al., 1992).

Apo E2 has only 1% of the binding activity to the LDL receptor compared with the normal apo E (Innerarity et al., 1984). However, residue Arg158 is not directly involved in the binding to the LDL receptor. Rather, it allows the protein to adopt a suitable conformation for binding (Innerarity et al., 1984). The apo E amino acids that are directly involved with binding lie in positions 140 to 150 (Innerarity et al., 1984), a series of mostly basic residues (Rall et al., 1982). The apo E2 phenotype exhibits approximately 60% of binding activity to HSPG compared to the normal apo E, showing a decreased, but not an absence of activity (Ji et al., 1994b). This likely explains the requirement of a recessive genotype and secondary factors to induce TIIIH characteristics. Interestingly, rare dominant apo E mutants were shown to have even further diminished, if not abolished, HSPG binding activity (Ji et al., 1994b), corresponding to their dominant effect for the development of TIIIH.

Another common APOE polymorphism is the SNP rs429358 which gives rise to the APOE4 gene isoform. This results in the amino acid change Cys112Arg and resulting protein named apo E4. The frequency of this polymorphism is slightly higher than APOE2, that is, up to 23% (Davignon et al., 1988). APOE4 is associated with higher LDL cholesterol levels (Sing and Davignon, 1985). The ancestral and most common protein isoform is apo E3 (gene isoform APOE3), where amino acids stay as Arg158 and Cys112.

1.1.4.2. Type IV hyperlipoproteinemia and very low density lipoprotein

Type IV hyperlipoproteinemia (TIVH) is characterized by an increased total TG level resulting from increased TG in VLDL (Levy and Fredrickson, 1968). Disorders associated with TIVH include femoral atherosclerosis (Ruhn et al., 1989) and increased liver steatosis (increased fat retention in the liver; Gariot et al., 1982). TIVH cases with CAD have been associated with an increased number and severity of coronary artery narrowing when compared to normolipidemic CAD cases, controls without CAD, and type II hyperlipoproteinemia cases (Cabin and Roberts, 1981). The peroxisomes in the hepatocytes of TIVH patients are increased in number compared to control liver samples (Gariot et al., 1986). TIVH has also been associated with increased insulin levels after a meal (Brunzell, 1981) or during a glucose tolerance test (Bassett et al., 1990).
TIVH can be influenced by lifestyle. Ethanol infusion was found to increase the total TG level in all lipoprotein classes, and VLDL was further found to increase in cholesterol and phospholipid content (Avogaro and Cazzolato, 1975). A high fructose diet induced an increased VLDL TG level as well as increased lipid deposition in hepatocytes (Sobrecases et al., 2010). Supplementations such as soya lecithin and omega-3 fish oils, and physical exercise, can decrease TG levels in TIVH cases (Brook et al., 1986; Lampman et al., 1980; Sanders et al. 1985; Singer et al., 1985).

Drugs can be used to treat TIVH. Fibrates such as gemfibrozil, bezafibrate and clofibrate decreases total TG, total apo B, VLDL TG and VLDL cholesterol levels (Bermúdez-Pirela et al., 2007; Bhatnagar et al., 1992; Bradford et al., 1992; Eisenberg et al., 1984; Olsson et al., 1985; Schwandt et al., 1982; Sirtori et al., 1992). Niacin derived drugs have also been shown to decrease total and VLDL TG levels (Tornvall and Walldius, 1991).

The increased VLDL in TIVH cases may result from the overproduction of VLDL particles (Packard et al., 1980). VLDL apo B in TIVH patients was found to be synthesized twice as fast, and have a longer half life due to decreased catabolism and the decreased rate and amount converted to LDL when compared to normolipidemic controls (Packard et al., 1980). The catabolism of TIVH VLDL may be due to defects in the enzymes processing it rather than the VLDL itself, as an in vitro study found that VLDL obtained from TIVH cases had the same rate of catabolism by the same amount of LPL compared to VLDL obtained from normolipidemic subjects (Taskinen et al., 1981).

VLDL synthesis consists of the integration of lipids with apo B in hepatocytes (Olofsson et al., 2000). The microsomal TG transfer protein is a multi-subunit complex including protein disulfide isomerise as a subunit (Wetterau et al., 1990). The microsomal TG transfer protein is involved with the early lipidation step of apo B100 on the rough endoplasmic reticulum (Mitchell et al., 1998). This forms a primordial VLDL particle on the endoplasmic reticulum membrane with a density similar to LDL or HDL, which can be further lipidated without the requirement of microsomal TG transfer protein (Gordon 1997). If apo B100 is insufficiently lipidated, it is rapidly degraded so that the protein does not accumulate (Liao et al., 2003). Sufficiently lipidated VLDL may be secreted from hepatocytes. Sar1 is a protein required for apo B100 translocation to the golgi apparatus from the endoplasmic reticulum via mechanisms involving the coat protein complex II (Gusarova et al., 2003). Also, coat protein complex I is
involved with the energy dependant anterograde transport of VLDL, with associated proteins ADP-ribosylation factor 1 and phospholipase D (Asp et al., 2000; Asp et al., 2005).

1.2. Atherosclerosis

Atherosclerosis is a large contributor to many types of cardiovascular events. The first stage of atherosclerosis occurs when LDL passes through arterial endothelial cells and becomes lodged in the intima of blood vessels (Glass and Witztum, 2001). The LDL can become oxidized, resulting in covalent changes on the apo B protein and the oxidation of lipids on the lipoprotein (Steinberg, 1997). This induces an inflammatory response, where monocytes are attracted to the location by the expression of monocyte chemo-attractant protein-1, and adhered by proteins such as vascular cell adhesion protein-1 (Ross, 1999). Once inside the intima, the monocytes differentiate into scavenger receptor-expressing macrophages that recognize oxidized LDL (Boullier et al., 2001). This leads to the engulfment of oxidized LDL by the macrophages, forming lipid-rich foam cells (the fatty streak), a reversible process (Glass and Witztum, 2001).

The atherosclerotic plaque progresses with the migration and proliferation of smooth myocytes, and the formation of proteoglycans, collagen and fibrin over the top of the fatty streak to form a fibrous capsule (Glass and Witztum, 2001). The internal foam cells undergo apoptosis, resulting in a lipid-rich necrotic core (Hegyi et al., 1996). The plaque may rupture, which may be caused from a combination of factors, such as the size of the lipid core, the strength of the fibrous capsule, and the long term mechanical forces imposed on the plaque (Zaman et al., 2000). Once ruptured, thrombosis will occur, occluding most of the artery to promote myocardial infarction (MI; Zaman et al., 200), commonly known as a heart attack.

1.2.1. Cardiovascular disease and genetics

Cardiovascular diseases are linked to both environmental and genetic factors. Large scale genome-wide association studies (GWAS) have revealed many genetic loci associated with CAD or MI. The Wellcome Trust Case Control Consortium (Burton et al., 2007) found SNPs such as rs383830, rs6922269, rs8055236, rs7250581 and rs688034 to moderately increase risk for CAD (genotypic p values ranged between $2.50 \times 10^{-5}$ and $3.75 \times 10^{-6}$ and homozygote odds ratio (OR) [95% confidence interval (CI)] ranged between 1.40 [1.05 - 1.86] to 2.23 [1.56 - 3.17]). All these SNPs were intergenic, except rs6922269, which was found in MTHFD1L (the gene for
methylenetetrahydrofolate dehydrogenase NADP$^+$-dependant 1-like protein). Samani et al. (2007) also found the SNP in \textit{MTHFD1L} associated with CAD, as well as other SNPs in \textit{PSRC1} (proline-serine-rich coiled-coil 1), \textit{MIA3} (melanoma inhibitory activity family member 3) and \textit{SMAD3} (SMAD family member 3) to associate with CAD and/or MI in the Wellcome Trust Case Control Consortium cohort and the German MI family study.

\subsection*{1.2.1.1. The 9p21 risk locus}

In both the GWAS conducted in the Wellcome Trust Case Control Consortium cohort and the German MI family study (Burton \textit{et al.}, 2007; Samani \textit{et al.}, 2007), the SNP rs1333049 was found to hold the most significant risk for CAD ($p = 1.16 \times 10^{-13}$, OR [95\% CI] = 1.90 [1.61 - 2.24] and $p = 6.12 \times 10^{-5}$, OR [95\% CI] = 1.28 [1.07 - 1.53], respectively). In fact, when 14 Caucasian GWAS cohorts were combined and meta-analyzed by the recently formed Coronary ARtery DIsease Genome-Wide Replication And Meta-Analysis consortium, rs1333049 was significantly associated with CAD ($p = 2.06 \times 10^{-20}$, OR [95\% CI] = 1.29 [1.22 - 1.36]), indicating true association at 100\% power (Preuss \textit{et al.}, 2010). Interestingly, this SNP was also found to associate with an increased gout risk in a Han Chinese population ($p = 0.01$, OR [95\% CI] = 1.26 [1.06 - 1.54]; Wang \textit{et al.}, 2011), indicating a possible link between cardiovascular disease and gout.

The rs1333049 SNP was found in high linkage disequilibrium (LD) with surrounding SNPs in a 58 kb region on chromosome 9p21 (McPherson \textit{et al.}, 2007). This region has been found to associate with various types of cardiovascular diseases in several populations, including individuals from Italy, Denmark, Canada, the United States of America, Iceland, China and South Korea (Helgadottir \textit{et al.}, 2007; McPherson \textit{et al.}, 2007; Paynter \textit{et al.}, 2009; Shen \textit{et al.}, 2008a; Shen \textit{et al.}, 2008b; Zhang \textit{et al.}, 2009). A part of this 58 kb region overlaps the gene \textit{CDKN2BAS} (Figure 4), which encodes cyclin dependant kinase inhibitor 2B anti-sense RNA 1 (CDKN2BAS). CDKN2BAS is a non-coding RNA prone to alternative splicing (Folkersen \textit{et al.}, 2009). Depending on its alternatively spliced transcripts, CDKN2BAS influences minimal promoter activity in transfection and luciferase assays (Jarinova \textit{et al.}, 2009). Interestingly, the expression of particular CDKN2BAS splice transcripts was influenced by the presence of risk alleles for CAD in the 58 kb region (Jarinova \textit{et al.}, 2009). The expression of CDKN2BAS \textit{per se} was also affected by particular variants in the risk locus (Harismendy \textit{et al.}, 2011). This
suggests that CDKN2BAS genetically affects CAD risk due to its ability to activate the promoters of nearby genes.

![Figure 4. Details of the genomic 9p21 locus. Single nucleotide polymorphisms in the 58 kb risk region for cardiovascular disease (red) form a high linkage disequilibrium block. CDKN2BAS (green) overlaps the risk region and two nearby genes. Nearby genes (blue) are MTAP, CDKN2A, CDKN2B and DMRTA1. Arrows indicate transcription direction. Cropped figure taken from Visel et al. (2010).](image)

Two genes belonging to the cyclin dependant kinase inhibitor family of proteins, namely CDKN2A and CDKN2B, are in a 35 kb region which overlaps the CDKN2BAS sequence (Figure 4). The protein products of CDKN2A and CDKN2B, namely p16INK4a and p15INK4b, respectively, bind to cyclin dependant kinase 4 and 6 to inhibit downstream effects of the G1 cell cycle phase that determines cell fate (Kim and Sharpless, 2006; Rosu-Myles and Wolff, 2008). Deregulation of this pathway may lead to various types of cancers, neoplasias and tumours (Kim and Sharpless, 2006; Rosu-Myles and Wolff, 2008). There is evidence that the 9p21 CAD risk region has influence on CDKN2A and CDKN2B expression. In the 9p21 risk region knockout mice (n = 6), CDKN2A and CDKN2B RNA expression levels were decreased compared to wild type mice (n = 6), and cells derived from knockout mice showed increased proliferation and the absence of senescence when compared with wild type mice (Visel et al., 2010). Furthermore, nine enhancer elements were found in the CAD risk locus in 9p21, and one of them was shown to interact with loci including CDKN2A and CDKN2B, as well as MTAP (see below for description; Harismendy et al., 2011).

Other genes close to the 58 kb region are MTAP (methylthioadenosine phosphorylase) and DMRTA1 (doublesex and mab3 related transcription factor-like family A1; Figure 4). The MTAP protein product catalyses the reaction where methylthioadenosine is cleaved into methylthioribose-L-phosphate and adenine, and is found to be fully or partially deleted in 22
malignant tumour cell lines (Nobori et al., 1996). The MTAP SNP rs7027989 has also been found to associate with MI in a male, but not female or combined sex, Han Chinese population (317 MI cases and 300 controls; Yang et al., 2009). The DMRTA1 protein product is expressed in the liver, kidneys, pancreas, prostate, and weakly in the ovaries (Ottolenghi et al., 2002). DMRTA1 has sequence similarities to the gene doublesex in Drosophila melanogaster, a gene determining sex differentiation during development (Burtis and Baker, 1989).

How the proteins of DMRTA1, MTAP, CDKN2A or CDKN2B affects cardiovascular risk is unknown. The 9p21 CAD risk region does not associate with plasma levels of cardiovascular disease risk factors; bilirubin, albumin, homocysteine, fibrinogen, uric acid (UA), LDL, HDL, Lp(a), apo AI and apo B100 (Broadbent et al., 2008; Paynter et al., 2009). The lack of association between the 58 kb risk locus with these factors indicate that its influence on cardiovascular disease is via mechanisms exclusive to these factors. It has been speculated that the cancer promoting properties of CDKN2A/p16INK4a and CDKN2B/p15INK4b may be involved with atherosclerotic cell proliferation, although evidence is still required for this hypothesis.

1.2.1.2. The apolipoprotein(a) gene (LPA) and cardiovascular risk

Lp(a) levels have been found to influence risk for many types of cardiovascular diseases, such as coronary, carotid, cerebral and peripheral vascular disorders, ischemic stroke, and abdominal aortic aneurysm (Cressman et al., 1992; Jones et al., 2007; Klein et al., 2008). This may in part be explained by the many types of genetic polymorphisms that exist within the gene encoding apo(a).

Apo(a) is encoded by the gene LPA, located at 6q26, and is estimated to contribute to up to 91% of variation in plasma Lp(a) levels (Boerwinkle et al., 1992). The variable size apo(a) protein isoforms resulting from the variable number of kringle IV type 2 repeats (Nordestgaard et al., 2010) are determined by a length polymorphism in the kringle IV repeat region of LPA (Lackner et al., 1993). This genetic polymorphism significantly associates with MI, and coronary artery and heart complications (Amemiya et al., 1996; Ducas et al., 2002; Kamstrup et al., 2009).

Other genotypic variations in LPA include a pentanucleotide repeat polymorphism (TTTTA)n in the promoter region. This polymorphism has been found to inversely correlate with Lp(a) levels and is estimated to explain 10% to 14% of variation in Lp(a) levels in Caucasian populations (Beneš et al., 2002; Kamstrup et al., 2009; Trommsdorff et al., 1995). It also
inversely correlates with MI and ischemic heart disease, independently of the kringle IV type 2 repeat region (Kamstrup et al., 2009), and has influence on the severity of the atherosclerotic disease phenotype (Amemiya et al., 1996).

Many SNPs have been found to contribute an estimated 30% to 40% of Lp(a) variance in Caucasian individuals (Clarke et al., 2009; Ronald et al., 2011). Of particular note, the two SNPs rs3798220 (Ile4399Met) and rs10455872 (intronic) show the strongest influence on Lp(a) levels (22% to 36% combined; Clarke et al., 2009; Ronald et al., 2011). Many studies have supported the association of these two SNPs with coronary and carotid complications (Clarke et al., 2009; Luke et al., 2007; Ronald et al., 2011; Shiffman et al., 2010).

1.3. Gout

Arthritic diseases affect bones and joints, and can result in the degeneration and/or the inflammation of the affected area. Gout results from the build up of monosodium urate crystals typically in the elbows, knees and phalange joints (Resnick, 1995). Other sites include the shoulder, sacroiliac joint, spine, mandibular condyles, vocal cords, chest wall, sternoclavicular joint and pelvic bones (Resnick, 1995). This results in an inflammatory response, and if left untreated, can cause visible tophus formation (Neogi, 2011). Thus, hyperuricemia (classified as serum UA levels greater than 0.42 mmol/L for men and 0.36 mmol/L for women) is associated with increased gout incidence (Campion et al., 1987).

There is genetic predisposition towards gout (see section 1.3.1), although environmental factors are often required to trigger the disorder. Diet is a significant influence. For example, the increased consumption of fructose in the form of sugary drinks, non-vegetable high purine foods, and beer increase the risk for gout or higher serum UA levels (Choi and Curhan, 2004; Choi et al., 2004; Choi et al., 2005; Choi et al., 2008; Choi et al., 2010), whereas dairy products and coffee lower the risk (Choi and Curhan, 2007; Choi et al., 2004; Choi et al., 2005).

The prevalence of gout is three to four fold higher in men than women (Neogi, 2011; Winnard et al., 2012), but gout incidence increases with increasing age for both sexes (Weaver, 2008). Similarly, UA levels are higher in men than women (for example, 0.29 mmol/L in men and 0.25 mmol/L in women in a North American population; Mikkelsen et al., 1965). The difference in serum UA levels between men and women can be attributed to the decreased urate reabsorption in renal tubules in women when compared to men (Antón et al., 1986).
Interestingly, due to the uricosuric effect of estrogen (Yahyaoui et al., 2008), post-menopausal women (n = 3047 naturally menopausal and 459 surgically menopausal) had higher serum UA levels (approximately 0.05 mmol/L) than pre-menopausal women (n = 3047, \( p < 0.001 \); Hak and Choi, 2008). Thus, the mean age of gout cases was significantly higher in women (70 years) than men (58 years, \( p < 0.001 \)), as found in a North American sample set (n = 4975 men and 1158 women; Harrold et al., 2006). From this, gout incidence equalizes between men and women in older populations (Neogi, 2011; Weaver, 2008).

The organic compound UA (168.11 g/mol) is an end product of purine metabolism in humans due to the absence of the enzyme uricase (Richette and Bardin, 2010). Humans are therefore more prone to developing hyperuricemia (Richette and Bardin, 2010). UA is a powerful scavenger of reactive oxygen species (Ames et al., 1981) and displays anti-oxidant effects in vivo (Glantzounis et al., 2005). UA is white, colourless, tasteless, has low solubility (1 g in 15000 parts of cold water; Budavari, 1996) and exists mostly as urate (salt derivatives) in the blood (Richette and Bardin, 2010). UA levels can be lowered by a variety of medications, including allopurinol (an inhibitor of xanthine oxidase), probenecid and benzbromarone (inhibitors of urate reabsorption; Neogi, 2011).

1.3.1. Uric acid transporters and genetics

UA accumulation in blood can arise from the unbalanced excretion and reabsorption of the compound in the kidneys. UA is freely filtered out of the blood via the glomerulus of the nephron (Wright et al., 2010). However, transporter proteins (Figure 5) found in the convoluted proximal tubule of the nephron allows for the reabsorption of UA back into the blood system (Wright et al., 2010). Dysfunction in these proteins contributes to abnormal UA exchange at the nephron, and may lead to gout development.

Figure 5. Examples of urate transporters in the proximal tubule of the nephron. Arrows indicate urate transport direction. GLUT9-1 = isoform 1 of GLUT9. GLUT9-2 = isoform 2 of GLUT9. ABCG2 = ATP-binding cassette G2.
URAT1, encoded by *SLC22A12*, is a member of the organic anion transporter family, a protein family that is involved with the filtering of endogenous compounds and xenobiotics (Anzai *et al.*, 2006). UA transport via URAT1 is shown in Figure 5. URAT1 is dependent on the exchange of anions such as chloride ions for the reabsorption of urate across the apical membrane of proximal tubule cells (Enomoto *et al.*, 2002). URAT1 mutant proteins with lowered UA transport activity have been found in Japanese hypouricemic subjects, and not in controls (Enomoto *et al.*, 2002).

ATP-binding cassette G2 is located at the apical membrane of human renal cells (Huls *et al.*, 2007), and with the requirement of ATP, has been shown to transport high concentrations of UA in human embryonic kidney 293 cells (Matsuo *et al.*, 2009) and *Xenopus laevis* oocytes (Woodward *et al.*, 2009). The SNP rs2231142 of the protein’s gene *ABCG2* results in the amino acid change Gln141Lys in the resultant protein, which showed reduced urate transport activity compared to wild type proteins in *Xenopus laevis* oocytes (Woodward *et al.*, 2009). Along with other SNPs in the gene, this SNP associated with higher serum UA levels in Caucasian, African descendant and Chinese populations (Brandstätter *et al.*, 2010; Dehghan *et al.*, 2008; Kolz *et al.*, 2009; Wang *et al.*, 2010; Woodward *et al.*, 2009). Furthermore, a significant susceptibility association of the minor allele of rs2231142 towards gout in Western Polynesian (WP), Caucasian, Japanese and African descendant populations has been found (Dehghan *et al.*, 2008, Matsuo *et al.*, 2009, Phipps-Green *et al.*, 2010). Thus, ATP-binding cassette G2 acts to secrete urate from proximal tubule cells into the lumen (Figure 5) for urinary excretion.

Transporter GLUT9, encoded by the gene *SLC2A9*, is a class II glucose and fructose transporter which has been identified to transport UA across the proximal tubule from the tubule lumen into the blood (Figure 5) at higher reaction rates than glucose or fructose transport (Anzai *et al.*, 2008; Vitart *et al.*, 2008). GLUT9 exists as two isoforms. Isoform 1 is located at the basolateral membrane, and isoform 2 (shorter by 28 amino acids and differing by the N-terminus domain) is located at the apical membrane (Augustin *et al.*, 2004). GLUT9 has been localized in articular chondrocytes (Mobasheri *et al.*, 2002), which may contribute to urate crystal deposition at these sites, although evidence is still required for its transport activity here. Nevertheless, many studies have shown *SLC2A9* as the strongest associative locus with serum UA levels. Of particular note are the two SNPs rs734553 and rs16890979 that display high LD with each other ($r^2 = 0.88$; Kolz *et al.*, 2009). These SNPs show strong association with lower serum UA levels.
in Caucasian and African descendant populations (Dehghan et al., 2008; Kolz et al., 2009). The minor allele of the SNP rs16890979 was associated with lowered gout risk for Caucasian, Māori and Pacific Island participants, although it was not associative in Japanese or African descendants (Dehghan et al., 2008; Hollis-Moffatt et al., 2009; Kolz et al., 2009; Urano et al., 2010). Many other SNPs within SLC2A9 were associative with serum UA levels and gout, including rs6449213, rs7442295, rs6855911, rs1014290, rs12510549, and rs737267 (Brandstätter et al., 2010; Döring et al., 2008; Urano et al., 2010; Vitart et al., 2008).

One particular study (Stark et al., 2009) focused on the SNPs rs734553 of SLC2A9 and rs2231142 of ABCG2 in gout as well as CAD and MI case and control sample sets. Results for these SNPs showed significant associations with gout (Stark et al., 2009), supportive of the studies mentioned above (Dehghan et al., 2008; Hollis-Moffatt et al., 2009; Kolz et al., 2009). However, no associations of the SNPs with CAD were found (Stark et al., 2009).

### 1.4. Common factors between gout and cardiovascular disease

Atherosclerosis has many risk factors, including, and overlapping with characteristics of other disorders such as type II diabetes mellitus (TIIDM) and metabolic syndrome. These include poor lipid profile, obesity, hypertension, sedentary lifestyle, increased age, the male gender, and a diet high in calories, fat and salt (Kannel and Sytkowski, 1987). Individuals with metabolic syndrome are nearly two times more likely to develop cardiovascular disease, and approximately five times more likely to develop TIIDM (Grundy, 2008). Insulin resistance is causal of TIIDM, is an inherent factor of metabolic syndrome, is associated with obesity (Goldstein, 2002), and increases the risk profile (such as increased dyslipidemia) of these disorders (Avramoglu et al., 2006). Thus, insulin resistance contributes to an increased risk of cardiovascular disease.

Gout itself has been shown to associate with cardiovascular events. Kuo et al. (2010) showed that gout cases (n = 1311) were more likely to die of general and cardiovascular mortality than normouricemic people (n = 12195) after adjustment for age, sex, metabolic syndrome and proteinuria. Hernández-Cuevas et al. (2009) showed that 90% of gout cases (n = 407) later in life went on to develop metabolic syndrome characteristics, metabolic syndrome itself, or other complications including ischemic heart disease and TIIDM. Novak et al. (2007) showed that gout cases (n = 1171) were more likely to consult medical services in most categories of health care, including services related to hyperlipidemia, hypertension and TIIDM,
compared with matched controls (n = 58550). Furthermore, the prevalence of these three factors along with several cardiovascular disorders were approximately two to three fold increased in gout cases compared to controls (Novak et al., 2007).

Serum UA levels has also been associated with many cardiovascular risk factors, such as age, blood pressure, hypertensive treatment, body mass index, waist circumference, a history of TIIDM, TG levels, tumour necrosis factor-α levels, γ-glutamyltransferase levels and cigarette smoking (Conen et al., 2004; Schulz et al., 2004; Strasak et al., 2008). UA levels positively correlates with insulin resistance and response to glucose (Facchini et al., 1991), and UA excretion in the tubules of the nephron is decreased by insulin infusion (Quinones Galvan et al., 1995). This implies that insulin sensitivity and levels could influence the development of gout via effects on UA levels and excretion.

UA has been directly linked to cardiovascular risks and outcomes. Baker et al. (2005) compared 21 studies and found that serum UA levels independently contributed to higher risk for cardiovascular disease in high risk populations (such as hypertensive and diabetic patients), although this association was less consistent in apparently healthy individuals. Furthermore, there is evidence that UA is directly involved with cardiovascular function. Lowering UA levels with allopurinol improved peripheral vasodilator capacity and blood flow in hyperuricemic subjects (Doehner et al., 2002). In patients with idiopathic dilated cardiomyopathy, lowering UA levels with allopurinol improved myocardial efficiency by decreasing oxygen consumption without decreased stroke work (blood consumption) and ventricular pressure (Cappola et al., 2001).

UA may contribute to the onset of cardiovascular disease via several mechanisms. Cell culture methods indicate that UA increases C-reactive protein expression and release from cells (Kang et al., 2005). C-reactive protein is an independent predictor of many forms of cardiovascular diseases (Ridker, 2003). UA also increase cell proliferation and migration (Kang et al., 2005), possibly linked with the proliferation and migration of smooth myocytes in the development of atherosclerosis. Additionally, despite displaying anti-oxidant properties (Ames et al., 1981; Glantzounis et al., 2005), UA is capable of becoming an oxidant and can contribute to the oxidation of lipids, proteins and lipoproteins (Bagnati et al., 1999; Sautin et al., 2007). Furthermore, UA can induce the expression of monocyte chemo-attractant protein-1 in rat
vascular smooth muscle cells with mechanisms including oxidation and reduction reactions (Kanellis et al., 2003).

1.5. Māori and Pacific health

Like indigenous populations from the United States of America (American Indians and Alaskan Natives), Canada (First Nations) and Australia (Aboriginal Australians), the Māori population of New Zealand has a lower life expectancy than the respective non-indigenous population (differences in life expectancy were approximately six years for the United States of America and Canada, eight years for New Zealand and 20 years for Australia; Bramley et al., 2004). This data is not surprising, as several population studies have found an increased prevalence of several disease states (including cardiovascular disease and gout) in Māori and Pacific people when compared to Caucasians.

Cardiovascular diseases are associated with the Māori and Pacific population. In the Auckland Coronary or Stroke study, individuals with coronary events were more likely to die from the event if they were Māori (n = 536, 68% death) or Pacific (n = 342, 64% death) compared to Europeans (n = 5845, 44% death; Bullen and Beaglehole, 1997). Bramley et al. (2004) showed that Māori had risk ratios for death by ischemic heart disease and diabetes of 1.9 and 5.7, respectively, when referenced against the non-indigenous New Zealand population. Pacific people in New Zealand were approximately six and three times more likely to be discharged from hospitals for cardiomyopathy and heart failure, respectively, when referenced against the census population (Sopoaga et al., 2010). In patients with TIIDM, the rate for a first cardiovascular event between the years 2000 and 2005 was investigated in Māori subjects (n = 7802), Pacific subjects (n = 8559), European subjects (n = 23708), and subjects of other ethnicities (n = 3352). The Māori sample set had a higher rate for the onset of a first cardiovascular event when referenced against European and other ethnicities (hazard ratio [95% CI] = 1.3 [1.19 - 1.41]; Kenealy et al., 2008). Interestingly, the Pacific population did not have an increased chance for having a first cardiovascular event when referenced against the same group (hazard ratio [95% CI] = 1.04 [0.95 - 1.13]; Kenealy et al., 2008).

There is high prevalence of gout in many Polynesian populations, such as the New Zealand Māori (6.1%) and Pacific (7.6%) population (Winnard et al., 2012) and the Taiwanese aboriginal population (11.7%; Chou and Lai, 1998). Pacific people in New Zealand were
approximately six times more likely to be discharged from hospitals with a diagnosis of gout when compared with the census population (Sopoaga et al., 2010). Gout prevalence in Caucasian populations are much lower. A Scottish population (n = 35251) showed a gout prevalence of 0.34% (Steven, 1992). Populations in Greece (n = 8740), England and Wales (from the timeframe of the years 2001 to 2007) showed gout prevalence of approximately 0.5% (Andrianakos et al., 2003; Elliot et al., 2009). In the United Kingdom (n = 2.5 million) and Germany (n = 2.4 million), gout was found to be prevalent at 1.4% in both populations (Annemans et al., 2008). The New Zealand Caucasian population compared to European populations was found to have a slightly higher prevalence at 3.2% (Winnard et al., 2012). Possible reasons for this higher prevalence may be due to differences in lifestyle factors (such as diet), or an increased ethnic admixture with Māori and Pacific people. Prevalence in other populations have been reported at 3% in the Togolese Republic (n = 3517; Mijiyawa, 1995), 2.0% for Asians in New Zealand (Winnard et al., 2012) and none in Saudi Arabians (n = 487; Al-Arfaj, 2001).

Several other related disease states and risk traits greatly affect Māori and Pacific people. The mean age for the onset of T2DM for Māori and Pacific people (n = 712, 56.8 years) has been found to be lower than that for New Zealand Europeans (n = 10841, 66.7 years, p < 0.001; Tomlin et al., 2006). In a study focused on Māori participants on the East Coast of the North Island (n = 249), a large proportion were found to be overweight (24.3%) or obese (67.2%; Tipene-Leach et al., 2004). The age-standardized prevalence for diabetes in this population was 11%, and for insulin resistance was up to 40% (Tipene-Leach et al., 2004). The individuals with insulin resistance (n = 91) were found to have a higher percentage of history for gout (20.9%) than people without insulin resistance (n = 112, 7.1%, OR [95% CI] = 4.2 [1.8 - 10.6]; Tipene-Leach et al., 2004). In an Auckland population with four groups of Pacific people (484 Samoan, 255 Tongan, 109 Niuean and 116 Cook Islanders), all Pacific groups had significantly increased body mass index than the sex-matched European group (n = 1745, p < 0.0001), and all Pacific groups except Cook Island males had increased waist circumference when compared with the sex-matched European group (p < 0.01; Sundborn et al., 2010). Additionally, all Pacific Island females had increased waist to hip ratios than European females (p < 0.0001; Sundborn et al., 2010). In rural Hawaii, Hawaiian Polynesians (n = 536) had the highest chance to have metabolic syndrome when referenced against Caucasians (n = 303, OR [95% CI] = 4.2 [2.4 - 7.3];
Grandinetti *et al.*, 2005). Hawaiian Polynesians also had the worst profiles for abdominal obesity and lowered HDL cholesterol levels when compared to Caucasian, Filipino (n = 197), Japanese (n = 211) and other mixed (n = 256) ethnicities (Grandinetti *et al.*, 2005). In a New Zealand population, Māori (n = 1006) and Pacific people (n = 996) had lower HDL cholesterol levels than Caucasians and Asians (n = 2021, Gentles *et al.*, 2007). Measures of HDL (apo AI) were found to be lower in Pacific Island societies compared with Western societies (Kottke *et al.*, 1990; Nestle and Zimmet, 1981). Interestingly, Samoans were found to have lower Lp(a) levels than several other ethnicities (Kamboh *et al.*, 2000). No known study has investigated Lp(a) levels in the Māori population.

Lipid levels in Pacific groups are influenced by changes in lifestyle and diet. For example, in the Tokelau migrant study conducted in the 1970s, Tokelauan migrants to New Zealand (n = 1158) had increased total energy intake resulting from higher alcohol, protein, carbohydrate and cholesterol consumption when compared to Tokelauan non-migrants who remained in Tokelau (n = 765; Stanhope *et al.*, 1981). Interestingly, Tokelauan migrant men were found to have increased serum total TG, total cholesterol, LDL cholesterol and lowered HDL cholesterol compared to non-migrant men (differences were 0.39 mmol/L, 0.47 mmol/L, 0.34 mmol/L and -0.06 mmol/L, respectively, no p values were presented; Stanhope *et al.*, 1981). Differences in lipid levels in women were not pronounced (Stanhope *et al.*, 1981). Migrant men from all three investigated locations of Tokelau (Fakaofo, Nukunonu and Atafu) further had an increased prevalence for TIVH (p < 0.05), and both migrant men and women (Fakaofo and Nukunonu only) had an increased prevalence for type IIa hyperlipoproteinemia (p < 0.05), when compared with sex-matched non-migrants (Stanhope *et al.*, 1981). In a cross sectional study, Hodge *et al.* (1997) investigated the lipid levels of areas in Western Samoa which differed by modernization. From 1978 to 1991, the prevalence of hypertriglyceridemia was increased in Samoa, but only in Apia, an urbanized area (n = 777, p < 0.001), and not Poutasi which had a medium level of modernization (n = 459, p = 0.162) or Tuasivi which was less modernized than either of the other two locations (n = 512, p = 0.276; Hodge *et al.*, 1997). This indicated that modernization had an effect on hypertriglyceridemia development. On the other hand, hypercholesterolemia was increased in all three locations between 1978 and 1991 (p ranged from less than 0.001 to 0.003; Hodge *et al.*, 1997).
The relatively poor health status of Māori and Pacific people highlights the importance for research into the health of these populations, especially for individuals who live a modernized or Westernized lifestyle. Investigating risk factors and possible predispositions in the diseases which are inflicted in these populations will help with formulating better prevention strategies, diagnostic skills or treatment options.

1.6. **Proposing further links between gout and cardiovascular disease**

The close relationship between gout and cardiovascular disease prompted the current project to elucidate further possible associations between these two disorders. New Zealand Māori/Pacific and Caucasian gout case and control sample sets were investigated and compared. The following aims were set:

- To investigate the lipid and lipoprotein levels of the sample sets, including Lp(a).
- To characterize the lipoprotein profile of the Māori and Pacific sample sets.
- To detect for association between gout and SNPs within *APOE* (rs7412 and rs42935), *LPA* (rs3798220 and rs10455872) and *CDKN2BAS* (rs1333049) in the sample sets.

It was hypothesized that gout cases in all populations would have increased abnormal lipid profiles when compared with gout controls. Additionally, as Māori and Pacific populations have increased gout prevalence and cardiovascular events, it was hypothesized that this population would have a higher frequency of abnormal lipid profiles than the Caucasian population. Characterizing the lipoprotein profile of the Māori and Pacific population would provide a deeper understanding of the lipoprotein and lipid details for this population. Investigating associations between gout and *APOE* isoforms would determine any relationship between TIIIH (indicating increased remnant lipoproteins) and gout. It was hypothesized that significant associations would be detected between rs7412 and rs429358 with gout, and genotype frequencies would differ between sample sets.

As the Lp(a) level distribution is similar across many ethnicities, it was hypothesized that the Māori and Pacific sample set would not significantly differ in Lp(a) levels, Lp(a) distribution, and the allele frequencies of rs3798220 and rs10455872 when compared to the Caucasian
sample set. However, due to links between gout and cardiovascular disease, associations of Lp(a) parameters with gout in Māori/Pacific and Caucasian populations were expected to be detected.

Like LPA and APOE SNPs, it was expected that significant associations between the SNP rs1333049 of CDKNBAS and gout would be detected, and genotype frequencies would differ between sample sets.
2. Methods

2.1. Subject recruitment

The American College of Rheumatology criterion was used to confirm gout cases. Gout cases were recruited in Auckland, Wellington and Christchurch by rheumatologists and research nurses as approved by the New Zealand Multi-region Ethics Committee (MEC/105/10/130). Demographic information for all cases was available as mentioned where relevant.

Two sets of gout controls were used. One set was called the New Zealand Caucasian, Māori and Pacific (NZCMP) controls (Table 3), and were recruited by research nurses mainly from Otago and Auckland areas, but also from other parts of New Zealand. The NZCMP controls were collected between October 1999 and August 2003. The NZCMP controls had limited demographic information (data was available only for the variable sex), therefore were only used in analyses where extended demographic information was not essential (namely in crude genotype analysis; see section 2.4). A subset of the NZCMP controls included a group of Māori and Pacific rheumatoid arthritis patients (10% to 20%; Table 3), as confirmed by the 1987 American College of Rheumatology criterion. These subjects were considered feasible as controls for gout due to the negative association between gout and rheumatoid arthritis co-occurrence (Wallace et al., 1979). The other set of controls were called the Aotearoa controls which were collected from the Auckland area from May 2009 (Table 3), with recruitment currently ongoing. Detailed demographic information was available for the Aotearoa controls, and this was used in analyses where relevant (when self-reported and unspecified heart problems is mentioned, otherwise see sections 2.3.7 and 2.4.5). Due to the more recent sample collection and the availability of extensive demographic information in the Aotearoa control group, only Aotearoa controls (and not NZCMP controls) were used in most analyses (see sections 2.3.1, 2.3.4, 2.3.6 and 2.4.5). Both sets of control groups self-reported their lack of gouty arthritis and were at least 17 years of age. The Lower South (Otago) Ethics Committee (OTA/99/11/098) approved gout controls.

Ancestry was self-reported in all subjects and was established by the ancestry of all four biological grandparents. For DNA genotyping analysis, four sample sets were formed: Māori,
Eastern Polynesian (EP), WP, and Caucasian. The separation of Polynesian subjects into EP and WP was due to genetic diversity between the two groups resulting from migration history (Renfrew, 2009; Trent et al., 1988). This was supported by significant differential allelic frequencies witnessed between this project’s EP and WP sample sets in the SNP rs2231142 of the ABCG2 gene (Phipps-Green et al., 2010). EP subjects included those who identified with Māori as well as Cook Island ancestry. Thus, there was an overlap of 147 cases and 326 controls between the Māori and EP sample sets in DNA genotyping analysis. WP subjects included individuals of Samoan, Tongan, Niuean or Tokelauan ancestry. A subject was considered Māori, EP or WP if at least one grandparent was identified as 50% of that ethnicity. For the EP and WP sample sets, if a subject identified to have both EP and WP ancestry, they were excluded in DNA genotyping analysis to avoid possible conflicting genetic contribution to results. For all lipid and lipoprotein analyses, an all Māori and Pacific group was formed due to low sample numbers. This included subjects with Māori, EP, WP and mixed Polynesian ancestry.

Table 3. Details of the number of subjects in various analyses.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Gout affliction</th>
<th>DNA genotyping</th>
<th>Lipoprotein(a) ELISA*</th>
<th>Basic lipid analyses*</th>
<th>FPLC and apo B analyses*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caucasian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>327</td>
<td>140</td>
<td>209 (194^)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>638 (133^)</td>
<td>133</td>
<td>143 (138^)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Māori</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>155</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>342 (187^, 69^)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Eastern Polynesian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>210</td>
<td>116</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>348 (195^, 71^)</td>
<td>173</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Western Polynesian</strong></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Cases</td>
<td>252</td>
<td>134</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>143 (107^, 15^)</td>
<td>120</td>
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<td></td>
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<td><strong>All Māori and Pacific</strong></td>
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<td></td>
</tr>
<tr>
<td>Cases</td>
<td>-</td>
<td>261</td>
<td>382 (340^)</td>
<td>39 (36)^</td>
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</tr>
<tr>
<td>Controls</td>
<td>-</td>
<td>312</td>
<td>341 (320^)</td>
<td>40 (37)^</td>
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</tr>
<tr>
<td><strong>Framingham Heart Study</strong></td>
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<td></td>
<td></td>
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</tr>
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<td>Cases</td>
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<tr>
<td>Controls</td>
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<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td><strong>Atherosclerosis Risk In Communities</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
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</tr>
<tr>
<td>Controls</td>
<td>6966</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*For control subjects, only Aotearoa controls were used (see section 2.1). ^The number of subjects after excluding subjects with high triglyceride levels (greater than 4.2 mmol/L) to exclude the inaccuracy of calculating low density lipoprotein levels via the Friedewald equation (Demacker, 2004). †The number of Aotearoa controls used in logistic regression analysis (see section 2.1 and 2.4.5). §The number of rheumatoid arthritis patients from the New Zealand Caucasian, Māori and Pacific control group, used as controls (see section 2.1). ‡Includes subjects of Māori ancestry, hence resulting in an overlap of 147 cases and 326 controls between this sample set and the Māori sample set for DNA genotyping analyses. †The number of subjects after excluding subjects with an apo B48 band in western blots, indicating the presence of chylomicrons. ELISA = enzyme-linked immunosorbant assay. FPLC = fast protein liquid chromatography. Apo B = apolipoprotein B.
Written informed consent was obtained from all subjects. The number of participants for various analyses can be found in Table 3.

2.2. Treatment and storage of blood

Originally, serum samples collected four years before the current project were used for the Lp(a) (see section 2.3.4) and Southern Community Laboratory (SCL; see section 2.3.6) measurements. However, these samples were stored at -20°C and inconsistent sample freezing was visibly noticeable, especially in the older samples (Figure 6). Therefore, in final results, only samples collected 1.5 years before the project took place were used for Lp(a) and SCL data.

There was also ongoing blood collection during the time of the project. For these, blood samples were collected in two serum separator tubes (SST) for serum, and one ethylenediaminetetraacetic acid (EDTA; acts as an anti-coagulant) tube for plasma. EDTA tubes were centrifuged at 1300 relative centrifugal force (rcf) for 10 minutes and the supernatant (plasma) for each sample was frozen in aliquots at -80°C. Some plasma samples were initially frozen at -20°C due to limited freezer space, but were eventually transferred to -80°C after approximately one month. The pellet (white blood cells) from EDTA tubes were used in DNA preparation for genotyping assays (see section 2.4.1). Serum from one SST was also stored in aliquots and frozen at -80°C. The second SST was sent to SCL (see section 2.3.6).

As the subject recruitment process was conducted at random and variable times of the day, and subjects were not asked to fast, all blood samples were assumed to be non-fasting.

Figure 6. Uneven freeze of serum samples stored at -20°C for up to four years. Left tube: visually even serum storage. Middle tube: subtle uneven serum storage. Right tube: obvious uneven serum storage. Notice the change in colour between the top and the bottom of the middle tube and the right tube.
2.3. Measurements for cardiovascular markers

2.3.1. Fast protein liquid chromatography

Fast protein liquid chromatography (FPLC) was used to separate lipoproteins (Linton et al., 1993) in Māori and Pacific plasma samples for analyses (Table 3). For control subjects, only Aotearoa (and not NZCMP) controls (see section 2.1) were subjected to FPLC.

Plasma was prepared by centrifugation at 16060 rcf for two minutes. Supernatant was diluted by a factor of two with filtered and degassed FPLC-phosphate buffer solution (Appendix A.I). Prepared sample was injected into a 400 µL loop of a BioLogic DuoFlow system (including a DuoFlow pump, auto-injection valve AVR7-3 and BioFrac fraction collector; Bio-Rad Laboratories, Inc). Filtered and degassed FPLC-phosphate buffer solution (Appendix A.I) was used as an isocratic running buffer at 0.5 mL/min. After 2 mL of buffer flow, sample (a total plasma volume of 200 µL) was auto-injected into a Superose™ 6 10/300 GL column (Tricorn™) within a flow volume of 0.8 mL. This was followed by a further buffer flow volume of 32 mL, which was divided into fractions of 0.5 mL. For each run, fraction numbers seven to 53 were collected in half of one 96 well storage plate (Thermal Scientific), so that each plate held fractions from two subjects. Plates were sealed with 96 cap sealing mats (Thermo Scientific) until further use. The BioLogic DuoFlow version 5.20 build 3 © 1998-2009 software (Bio-Rad Laboratories, Inc) was used for viewing the 280 nm ultraviolet chromatogram traces (Appendix B.I).

TG and cholesterol assays were performed on the FPLC fractions at most one day after the fractions had been collected (see section 2.3.2). Fractions were then frozen at -80ºC until thawed for apo B measurements (see section 2.3.4).

2.3.2. Triglyceride and cholesterol assays

Collected FPLC fractions and corresponding diluted whole plasma samples were assayed for TG and cholesterol content using colourimetric assays. Dilutions of Precipath L (Cobas®, Roche/Hitachi) were used to make a standard curve (Appendix C). The FPLC fractions were assayed once, whereas the whole plasma samples and standard curve points were tested in triplicate. Each sample at 100 µL was incubated with 100 µL of TG (glycerol phosphate oxidase - phenol and aminophenazone) reagent (Roche/Hitachi) or cholesterol (cholesterol oxidase -
phenol and aminophenazone) reagent (Roche/Hitachi) for five to 20 minutes at 37°C in a 96 well plate (Nunc™, Thermo Scientific). The plate was read using an ELX808 ultra microplate reader (Bio-Tek® instruments, Inc) with Gen5™ version 1.05.11 © 2006-2008 software at 490 nm.

2.3.3. Lipoprotein gel electrophoresis

Plasma samples from subjects who underwent FPLC analysis were subjected to lipoprotein gel electrophoresis. This was to assay for abnormal lipoprotein banding patterns indicative of hyperlipoproteinemia, and to detect for the presence of a CM band, indicative of a non-fasting status. The TITAN GEL lipoprotein electrophoresis system (Helena Laboratories, Texas) was used following product protocol. Briefly, samples were loaded at 3 µL and electrophoresis was conducted at 80 volts for 20 minutes. Fat Red 7B was used to stain the gels.

2.3.4. Lipoprotein(a) and apolipoprotein B measurements

An enzyme-linked immunosorbant assay (ELISA) was used to measure Lp(a) in plasma or serum samples, and apo B in plasma and FPLC fractions (Marcovina et al., 1995). For the Lp(a) ELISA, serum samples that were collected before the project started and stored at -20°C were used. For control subjects, only Aotearoa (and not NZCMP) controls were used (see section 2.1). A chipping method was utilized to obtain serum. This involved chipping approximately 10 µL off the top of the frozen serum in the storage tube and thawing only that piece for measurement. This was conducted in the hope to prevent an unnecessary freeze-thaw of the sample. During this time, more recently frozen samples were chipped and assayed before older samples. However, as older samples were uncovered, uneven freezing of sera became noticeable (Figure 6). The chipping method was therefore abandoned and assays were repeated with the serum samples thawed by the whole tube. Subject numbers can be found in Table 3. For the apo B ELISA, whole plasma samples from subjects whom underwent FPLC analysis were measured. Also, the VLDL fractions separated by the FPLC were pooled and measured for apo B content.

Standards (Appendix C) and positive quality controls were prepared from lyophilized plasma. For Lp(a), these were prepared by the Northwest Lipid Research Labs (Marcovina et al., 1995). The quality controls had the following known Lp(a) concentrations: L1: 10.6 nmol/L, L2: 25.5 nmol/L, L3: 40.2 nmol/L and L4: 66.7 nmol/L. For apo B, Precipath L (Cobas®, Roche/Hitachi) was used for the standards, and Cfas Lipid (Cobas®, Roche/Hitachi) with a
known apo B concentration of 2185 nmol/L was used as a positive quality control. These materials were resuspended and diluted with MilliQ® water before use. Plasma, serum or FPLC fraction samples were plated undiluted or up to a dilution factor of 8000, dependent on whether or not the concentration of the sample fell in the range of the standard curve. Diluted samples were diluted with plasma dilution buffer (Appendix A.II).

For Lp(a), the primary antibody used to coat the plate was monoclonal antibody a6, specific for the kringle IV type 2 repeat of Lp(a) (Marcovina et al., 1995). This was diluted to 1.5 µL/mL with antibody coating buffer (Appendix A.II). For apo B, the primary antibody used was a polyclonal antibody (Roche) which recognizes multiple epitopes of the apo B protein, diluted one in 10000 with antibody coating buffer (Appendix A.II). Plates were incubated with diluted primary antibody and then washed with sterile phosphate buffer solution (Appendix A.II). Wells were incubated with freshly prepared blocking buffer (Appendix A.II), then washed as before. Standards, quality controls and serum, plasma or FPLC fraction samples were incubated. Plates were washed with wash buffer (Appendix A.II) and incubated with the secondary antibody conjugated to horseradish peroxidase (hrp; a catalyst for the development of the colourimetric reaction). For Lp(a), this was monoclonal a1-1-hrp, specific for binding kringle IV type 8 of apo(a) (Marcovina et al., 2000), in a 1 in 2500 dilution. For apo B, this was monoclonal 1D1-hrp, specific for binding apo B amino acids 474 to 539 (Pease et al., 1990) in a 1 in 2000 dilution. Plates were further washed with wash buffer (Appendix A.II). Substrate solution (Appendix A.II) was added to the plates and left to develop in the dark for five to 15 minutes. The developing reaction was stopped with 2N H₂SO₄.

For both Lp(a) and apo B ELISAs, 96 well EIA/RIA plates 3590 (Costar®) were used for reactions. ImmunoWash 1575 (Bio-Rad) was used to wash plates, with a protocol of using 300 µL wash solution per well, conducted three times per well per wash. All solutions with a volume of 100 µL were incubated in each well at 28°C for one hour, except for the primary antibody where the incubation was overnight at 4°C, the blocking buffer where a volume of 300 µL was used, and the secondary antibody where the incubation period was 1.5 hours. During all incubations, plates were sealed with non-sterile plate sealer (Corning®). The Lightning-Link™ HRP conjugation kit (Innova Biosciences) was used to label antibodies with hrp.

Absorbance was read with an ELX808 ultra microplate reader (Bio-Tek® instruments, Inc) at 490 nm with Gen5™ version 1.05.11 © 2006-2008 software.
2.3.5. Western blot for lipoprotein(a) and apolipoprotein B

Selected plasma or serum samples that were measured for Lp(a) levels underwent western blotting to check for the degradation of Lp(a). As samples with low Lp(a) molecular weight (corresponding to high Lp(a) levels; Utermann et al., 1987) are more likely to degrade (Kronenberg et al., 1996), samples with high Lp(a) levels were selected for western blotting.

Western blotting against apo B was also conducted on plasma samples from subjects with FPLC analysis to check for the fasting status of the samples. As apo B100 (corresponding to VLDL and LDL) and apo B48 (corresponding to CM) have different electrophoretic mobilities (Kane, 1983), the presence of CM, and hence fasting status, can be detected.

2.3.5.1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis

A spacer plate and a short plate (Bio-Rad) were clamped together and 6.5 mL of resolving gel solution (Appendix A.III) was pipetted into the space between the plates. Up to 0.4 mL of bulk methanol was layered over the top to rupture air bubbles. The gel was left to set for 20 minutes and the methanol was tipped out. 2 mL of stacking gel solution (Appendix A.III) was pipetted over the top of the resolving gel and a 10 well comb was placed. The stacking gel was left to set for 20 minutes and then the combs were removed.

For western blots against Lp(a), plasma or serum samples were diluted to an Lp(a) concentration of 10 nmol/L with MilliQ® water and 1× reducing sample buffer (Appendix A.III), which dissociates apo(a) from LDL. Plasma from human Lp(a) transgenic mice (Linton et al., 1993) acted as a positive control. It was treated the same way, except that it was not diluted with MilliQ® water due to relatively lower levels of apo(a) or Lp(a) in transgenic mice. For western blots against apo B, 1 µL of plasma sample was diluted with 19 µL of water and 1× non-reducing sample buffer (Appendix A.III). Plasma from human apo B transgenic mice (Linton et al., 1993) was used as a positive control and treated the same way, except 2 µL of plasma and 18 µL of water was used instead. The band from this sample gave indication to the apo B100 position. All samples were put in a 95°C water bath for five minutes to denature protein. 20 µL of sample for blots against Lp(a) and 24 µL of sample for blots against apo B were loaded into the wells of the sodium dodecyl sulfate polyacrylamide gel. Gels were electrophoresed in MiniPROTEAN® 3 cell tanks (Bio-Rad), first at 50 volts for 30 minutes through the stacking gel, then at 0.80 Amps for 90 minutes through the resolving gel.
2.3.5.2. Transfer and immunoblot

Woollen transfer pad (Bio-Rad), 3 MM blotting filter paper (Whatman®) and Protran® nitrocellulose transfer membrane were pre-soaked in transfer buffer (Appendix A.III). Air bubbles were rolled out from the transfer pads with a thin glass cylinder. A clamped sandwich was created from the following layers: two transfer pad layers, filter paper, sodium dodecyl sulfate polyacrylamide gel, transfer membrane, filter paper and two transfer pad layers. The apparatus was placed in a Trans-blot® cell tank (Bio-Rad) and electrophoresed at 0.8 Amps overnight at 4°C with transfer buffer (Appendix A.III). The tank was stirred with a magnetic stirrer, and cooled with cold running water through a pipe.

The membrane was blocked with blocking solution (Appendix A.III) for 30 minutes to reduce the binding of non-specific proteins. For Lp(a) samples, the monoclonal antibody a5-hrp specific for kringle IV types 1 and 2 (Marcovina et al., 1995), was diluted 1 in 1500 with 4 mL of blocking solution (Appendix A.III). For FPLC samples, the polyclonal anti-apo B antibody (Roche) labelled with hrp was diluted 1 in 2000 with 4 mL of blocking solution (Appendix A.III). Both antibodies were labelled with the hrp using the Lightning-Link™ HRP conjugation kit (Innova Biosciences). The membrane was mixed with the corresponding diluted antibody at 4°C overnight. The membrane was rinsed twice with Tris-buffered saline (Appendix A.III) for five minutes each. The membranes were developed by the addition of 1 mL of electrochemiluminescence reagent A (Appendix A.III) and 1 mL of electrochemiluminescence reagent B (Appendix A.III). Bands were visualized in a LAS-3000 (Fujifilm) cooled to -30°C via chemiluminescence with the image reader software LAS-3000 version 2.2 © 2002-2005 (Fujifilm Co, Ltd).

2.3.6. Measurements by Southern Community Laboratories

Serum samples (Table 3) were sent to SCL for the colourimetric measurement of total TG, total cholesterol, HDL cholesterol and UA levels. HDL cholesterol was measured with the polyethylene glycol precipitation method (Viikari, 1976). This first precipitates other lipoproteins from the sample, then cholesterol in remaining lipoproteins (HDL) is subsequently assayed. LDL cholesterol values were calculated via the Friedewald equation (LDL cholesterol = total cholesterol – HDL cholesterol – TG/2.2 in mmol/L; Friedewald et al., 1972). If the TG level was over 4.2 mmol/L, LDL levels were not calculated as the Friedewald equation becomes
inaccurate at such levels (Demacker, 2004). UA was measured with the uricase and peroxidase colourimetric method (Domagk and Schlicke, 1968). For serum samples stored at -20°C for 1.5 years, samples were thawed and sent to SCL on the same day that the Lp(a) ELISA was performed (see section 2.3.4). For control subjects, only Aotearoa (and not NZCMP) controls were used for analysis (see section 2.1) as it was deemed that lipid measurements might not be accurate for samples which have been stored for a longer period of time at -20°C. For freshly recruited samples, the second SST was sent directly to SCL without a freeze-thaw.

2.3.7. Statistical analyses on cardiovascular markers

All lipid, lipoprotein and apolipoprotein values were compared using the Mann-Whitney (MW) test via the Intercooled Stata™ software version 8.0 (College Station, TX 77845, United States of America). The MW test was used due to the deviation from a normal distribution in many datasets. The student’s t test was also conducted on values after log transformation to reduce the skewness in data. Values were compared between gout cases and controls in both sample sets, and between sample sets (Caucasian and Māori/Pacific).

Lp(a) levels were further compared between cases and controls of self-reported and unspecified heart problems, and after subgrouping into LPA and APOE alleles. TG and UA levels were also investigated against APOE isoforms, due to associations found by other researchers (Alvim et al., 2010; Cardona et al., 2003; Liberopoulos et al., 2005). Correlation analysis was conducted between TG, HDL, Lp(a) and UA levels using the Pearson’s correlation coefficient. This was conducted as past studies have found associations between these factors (Chun et al., 2001; Ritter et al., 1997).

The inter-assay and intra-assay coefficient of variation (standard deviation (SD) divided by mean) was calculated for Lp(a) and apo B measurements. The inter-assay measurement was calculated for quality controls, where values from the same sample across separate assays were compared. The intra-assay measurement was calculated for standards, positive quality controls and plasma or serum samples, where values derived from triplicate measurements of the same sample within the same assay were compared.

For the subgroup of samples that underwent FPLC analysis, the percentage recovery of plasma lipids from the FPLC was calculated. To do this, the mean concentration of TG and cholesterol from FPLC fractions (as measured with the TG and cholesterol assays) was used as
the nominator, whereas the TG and cholesterol values obtained from whole plasma (as measured with the TG and cholesterol assays) was used as the denominator. Lipid ratios involving VLDL and LDL were compared between cases and controls, as were apo B measurements in VLDL fractions and whole plasma. For the VLDL apo B to total apo B ratio, total apo B was adjusted for the percentage lipid recovery from the FPLC. This was done for each individual sample by multiplying the total apo B value by the percentage recovery of plasma lipids from the FPLC. One way analysis of variance was used to investigate apo B measurements across quartiles of the VLDL TG to total TG ratio.

Classification criteria for TIVH were explored. The characterizing lipid anomaly in TIVH is increased blood TG concentration due to increased VLDL TG (Levy and Fredrickson, 1968). Thus, various combinations accounting for total plasma TG (plasma TG and plasma apo B levels), VLDL (VLDL TG level, VLDL TG to total TG ratio, and VLDL apo B to total apo B ratio) and LDL (LDL TG level, LDL TG to total TG ratio, and LDL cholesterol level) were used to classify TIVH (Table 4). LDL was included in the analysis to exclude possible increases in total TG due to LDL rather than VLDL. The 75th percentile was used as a cut off point for determining TIVH status (Table 5). However, CM can also contribute to increased TG levels due to either a non-fasting status after a fatty-rich meal, or the presence of type V hyperlipoproteinemia. Type V hyperlipoproteinemia is characterized by an increase in both VLDL and CM in the blood (Levy and Fredrickson, 1968). To account for the misclassification of TIVH due to increased CM, analysis was conducted after samples with a CM band (three cases and three controls), as determined by western blots (see section 2.3.5), were removed.

Results were compared with lipoprotein gel banding pattern results. The percentage match rate between each TIVH criterion and lipoprotein gel results was calculated. Any increase in intensity at the pre-β area of the lipoprotein gel, which signified increased VLDL, was considered a match with subjects classified with TIVH.

Logistic regression was conducted on FPLC variables using the software Intercooled Stata™ version 8.0 (College Station, TX 77845, United States of America), namely VLDL apo B, VLDL apo B to total apo B ratio, plasma apo B, plasma TG, VLDL TG to total TG ratio, and LDL TG to total TG ratio. Significant differences in possible confounding covariates between gout cases and controls were tested for. These included age, sex, waist circumference, the presence of hypertension, TIIDM, and unspecified heart and kidney problems, the levels of
serum Lp(a) and UA, the consumption of fruit, sugar drinks and alcohol, the number of Māori and Pacific grandparents, and Eastern, Western or mixed Polynesian ethnicity. All these variables were self-reported except for Lp(a) and UA levels. The Fisher’s exact test (for binomial outcomes), the MW test (for continuous variables deviating from a normal distribution) and the Chi-squared test (for three or more outcomes) were used to test for significance.

Table 4. Various investigated criteria for type IV hyperlipoproteinemia classification.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Total TG component</th>
<th>VLDL component</th>
<th>LDL component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a*</td>
<td>Plasma TG &gt; 75th percentile</td>
<td>VLDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt; &gt; 75th percentile</td>
<td>LDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt; &lt; 75th percentile</td>
</tr>
<tr>
<td>1b*</td>
<td>Plasma TG &gt; 75th percentile</td>
<td>VLDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt; &gt; 75th percentile</td>
<td>LDL TG &lt; 75th percentile</td>
</tr>
<tr>
<td>1c*</td>
<td>Plasma TG &gt; 75th percentile</td>
<td>VLDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt; &gt; 75th percentile</td>
<td>LDL chol &lt; 75th percentile</td>
</tr>
<tr>
<td>1d*</td>
<td>Plasma TG &gt; 75th percentile</td>
<td>VLDL&lt;sub&gt;TG&lt;/sub&gt; TG &gt; 75th percentile</td>
<td>LDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt; &lt; 75th percentile</td>
</tr>
<tr>
<td>2*</td>
<td>Plasma TG &gt; 75th percentile</td>
<td>VLDL&lt;sub&gt;apo B&lt;/sub&gt; : total&lt;sub&gt;apo B&lt;/sub&gt; &gt; 75th percentile</td>
<td>LDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt; &lt; 75th percentile</td>
</tr>
<tr>
<td>3a#</td>
<td>Plasma TG &gt; 75th percentile</td>
<td>-</td>
<td>LDL chol &lt; 75th percentile</td>
</tr>
<tr>
<td>3b#</td>
<td>Apo B &gt; 75th percentile</td>
<td>-</td>
<td>LDL chol &lt; 75th percentile</td>
</tr>
<tr>
<td>3c#</td>
<td>Plasma TG and apo B &gt; 75th percentile</td>
<td>-</td>
<td>LDL chol &lt; 75th percentile</td>
</tr>
</tbody>
</table>

*Based on values derived from fast protein liquid chromatography methodology. #Based on values derived from standard lipid measurements. TG = triglyceride. VLDL = very low density lipoprotein. LDL = low density lipoprotein. Chol = cholesterol. Apo B = apolipoprotein B. See Table 5 for the 75th percentile values.

Table 5. The 75th percentile values used in type IV hyperlipoproteinemia classification criteria.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total TG (mmol/L)</td>
<td>2.33</td>
</tr>
<tr>
<td>Total apo B (nmol/L)</td>
<td>1495.3</td>
</tr>
<tr>
<td>VLDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt;</td>
<td>0.55572</td>
</tr>
<tr>
<td>VLDL TG (mmol/L)</td>
<td>0.99</td>
</tr>
<tr>
<td>VLDL&lt;sub&gt;apo B&lt;/sub&gt; : total&lt;sub&gt;apo B&lt;/sub&gt;</td>
<td>0.07028</td>
</tr>
<tr>
<td>LDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt;</td>
<td>0.43430</td>
</tr>
<tr>
<td>LDL TG (mmol/L)</td>
<td>0.59</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.40</td>
</tr>
</tbody>
</table>

NB. TG = triglyceride. Apo B = apolipoprotein B. VLDL = very low density lipoprotein. LDL = low density lipoprotein.
2.4. DNA genotyping

2.4.1. Sample preparation

White blood cells from EDTA tubes (see section 2.2) were incubated with guanidine hydrochloride reagents (Appendix A.IV) at 60°C for one hour to precipitate DNA. After cooling to room temperature, samples were mixed with 2 mL chloroform (chilled to 4°C), left to settle for one minute, and spun at 12600 rcf for three minutes. The upper layer was mixed with 10 mL ethanol (chilled to 4°C) to precipitate the DNA, and spun at 18144 rcf for 15 minutes. The pellet was washed twice with 4 mL ethanol (70%) and centrifuged at 18144 rcf for five minutes. The pellet was incubated with 200 µL Tris-EDTA buffer (Appendix A.IV) at 55°C for one hour. The DNA concentration was quantified with a NanoDrop 1000 spectrophotometer and the software NanoDrop version 3.0.1 (NanoDrop Technologies, Wilmington, Delaware). A DNA concentration of 6 to 8 ng/mL was used for genotyping (see section 2.4.2), diluted with Tris-EDTA buffer (Appendix A.IV).

2.4.2. Taqman® single nucleotide polymorphism genotyping

Taqman® SNP genotyping was performed using a LightCycler® 480 Real-Time Polymerase Chain Reaction System (Roche Applied Science, Indianapolis, United States of America) in 384 well plates for chosen SNPs. Five SNPs from LPA, APOE and CDKN2BAS were genotyped (Table 6). The two SNPs from LPA were chosen because they were found to have a strong association with coronary disease, carotid disease and high Lp(a) levels (OR ranged from 1.70 to 3.14; Clarke et al., 2009; Luke et al., 2007; Ronald et al., 2011; Shiffman et al., 2010). The two SNPs from APOE were chosen to type for the APOE isoform in subjects. The CDKN2BAS SNP was chosen due to associations found with cardiovascular disease and gout (Burton et al., 2007; Samani et al., 2007; Wang et al., 2011). Genotypes were visualized with scatter graphs (Appendix B.II) and assigned to samples with the LightCycler® 480 software version 1.5.0 SP4 (Roche).

2.4.3. Allelic association analyses

Genotype data were analyzed via the SHEsis analysis tool (Shi and He, 2005) for single site analysis between gout cases and controls. Additionally for the SNP rs1333049 in
CDKN2BAS, the same analysis was conducted between subjects with and without self-reported and unspecified heart problems. This was done due to the extensive associations found between rs1333049 and cardiovascular diseases (see section 1.2.1.1).

The \( p \) values of allelic association with gout in the Māori, EP and WP sample sets were adjusted for Caucasian admixture. This was to rule out Caucasian genetic influence from the Māori, EP and WP sample sets so that the association results can be completely attributed to the respective ethnicities. The genotypes for each SNP, along with the genotypes of unlinked genomic markers (Appendix C), were used for this process (Pritchard et al., 2000). The markers were genotyped by previous and current Merriman laboratory members in the Department of Biochemistry in the University of Otago. This adjustment was done individually for each SNP in each sample set. All Māori, EP and WP gout case and control genotypes were used, whereas only 505 of the 638 Caucasian control genotypes were used. All markers had minor allele frequencies that differed by greater than 0.05 between the Caucasian control frequencies and relevant Māori, EP or WP case frequencies. As a result, a total of 25 markers were used for the Māori population, and 23 markers were used for the EP and WP populations. The Structure software version 2.3 (http://pritch.bsd.uchicago.edu/software.html) was used for the appropriate clustering of each sample set into ancestral groups with the settings of 30000 burnin period, 1000000 Markov chain Monte Carlo replications after burnin, and two assumed number of populations. The \( p \) values adjusted for Caucasian admixture were calculated by STRAT (http://pritch.bsd.uchicago.edu/software.html) using the output from Structure.

For each SNP of each sample set, the Hardy-Weinberg equilibrium was noted using the SHEsis analysis tool (Shi and He, 2005). Deviations from the Hardy-Weinberg equilibrium (\( p < 0.05 \)) may indicate a non-random sample set (Crow and Dove, 1988) or highlight genotyping errors, such as inaccurate genotype calls (Hosking et al., 2004).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Associated gene</th>
<th>Assay identification code</th>
<th>Chromosome</th>
<th>SNP type</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7412</td>
<td>APOE</td>
<td>C_904973_10</td>
<td>6</td>
<td>Missense polymorphism</td>
</tr>
<tr>
<td>rs429358</td>
<td>APOE</td>
<td>C_3084793_20</td>
<td>6</td>
<td>Missense polymorphism</td>
</tr>
<tr>
<td>rs1333049</td>
<td>CDKN2BAS</td>
<td>C_1754666_10</td>
<td>9</td>
<td>Intergenic</td>
</tr>
<tr>
<td>rs3798220</td>
<td>LPA</td>
<td>C_25930271_10</td>
<td>19</td>
<td>Missense polymorphism</td>
</tr>
<tr>
<td>rs10455872</td>
<td>LPA</td>
<td>C_30016089_10</td>
<td>19</td>
<td>Intronic</td>
</tr>
</tbody>
</table>

NB: SNP = single nucleotide polymorphism.
Post hoc power calculations were conducted on allelic association analysis at the significance level of 0.05. As the SNP rs1333049 of CDKN2BAS was genotyped with the aim to replicate the association with gout (p = 0.01, OR [95% CI] = 1.26 [1.06 - 1.54]) as shown by the study conducted by Wang et al. (2011), the power calculation for this SNP was conducted with the OR set at 1.26. In allelic association analysis with self-reported and unspecified heart problems for rs1333049, power calculations were conducted for a range of OR, due to more than one study showing association between this SNP and cardiovascular disease. No known studies have investigated the association between gout and the SNPs rs3798220, rs10455872, rs429358 and rs7412 of LPA and APOE. Therefore, the power to detect for association with gout was calculated for a range of OR for these SNPs.

2.4.4. Genotypes from other studies

Data from the Framingham Heart Study (FHS; http://www.framinghamheartstudy.org/) and the Atherosclerosis Risk in Communities (ARIC; Szklo et al., 1989) study were used to compare genotype frequencies with those of the current project.

The FHS is directed by the National Heart, Lung, and Blood Institute and Boston University (United States of America). Research by the FHS focuses on cardiovascular diseases, but the sample set is also publically available for general biomedical research. The FHS includes several samples sets. The first sample set was named the Original Cohort and consists of randomly sampled individuals from Framingham, Massachusetts, in 1948. The current project utilized data from the Offspring Study and GenIII sample sets (Table 3). The Offspring Study participants were either the offspring of the Original Cohort sample set, or the offspring’s spouse, and were founded in 1971. The GenIII participants were the children of the Offspring Study who were aged over 19 years by 2005.

The ARIC study, like the FHS, is sponsored by the National Heart, Lung, and Blood Institute, and focuses on cardiovascular research. Subjects were recruited from the United States of America: Forsyth County, Washington County, Jackson, and the northwest suburbs of Minneapolis. There were two sample sets: the Cohort Component, founded in 1987 with subjects aged between 45 years to 65 years, and the Community Surveillance Component, currently ongoing since 2006, with a subject age range of 35 years to 84 years. For the current project, both sample sets were used (Table 3).
The approval number was #834 for accessing data from the FHS and ARIC study, under the project name “The Genetic Basis of Gout.” Subjects from the FHS and ARIC studies who were familially related were removed from analysis. All subjects included were Caucasian. FHS and ARIC data were accessed via BC\SNPmax version 3.3 (Biocomputing Platforms Ltd, Espoo, Finland). The GWAS case-control analysis PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) software accessed via BC\SNPmax version 3.3 was used to generate allelic and genotypic data (Purcell et al., 2007). For the SNP rs1333049, the sub-dataset from the FHS 500k Affymetrix chip was used in the FHS sample set.

SNPs in APOE (rs429358 and rs7412) and LPA (rs3798220 and rs10455872) were not genotyped in either FHS or ARIC sample sets, so imputation was attempted. Imputation is an in silico method which uses known genotypes of surrounding SNPs and racially-matched known haplotypes to calculate the predicted genotype frequency for the SNP of interest. Using BC\SNPmax version 3.3 to access the GWAS analysis software IMPUTE version 2 tool (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html), the genotypes for FHS and ARIC sample sets were imputed separately for each of the SNPs. Reference SNPs were taken within the region of chromosome 6 positions 160881000 to 161087500 for LPA and chromosome 19 positions 50102000 to 50104000 for APOE. For both genes, the haplotype reference set used was 1000 Genomes (August 2009) and the population used was CEU (Utah residents with Northern or Western European ancestry). All other settings were default.

The LD between the SNPs in APOE (rs429358 and rs7412) and LPA (rs3798220 and rs10455872) with surrounding SNPs was investigated using information obtained from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) phase I, II and III, release number 28. For LPA, SNPs were taken from the region of chromosome 6 positions 160872506 to 161007397. For APOE, SNPs were taken from the region of chromosome 19 positions 50100000 to 50106000. LD plots were generated with the software Haploview 4.2 (Barrett et al., 2005).

2.4.5. Logistic regression analysis on genotype data

Multivariate logistic regression was performed using the Intercooled Stata™ software version 8.0 (College Station, TX 77845, United States of America). The following self-reported factors were adjusted for: sex, age, waist circumference, and the presence of TIIDM, unspecified hyperlipidemia and unspecified heart problems. Data on these demographics were present in all
cases, but only in Aotearoa (and not NZCMP) controls (see section 2.1). Therefore, for controls, logistic regression could only be performed in a subset group (Table 3).
3. Results

3.1. Lipid analyses in gout case and control sample sets

From SCL results of Māori and Pacific samples, there were significantly higher levels of total cholesterol \( (p = 0.0004) \), LDL cholesterol \( (p = 0.0090) \) and total TG \( (p = 1.80 \times 10^{-8}) \) in gout cases when compared to controls \( \text{total cholesterol} = 5.17 \text{ mmol/L in cases and } 4.88 \text{ mmol/L in controls, LDL cholesterol} = 3.09 \text{ mmol/L in cases and } 2.88 \text{ mmol/L in controls, and total TG} = 2.50 \text{ mmol/L in cases and } 1.96 \text{ mmol/L in controls; Table 7} \). There were significantly lower levels of HDL cholesterol \( (p = 0.0095) \) in cases \( (1.07 \text{ mmol/L}) \) when compared to controls \( (1.12 \text{ mmol/L}; \text{ Table 7}) \). As expected, cases had significantly higher UA levels than controls \( (0.45 \text{ mmol/L in cases and } 0.36 \text{ mmol/L in controls, } p = 2.00 \times 10^{-26}; \text{ Table 7}) \).

From SCL results of Caucasian samples, there were no significant differences in total or HDL cholesterol between gout cases and controls \( (p = 0.5115 \text{ and } 0.3602, \text{ respectively}) \). Total TG was significantly higher \( (p = 4.00 \times 10^{-6}) \) and LDL cholesterol was significantly lower \( (p = 0.0027) \) in cases than controls \( \text{total TG} = 2.33 \text{ mmol/L in cases and } 1.73 \text{ mmol/L in controls, and LDL cholesterol} = 2.82 \text{ mmol/L in cases and } 3.09 \text{ mmol/L in controls} \). As expected, gout cases had significantly higher UA levels than controls \( (0.39 \text{ mmol/L in cases and } 0.29 \text{ mmol/L in controls, } p = 5.26 \times 10^{-26}; \text{ Table 7}) \).

All Māori and Pacific gout cases had lower HDL cholesterol \( (p = 5.21 \times 10^{-5}) \) and higher LDL cholesterol \( (p = 0.0007) \) than Caucasian gout cases (Table 7). All Māori and Pacific gout controls had lower LDL cholesterol \( (p = 0.0156) \) than Caucasian gout controls but no differences in HDL cholesterol (Table 7). Both Māori and Pacific gout case and control groups had higher UA levels than the respective Caucasian groups \( (p = 1.82 \times 10^{-7} \text{ and } 2.92 \times 10^{-16}, \text{ respectively; Table 7}) \).

Correlation analysis was conducted between TG to HDL and UA levels. TG levels negatively correlated with HDL levels in Māori/Pacific and Caucasian gout case and control sample sets \( \text{Pearson’s correlation coefficient ranged from } -0.5282 \text{ to } -0.3811, \text{ } p \text{ ranged from } 3.49 \times 10^{-17} \text{ to } 3.74 \times 10^{-11}; \text{ Table 8}) \). For Caucasian cases and controls and Māori/Pacific controls, TG positively correlated with UA levels \( \text{Pearson’s correlation coefficient ranged from} \)
0.2282 to 0.2952, \( p \) ranged from 2.75 \( \times 10^{-8} \) to 0.0009; Table 8). Strikingly, there was no sign of correlation between TG and UA levels in Māori and Pacific cases (Pearson’s correlation coefficient = 0.0036, \( p = 0.9448 \); Table 8).

Table 7. Southern Community Laboratories results in gout case and control sample sets.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Statistic</th>
<th>Total cholesterol</th>
<th>Total triglyceride</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
<th>Uric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Māori and Pacific cases</td>
<td>Mean</td>
<td>5.17</td>
<td>2.50</td>
<td>1.07</td>
<td>3.09</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.10</td>
<td>1.75</td>
<td>0.34</td>
<td>0.98</td>
<td>0.11</td>
</tr>
<tr>
<td>Māori and Pacific controls</td>
<td>Mean</td>
<td>4.88</td>
<td>1.96</td>
<td>1.12</td>
<td>2.88</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.04</td>
<td>1.22</td>
<td>0.32</td>
<td>0.91</td>
<td>0.09</td>
</tr>
<tr>
<td>Caucasian cases</td>
<td>Mean</td>
<td>5.06</td>
<td>2.33</td>
<td>1.19</td>
<td>2.82</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.12</td>
<td>1.55</td>
<td>0.38</td>
<td>1.00</td>
<td>0.11</td>
</tr>
<tr>
<td>Caucasian controls</td>
<td>Mean</td>
<td>5.00</td>
<td>1.73</td>
<td>1.15</td>
<td>3.09</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.02</td>
<td>0.99</td>
<td>0.33</td>
<td>0.83</td>
<td>0.08</td>
</tr>
<tr>
<td>Māori and Pacific cases versus controls</td>
<td>( p ) (MW)</td>
<td>0.0003</td>
<td>7.01 ( \times 10^{-8} )</td>
<td>0.0163</td>
<td>0.0062</td>
<td>3.20 ( \times 10^{-28} )</td>
</tr>
<tr>
<td></td>
<td>( p ) (t test)*</td>
<td>0.0004</td>
<td>1.80 ( \times 10^{-8} )</td>
<td>0.0095</td>
<td>0.0090</td>
<td>2.00 ( \times 10^{-26} )</td>
</tr>
<tr>
<td>Caucasian cases versus controls</td>
<td>( p ) (MW)</td>
<td>0.6059</td>
<td>4.54 ( \times 10^{-6} )</td>
<td>0.3220</td>
<td>0.0027</td>
<td>6.42 ( \times 10^{-20} )</td>
</tr>
<tr>
<td></td>
<td>( p ) (t test)*</td>
<td>0.5115</td>
<td>4.00 ( \times 10^{-6} )</td>
<td>0.3602</td>
<td>0.0024</td>
<td>5.26 ( \times 10^{-26} )</td>
</tr>
<tr>
<td>Cases: Caucasian versus Māori and Pacific</td>
<td>( p ) (MW)</td>
<td>0.0746</td>
<td>0.2435</td>
<td>0.0001</td>
<td>0.0010</td>
<td>3.47 ( \times 10^{-8} )</td>
</tr>
<tr>
<td></td>
<td>( p ) (t test)*</td>
<td>0.0684</td>
<td>0.1972</td>
<td>5.21 ( \times 10^{-5} )</td>
<td>0.0007</td>
<td>1.82 ( \times 10^{-7} )</td>
</tr>
<tr>
<td>Controls: Caucasian versus Māori and Pacific</td>
<td>( p ) (MW)</td>
<td>0.0841</td>
<td>0.0951</td>
<td>0.5070</td>
<td>0.0151</td>
<td>1.03 ( \times 10^{-14} )</td>
</tr>
<tr>
<td></td>
<td>( p ) (t test)*</td>
<td>0.0743</td>
<td>0.0858</td>
<td>0.3661</td>
<td>0.0156</td>
<td>2.92 ( \times 10^{-16} )</td>
</tr>
</tbody>
</table>

*On log transformed values. All values are presented as mmol/L. HDL = high density lipoprotein. LDL = low density lipoprotein. SD = standard deviation. MW = Mann-Whitney.

Table 8. Correlation analysis of various factors with triglyceride levels.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Gout affliction</th>
<th>High density lipoprotein</th>
<th>Uric acid</th>
<th>Lipoprotein(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r^* )</td>
<td>( p ) (MW)</td>
<td>( r^* )</td>
<td>( p ) (MW)</td>
</tr>
<tr>
<td>Caucasian cases</td>
<td>-0.4369</td>
<td>3.74 ( \times 10^{-11} )</td>
<td>0.2282</td>
<td>0.0009</td>
</tr>
<tr>
<td>Controls</td>
<td>-0.5282</td>
<td>1.20 ( \times 10^{-11} )</td>
<td>0.2781</td>
<td>0.0008</td>
</tr>
<tr>
<td>Māori and Pacific cases</td>
<td>-0.3811</td>
<td>1.19 ( \times 10^{-14} )</td>
<td>0.0036</td>
<td>0.9448</td>
</tr>
<tr>
<td>Controls</td>
<td>-0.4351</td>
<td>3.49 ( \times 10^{-17} )</td>
<td>0.2952</td>
<td>2.75 ( \times 10^{-8} )</td>
</tr>
</tbody>
</table>

*Pearson’s correlation coefficient. For high density lipoprotein and uric acid analysis, \( n = 209 \) and 143 Caucasian cases and controls, respectively, and \( n = 382 \) and 341 Māori and Pacific cases and controls, respectively. For lipoprotein(a) analysis, \( n = 138 \) and 126 Caucasian cases and controls, respectively, and \( n = 256 \) and 309 Māori and Pacific cases and controls, respectively.
As lipid levels have been associated with APOE isoforms, TG levels were compared between subjects grouped by APOE isoforms. The frequencies of the APOE alleles in this analysis (Table 9) were similar to the full genotype analysis frequencies (see section 3.2.3), where Caucasians had relatively higher, equal and lower frequencies of APOE3, APOE2 and APOE4 alleles, respectively, when compared to Māori and Pacific subjects. In Māori and Pacific gout cases, carriers of the APOE2 and APOE4 isoforms had significantly higher TG levels (2.91 mmol/L and 2.82 mmol/L, respectively) than APOE3 homozygotes (2.30 mmol/L, p = 0.0475 and 0.0489 between APOE3 homozygotes and APOE2 or APOE4 carriers, respectively; Table 9). For Māori/Pacific gout controls and Caucasian gout cases, TG levels were significantly higher in APOE4 carriers (2.13 mmol/L and 2.66 mmol/L, respectively) than APOE3 homozygotes (1.70 mmol/L and 2.46 mmol/L, respectively, p = 0.0035 and 0.0490, respectively; Table 9). Additionally for the Māori and Pacific gout controls, the TG level in the APOE4 carriers was significantly higher than in the APOE2 carriers (where the TG level was 1.65 mmol/L; p = 0.0138). No other differences were found in the TG level between different APOE isoform groups (Table 9). A small number of subjects were carriers of both APOE2 and APOE4 alleles (compound heterozygotes; Table 9). To rule out possible conflicting effects between the two genotypes, analysis was repeated after the exclusion of these subjects. The p value when comparing APOE4 carriers and APOE3 homozygotes in Caucasian gout cases shifted to non-significance (p = 0.0643). Otherwise, no changes in significance were witnessed.

To increase subject numbers for TG level comparisons between APOE genotype isoforms, the Māori/Pacific and Caucasian gout cases were combined into one group, and the Māori/Pacific and Caucasian gout controls were combined into another group. This was justified by a lack of significant differences in the TG level between these groups (p = 0.2435 and 0.0951, respectively; Table 7). Results showed a significantly higher TG level in APOE4 carriers when compared with APOE3 homozygotes in both combined case and control groups (APOE4 carriers in cases = 2.78 mmol/L, APOE3 homozygotes in cases = 2.37 mmol/L, p = 0.0063; APOE4 carriers in controls = 2.08 mmol/L, APOE3 homozygotes in controls = 1.66 mmol/L, p = 0.0002; Table 9). Results did not significantly change when APOE2 and APOE4 compound heterozygotes were excluded. In combined controls, APOE4 carriers also had a higher TG level than APOE2 carriers (TG = 1.71 mmol/L in APOE2 carriers; p = 0.0443; Table 9).
Few studies have reported UA levels within groups of different APOE genotypes, therefore UA levels were compared between APOE isoform groups. Māori and Pacific gout cases that were APOE4 carriers had significantly higher UA levels (0.46 mmol/L) than APOE3 homozygotes (0.42 mmol/L; $p = 0.0225$; Table 9). Excluding APOE2 and APOE4 compound heterozygotes from analysis did not change the significance of the $p$ value, nor did the $p$ value change after adjusting for TG levels. Otherwise, no significant differences in UA levels were found between the APOE isoforms in gout cases or controls (Table 9).

Table 9. Triglyceride and uric acid levels when stratified by APOE isoforms.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Māori and Pacific</th>
<th>Caucasian</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td>Triglyceride (mmol/L, frequency)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE3</td>
<td>2.30 (0.406)</td>
<td>1.70 (0.535)</td>
<td>2.46 (0.610)</td>
</tr>
<tr>
<td>APOE2</td>
<td>2.91 (0.496)*</td>
<td>1.65 (0.196)</td>
<td>2.17 (0.132)</td>
</tr>
<tr>
<td>APOE4</td>
<td>2.82 (0.496)*</td>
<td>2.13 (0.515)</td>
<td>2.66 (0.287)*</td>
</tr>
<tr>
<td>Uric acid (mmol/L, frequency)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE3</td>
<td>0.42 (0.406)</td>
<td>0.35 (0.535)</td>
<td>0.41 (0.610)</td>
</tr>
<tr>
<td>APOE2</td>
<td>0.43 (0.150)</td>
<td>0.35 (0.196)</td>
<td>0.37 (0.132)</td>
</tr>
<tr>
<td>APOE4</td>
<td>0.46 (0.496)*</td>
<td>0.35 (0.515)</td>
<td>0.39 (0.287)</td>
</tr>
</tbody>
</table>

*Mann-Whitney $p < 0.05$ when compared with APOE3 homozygotes (APOE3).
*Mann-Whitney $p < 0.01$ when compared with APOE3.
*Mann-Whitney $p < 0.05$ when compared with APOE2 carriers (APOE2).
Analysis displayed in this table included subjects who were compound heterozygotes for APOE2 and APOE4, except when comparing APOE2 with APOE4 carriers (APOE4). When the APOE2 and APOE4 compound heterozygotes were removed from analysis, the significance in results did not change, except for Caucasian cases when comparing APOE4 to APOE3 where the Mann-Whitney $p$ became 0.0643. For APOE3, APOE2 and APOE4, $n = 95, 35$ and $116$ for Māori and Pacific cases, $n = 83, 46$ and $121$ for Māori and Pacific controls, $n = 83, 18$ and $39$ for Caucasian cases, and $n = 68, 24$ and $38$ for Caucasian controls, all respectively. For compound heterozygotes of APOE2 and APOE4, $n (frequency) = 12 (0.051)$ for Māori and Pacific cases, $15 (0.064)$ for Māori and Pacific controls, $4 (0.029)$ for Caucasian cases, and $6 (0.048)$ for Caucasian controls.

### 3.2. Lipoprotein analyses in Māori and Pacific subjects

Plasma from a subset of Māori and Pacific subjects underwent FPLC analysis. FPLC was not conducted on Caucasian samples due to time constraints and the precedence to focus on Māori and Pacific samples. TG and cholesterol content was measured in collected FPLC fractions and visualized with traces (Appendix B.I). Total TG and cholesterol concentrations of FPLC fractions and plasma samples were compared to values obtained from serum samples.
measured by SCL. SCL concentrations were generally higher than FPLC concentrations, although with high variability (SD were from 24.4% to 39.3%; Table 10). When FPLC and SCL lipid ratios were compared (namely the HDL cholesterol or LDL cholesterol to total cholesterol ratios), values were more similar, albeit still with high variability (SD were from 24.7% to 27.0%; Table 10). Total plasma TG and cholesterol concentrations from TG and cholesterol assays had values closer to SCL results, and had less variability between samples (SD were from 12.9% to 20.6%; Table 10). The mean percentage recovery of TG and cholesterol from the FPLC was calculated to be 68.9% (SD = 11.7%) and 72.6% (SD = 13.3%), respectively.

As FPLC is not a standardized method for lipid quantification, lipid ratios rather than absolute lipid values were used for further analyses between Māori and Pacific gout cases and controls. Significant differences were found in the VLDL TG to total TG ratio, LDL TG to total TG ratio and LDL TG to VLDL TG ratio between cases and controls (p values ranged from 0.0002 to 0.0114; Table 11). Compared to controls, cases had a higher percentage of TG in VLDL fractions (59.6% in cases compared to 45.4% in controls), and less TG in LDL fractions (27.7% in cases compared to 38.0% in controls; Table 11). This could be visualized with TG lipoprotein traces of FPLC fractions (Figure 7). There was a significant correlation between the VLDL TG to total TG ratio and the plasma total TG level in cases (Pearson’s correlation coefficient = 0.7168, p < 0.01) and controls (Pearson’s correlation coefficient = 0.5887, p < 0.01). This indicated that the relative amount of VLDL TG is proportional to the total TG level, and that VLDL TG was likely to have an influence on increasing total TG levels, especially for cases. There were no significant differences in the VLDL cholesterol to total cholesterol (p = 0.1253) or to total TG (p = 0.6848) ratios between cases and controls (Table 11).

<p>| Table 10. Percentage of Southern Community Laboratories lipid values to fast protein liquid chromatography and triglyceride/cholesterol assays values. |
|---------------------------------|----------------|----------|---------|---------|---------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Assay</th>
<th>Statistic</th>
<th>Total chol</th>
<th>Total TG</th>
<th>HDL chol</th>
<th>LDL chol</th>
<th>Total_chol : HDL_chol</th>
<th>LDL_chol : total_chol</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPLC</td>
<td>Mean</td>
<td>131.1</td>
<td>153.3</td>
<td>118.5</td>
<td>117.5</td>
<td>112.8</td>
<td>92.2</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>29.3</td>
<td>39.3</td>
<td>24.4</td>
<td>33.6</td>
<td>24.7</td>
<td>27.0</td>
</tr>
<tr>
<td>TG and chol assays</td>
<td>Mean</td>
<td>92.5</td>
<td>103.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>12.9</td>
<td>20.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

NB: chol = cholesterol. TG = triglyceride. HDL = high density lipoprotein. LDL = low density lipoprotein. FPLC = fast protein liquid chromatography. SD = standard deviation.
Table 11. Fast protein liquid chromatography lipid ratio comparisons in Māori and Pacific gout cases and controls.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Statistic</th>
<th>VLDL&lt;sub&gt;chol&lt;/sub&gt;:total&lt;sub&gt;chol&lt;/sub&gt;</th>
<th>VLDL&lt;sub&gt;chol&lt;/sub&gt;:total&lt;sub&gt;TG&lt;/sub&gt;</th>
<th>LDL&lt;sub&gt;TG&lt;/sub&gt;:VLDL&lt;sub&gt;TG&lt;/sub&gt;</th>
<th>VLDL&lt;sub&gt;TG&lt;/sub&gt;:total&lt;sub&gt;TG&lt;/sub&gt;</th>
<th>LDL&lt;sub&gt;TG&lt;/sub&gt;:total&lt;sub&gt;TG&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>Mean</td>
<td>0.158</td>
<td>0.127</td>
<td>0.565</td>
<td>0.596</td>
<td>0.277</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.134</td>
<td>0.037</td>
<td>0.419</td>
<td>0.158</td>
<td>0.110</td>
</tr>
<tr>
<td>Controls</td>
<td>Mean</td>
<td>0.109</td>
<td>0.137</td>
<td>5.549</td>
<td>0.454</td>
<td>0.380</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.069</td>
<td>0.052</td>
<td>28.468</td>
<td>0.161</td>
<td>0.157</td>
</tr>
<tr>
<td>p (MW)</td>
<td></td>
<td>0.2918</td>
<td>0.2764</td>
<td>0.0004</td>
<td>0.0004</td>
<td>0.0011</td>
</tr>
<tr>
<td>p (t test)*</td>
<td></td>
<td>0.1253</td>
<td>0.6848</td>
<td>0.0006</td>
<td>0.0114</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

*On log transformed values. VLDL = very low density lipoprotein. Chol = cholesterol. LDL = low density lipoprotein. TG = triglyceride. SD = standard deviation. MW = Mann-Whitney. The high variability for LDL<sub>TG</sub>:VLDL<sub>TG</sub> in gout controls was due to an extreme outlier with a LDL<sub>TG</sub>:VLDL<sub>TG</sub> value of 181.000. After excluding the outlier, mean = 1.050 and SD = 0.948.

Figure 7. Representative triglyceride lipoprotein traces of a gout case and a gout control. Lipoproteins in plasma samples were separated (as indicated) by fast protein liquid chromatography and collected into 0.5 mL fractions. Each fraction was assayed for triglyceride (TG) concentration. See sections 2.3.1 and 2.3.2 for details. Notice the difference in TG levels between the case and control fractions of very low density lipoprotein (VLDL) and low density lipoprotein (LDL). HDL = high density lipoprotein.

VLDL particles contain only one copy of apo B each (Elovson et al., 1988), therefore the concentration of apo B in eluted FPLC VLDL fractions would be reflective of the number of VLDL particles. Increased TG levels in gout cases, especially from the VLDL fractions of the FPLC, prompted the investigation of apo B levels in total plasma and VLDL fractions. This would allow for insight into whether VLDL particles were more TG-rich, or increased in number. The apo B quality control Cfas Lipid (Cobas®, Roche/Hitachi) had a mean
concentration of 2005.4 nmol/L across assays (SD = 305.4 nmol/L), and an inter-assay coefficient of variation of 0.161 (Table 12). The mean intra-assay coefficient of variation for the apo B results was 0.038.

The total plasma apo B level was significantly increased ($p = 2.86 \times 10^{-5}$) in gout cases compared to controls (Table 13). There were no significant differences between cases and controls for the VLDL TG to VLDL apo B ratio ($p = 0.3345$), indicating that VLDL TG increased as VLDL apo B increased for both case and control sample sets. This also indicated that the increased TG level in VLDL particles in cases was reflected by a higher number of VLDL particles, rather than TG-enriched particles. Added to this, VLDL apo B content was increased ($p = 0.0394$) in gout cases (97.6 nmol/L) when compared to controls (51.4 nmol/L; Table 13). To account for variability in FPLC elution, VLDL apo B was also expressed as a ratio to total plasma apo B that had been adjusted for the recovery of lipids from the FPLC. However, no significant differences were found between cases and controls for the VLDL apo B to total apo B ratio ($p = 0.1467$; Table 13).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Quality control</th>
<th>Mean*</th>
<th>SD*</th>
<th>Minimum*</th>
<th>Maximum*</th>
<th>Known*</th>
<th>CV^</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apolipoprotein B</td>
<td>Cfas Lipid</td>
<td>2005.4</td>
<td>305.4</td>
<td>1730.4</td>
<td>2455.9</td>
<td>2185.0</td>
<td>0.161</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td></td>
<td>7.2</td>
<td>1.2</td>
<td>4.9</td>
<td>10.0</td>
<td>10.6</td>
<td>0.160</td>
</tr>
<tr>
<td>L2</td>
<td></td>
<td>21.4</td>
<td>2.3</td>
<td>16.2</td>
<td>26.3</td>
<td>25.5</td>
<td>0.107</td>
</tr>
<tr>
<td>L3</td>
<td></td>
<td>38.8</td>
<td>5.4</td>
<td>30.4</td>
<td>53.7</td>
<td>40.2</td>
<td>0.139</td>
</tr>
<tr>
<td>L4</td>
<td></td>
<td>57.8</td>
<td>7.2</td>
<td>44.0</td>
<td>71.3</td>
<td>66.7</td>
<td>0.124</td>
</tr>
</tbody>
</table>

*Concentration of the quality control across assays in nmol/L. ^Known concentration of the quality control in nmol/L. *Inter-assay coefficient of variation. The mean intra-assay coefficient of variation for apolipoprotein B was 0.038, and for lipoprotein(a) was 0.046. Cfas Lipid was obtained from Cobas®, Roche/Hitachi. L1, L2, L3 and L4 were obtained from the Northwest Lipid Research Labs (Marcovina et al., 1995). SD = standard deviation.

Across VLDL TG to total TG ratio quartiles, there was a significant increase in the absolute apo B concentration in VLDL fractions for gout cases ($p = 0.0001$) and controls ($p = 0.0219$; Figure 8). Across the same quartiles, there was also a significant increase in VLDL apo B to total apo B ratio in cases ($p = 0.0003$) and controls ($p = 0.0037$; Figure 8). This indicated that individuals with a higher TG level in VLDL, and hence increased total TG, was reflected by
an increased number of VLDL particles rather than TG-enriched VLDL particles. As determined by the Pearson’s correlation coefficient (ranging from 0.4864 to 0.5790), these positive associations were significantly correlated ($p < 0.01$; Figure 8).

Table 13. Apolipoprotein B measurements in Māori and Pacific gout cases and controls.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Statistic</th>
<th>Total apo B (nmol/L)</th>
<th>VLDL apo B (nmol/L)</th>
<th>VLDL apo B : total apo B</th>
<th>VLDL apo B : VLDL TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>Mean</td>
<td>1621.7</td>
<td>97.6</td>
<td>0.0978</td>
<td>45.4</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>377.6</td>
<td>84.5</td>
<td>0.0915</td>
<td>48.2</td>
</tr>
<tr>
<td>Controls</td>
<td>Mean</td>
<td>1291.3</td>
<td>51.4</td>
<td>0.0528</td>
<td>40.3</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>319.8</td>
<td>40.1</td>
<td>0.0346</td>
<td>37.0</td>
</tr>
<tr>
<td>p (MW)</td>
<td></td>
<td>0.0001</td>
<td>0.0174</td>
<td>0.1057</td>
<td>0.5239</td>
</tr>
<tr>
<td>p (t test)*</td>
<td></td>
<td>$2.86 \times 10^{-5}$</td>
<td>0.0394</td>
<td>0.1467</td>
<td>0.3345</td>
</tr>
</tbody>
</table>

*On log transformed values. Apo B = apolipoprotein B. VLDL = very low density lipoprotein. TG = triglyceride. SD = standard deviation. MW = Mann-Whitney.

3.2.1. Multivariate analysis on lipids, lipoproteins and apolipoprotein B

Logistic regression accounting for possible confounders was conducted on lipoprotein and apo B parameters. Covariates found to be significantly different between gout cases and controls were adjusted for in the multivariate model. Specifically, these covariates were serum UA levels, plasma Lp(a) levels, the presence of hypertension, the number of Māori and Pacific grandparents, and fruit consumption (Table 14). Variables not found to be significantly different between cases and controls were age, sex, the presence of TIIDM and unspecified heart problems, waist circumference, alcohol and sugar drink consumption, and ethnicity (EP, WP or mixed Polynesian; Table 14), therefore these variables were not included in the multivariate model.

After the multivariate adjustment for covariates, the plasma levels of total TG ($p = 0.0188$, OR [95% CI] = 1.009 [1.001 - 1.016]), total apo B ($p = 0.0039$, OR [95% CI] = 1.004 [1.001 - 1.006]), VLDL TG to total TG ratio ($p = 0.0178$, OR [95% CI] = 1.057 [1.009 - 1.107]) and VLDL apo B ($p = 0.0178$, OR [95% CI] = 1.020 [1.003 - 1.036]) remained significantly higher in gout cases than controls (Table 15). The LDL TG to total TG ratio ($p = 0.0536$, OR [95% CI] = 0.003 [5.96 × 10^{-6} - 1.114]) and the VLDL apo B to total apo B ratio ($p = 0.0819$, OR [95% CI] = 1.011 [0.998 - 1.023]) were also significantly different between cases and controls.
[95% CI] = 3.99 × 10^3 [0.191 - 8.32 × 10^{11}] were approaching a significant difference between cases and controls (Table 15). There were no significant differences between cases and controls for the VLDL TG to VLDL apo B ratio ($p = 0.5687$; Table 15).

![Figure 8](image-url)

Figure 8. Apolipoprotein B measurements and analysis of variance across very low density lipoprotein triglyceride to total triglyceride ratio quartiles. The numbers in columns represent the mean value for the corresponding group. $r$ = Pearson’s correlation coefficient. For all $r$, $p < 0.01$. Apo B = apolipoprotein B. ANOVA = analysis of variance. VLDL = very low density lipoprotein. TG = triglyceride.

3.2.2. Type IV hyperlipoproteinemia classification

With lipid and apolipoprotein results, TIVH criteria were established and compared. This involved incorporating various combinations of three particular components: total TG, VLDL
and LDL. Unfortunately, CM lipid and CM apo B values were not assayed for, therefore western blotting was conducted to investigate the presence of the CM specific apo B48 band in plasma (Figure 9). Only a total of three gout cases and three gout controls had an apo B48 band. To account for this, these subjects were excluded from analysis.

Table 14. Clinical details for Māori and Pacific gout cases and controls with fast protein liquid chromatography analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Statistic</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>43.2</td>
<td>42.1</td>
</tr>
<tr>
<td>Sex</td>
<td>% male</td>
<td>79.5</td>
<td>71.8</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>Mean</td>
<td>109.9</td>
<td>108.1</td>
</tr>
<tr>
<td>Heart problems</td>
<td>% yes</td>
<td>5.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Kidney problems</td>
<td>% yes</td>
<td>0.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Hypertension</td>
<td>% yes</td>
<td>38.5</td>
<td>15.4</td>
</tr>
<tr>
<td>Type II diabetes mellitus</td>
<td>% yes</td>
<td>13.2</td>
<td>7.5</td>
</tr>
<tr>
<td>Plasma lipoprotein(a) (nmol/L)</td>
<td>Median</td>
<td>8.3</td>
<td>12.1</td>
</tr>
<tr>
<td>Serum uric acid (mmol/L)</td>
<td>Mean</td>
<td>0.48</td>
<td>0.37</td>
</tr>
<tr>
<td>Fruit consumption (pieces per day)</td>
<td>Mean</td>
<td>1.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Sugar drink consumption (drinks per day)</td>
<td>Mean</td>
<td>3.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Alcohol consumption (drinks per week)</td>
<td>Mean</td>
<td>7.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Number of Māori and Pacific grandparents</td>
<td>Mean</td>
<td>3.8</td>
<td>3.3</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>% Western Polynesian</td>
<td>66.7</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td>% Eastern Polynesian</td>
<td>33.3</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td>% mixed Polynesian</td>
<td>0.0</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Mann-Whitney test. ⁷Fisher’s exact test. ⁸Chi squared test. All variables were self-reported except for plasma lipoprotein(a) and serum uric acid levels. For heart and kidney problems, the type of problem was unspecified.

Table 15. Logistic regression analysis between Māori and Pacific gout cases and controls on lipoprotein parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL apo B</td>
</tr>
<tr>
<td>Total apo B</td>
</tr>
<tr>
<td>Total TG</td>
</tr>
<tr>
<td>VLDL TG : total TG</td>
</tr>
<tr>
<td>VLDL apo B : total apo B</td>
</tr>
<tr>
<td>LDL TG : total TG</td>
</tr>
<tr>
<td>VLDL TG : VLDL apo B</td>
</tr>
</tbody>
</table>

NB: covariates adjusted for were the presence of hypertension, serum uric acid level, plasma lipoprotein(a) level, fruit consumption, and the number of Māori and Pacific grandparents (see section 3.2.1). VLDL = very low density lipoprotein. Apo B = apolipoprotein B. TG = triglyceride. LDL = low density lipoprotein.
The percentages of subjects classified with TIVH in the various criteria are shown in Table 16. For criterion 1a, 47.2% and 10.8% of gout cases and controls were classified with TIVH, respectively ($p = 0.0014, \text{OR [95\% CI]} = 7.382 [2.165 - 25.172]$). When the LDL TG to total TG ratio was substituted by LDL TG (criterion 1b), the percentages classified with TIVH decreased to 36.1% in cases and 8.1% in controls, indicating that this was a more stringent criterion. When LDL cholesterol was used (criterion 1c), the percentage of subjects classified with TIVH decreased even further to 30.6% in cases and 5.4% in controls. In criterion 1d where the absolute value of VLDL TG was used instead of the VLDL TG to total TG ratio, the number of controls classified with TIVH increased in comparison to criterion 1a to 18.9%, whereas one less case was classified (44.4%).

In criterion 2, VLDL apo B to total apo B ratio was used for the VLDL component of the classification system. With this, one less gout case was classified when compared with criterion 1a. Furthermore, two cases were differentially classified between the two criteria, where one subject was classified as TIVH by criterion 1a but not by criterion 2, and vice versa. The percentage of gout controls classified with TIVH by criterion 2 remained the same as criterion 1a (Table 16). However, there were two differentially classified controls between the two criteria, with one subject classified as TIVH by criterion 1a but not by criterion 2, and vice versa.
Table 16. Percentage of Māori and Pacific gout cases and controls classified with type IV hyperlipoproteinemia by various criteria.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Components*</th>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
<th>Odds ratio [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Total TG, VLDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt;, LDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt;</td>
<td>47.2</td>
<td>10.8</td>
<td>0.0014</td>
<td>7.382 [2.165 - 25.172]</td>
</tr>
<tr>
<td>1b</td>
<td>Total TG, VLDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt;, LDL TG</td>
<td>36.1</td>
<td>8.1</td>
<td>0.0076</td>
<td>6.406 [1.640 - 25.016]</td>
</tr>
<tr>
<td>1c</td>
<td>Total TG, VLDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt;, LDL cholesterol</td>
<td>30.6</td>
<td>5.4</td>
<td>0.0121</td>
<td>7.700 [1.568 - 37.821]</td>
</tr>
<tr>
<td>1d</td>
<td>Total TG, VLDL TG, LDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt;</td>
<td>44.4</td>
<td>18.9</td>
<td>0.0220</td>
<td>3.429 [1.196 - 9.828]</td>
</tr>
<tr>
<td>2</td>
<td>Total TG, VLDL&lt;sub&gt;apoB&lt;/sub&gt; : total&lt;sub&gt;apoB&lt;/sub&gt;, LDL&lt;sub&gt;TG&lt;/sub&gt; : total&lt;sub&gt;TG&lt;/sub&gt;</td>
<td>44.4</td>
<td>10.8</td>
<td>0.0026</td>
<td>6.600 [1.932 - 22.544]</td>
</tr>
<tr>
<td>3a</td>
<td>Total TG, LDL cholesterol</td>
<td>33.3</td>
<td>16.2</td>
<td>0.0949</td>
<td>2.583 [0.847 - 7.882]</td>
</tr>
<tr>
<td>3b</td>
<td>Total apo B, LDL cholesterol</td>
<td>27.8</td>
<td>10.8</td>
<td>0.0751</td>
<td>3.173 [0.893 - 11.280]</td>
</tr>
<tr>
<td>3c</td>
<td>Total TG and apo B, LDL cholesterol</td>
<td>19.4</td>
<td>5.4</td>
<td>0.0873</td>
<td>4.224 [0.814 - 21.923]</td>
</tr>
</tbody>
</table>

*For the full details of the criteria, see Table 4. n = 37 cases and 36 controls after the exclusion of subjects with an apolipoprotein B48 band as determined by western blots (see section 2.3.5). CI = confidence interval. TG = triglyceride. VLDL = very low density lipoprotein. LDL = low density lipoprotein. Apo B = apolipoprotein B.

Criteria 3a, 3b and 3c investigated TIVH classification using values that did not require FPLC methodology. None of these criteria showed significant differences between gout cases and controls for the number of subjects classified with TIVH (p > 0.05, OR ranged from 2.583 to 4.224; Table 16). Furthermore, a large number of the subjects classified as TIVH by these criteria were different subjects to the ones classified as TIVH by criterion 1a. For criterion 3a, there were seven differentially classified cases and five differentially classified controls when compared to criterion 1a. The use of total apo B (criterion 3b) as a surrogate for total TG (criterion 3a) gave 15 differentially classified cases and five differentially classified controls when compared to criterion 1a. Even when criterion 3b was compared to criterion 3a, there were eight differentially classified cases and six differentially classified controls. Criterion 3c, which used both total apo B and total TG measurements, classified fewer subjects with TIVH than any other criterion (Table 16). This was due to the stringent nature of the criterion resulting from the differentially classified subjects between criteria 3a and 3b. A total of 12 cases and four controls were differentially classified between the criteria 1a and 3c. A total of five cases and four controls were differentially classified between the criteria 3a and 3c.
Plasma samples were run on lipoprotein gels to investigate lipoprotein banding patterns and to detect for the presence of CM. A variety of banding patterns were observed (Figure 10). For example, some samples had little or no pre-β band, some samples had an increased pre-β band, with or without the β band, and some samples had smearing between the pre-β and β band (Figure 10). For purposes of comparison to the TIVH criteria outlined in Table 4, samples with an increased intensity in the pre-β area, relative to the β area, were considered to have an increased VLDL TG level and hence classified as TIVH (Figure 10), as the stain used (Fat Red 7B) has a high affinity to TG. The percentage match rate is shown in Table 17. Criterion 3a had the highest match percentage (79.5%) with lipoprotein gel results. The lowest match percentage was 65.8% for criterion 3b.

Many samples also appeared to have bands at the origin (Figure 10), indicating the presence of CM. However, these samples did not match well with samples from subjects identified to have the presence of apo B48 in the western blot (Figure 10).

Caution needs to be placed on lipoprotein gel results. Banding patterns were very difficult to interpret; pattern interpretation was easily subjective to opinion and open to bias when judged by the eye.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Percentage match</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>72.6</td>
</tr>
<tr>
<td>1b</td>
<td>74.0</td>
</tr>
<tr>
<td>1c</td>
<td>75.3</td>
</tr>
<tr>
<td>1d</td>
<td>78.1</td>
</tr>
<tr>
<td>2</td>
<td>74.0</td>
</tr>
<tr>
<td>3a</td>
<td>79.5</td>
</tr>
<tr>
<td>3b</td>
<td>65.8</td>
</tr>
<tr>
<td>3c</td>
<td>72.6</td>
</tr>
</tbody>
</table>
3.2.3. APOE genotypic isoform analysis

For the EP sample set, the SNP rs429358 of APOE (corresponding to the APOE4 isoform) nearly reached significance for association with gout after adjustment for Caucasian ancestry ($p = 0.052$, OR [95% CI] = 1.35 [1.04 - 1.75]; Table 18). Otherwise, no significant associations with gout were found in the two APOE SNPs (rs429358 and rs7412) in any sample set, even after stratification analysis in the Māori, EP and WP groups (Table 18). All sample sets were in Hardy-Weinberg equilibrium ($p > 0.05$). Interestingly, the minor allele C frequency for rs429358 was notably higher in the Māori, EP and WP sample sets (24% to 36%) compared to
the Caucasian sample set (approximately 16%; Table 18). The Caucasian allele C frequencies from several studies summarized by Davignon et al. (1988) were approximately 13% to 16% (although a Finland sample set did reach 23%), and that reported by the 1000 genomes dataset (http://asia.ensembl.org/index.html) was 9% (Table 19). The variation in the allele C frequency of rs429358 in Caucasian populations is unclear.

Table 18. Association analyses with gout for single nucleotide polymorphisms in APOE.

<table>
<thead>
<tr>
<th>rs429358</th>
<th>Sample set</th>
<th>Genotype (n, freq)</th>
<th>Allele C (n, freq)</th>
<th>OR[95% CI]</th>
<th>p*</th>
<th>p#</th>
<th>OR[95% CI]^</th>
<th>p^</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>TC</td>
<td>CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>225(0.688)</td>
<td>94(0.287)</td>
<td>8(0.024)</td>
<td>110(0.168)</td>
<td>1.13[0.87-1.45]</td>
<td>0.364</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>459(0.721)</td>
<td>162(0.254)</td>
<td>16(0.025)</td>
<td>194(0.152)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Māori</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>72(0.468)</td>
<td>65(0.422)</td>
<td>17(0.110)</td>
<td>99(0.321)</td>
<td>1.14[0.85-1.52]</td>
<td>0.392</td>
<td>0.616</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>169(0.500)</td>
<td>139(0.411)</td>
<td>30(0.089)</td>
<td>199(0.294)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eastern Polynesian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>87(0.416)</td>
<td>95(0.455)</td>
<td>27(0.129)</td>
<td>149(0.356)</td>
<td>1.35[1.04-1.75]</td>
<td>0.024</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>173(0.504)</td>
<td>140(0.408)</td>
<td>30(0.087)</td>
<td>200(0.292)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Western Polynesian</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>146(0.586)</td>
<td>87(0.349)</td>
<td>16(0.064)</td>
<td>119(0.239)</td>
<td>1.01[0.71-1.42]</td>
<td>0.966</td>
<td>0.635</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>84(0.596)</td>
<td>47(0.333)</td>
<td>10(0.071)</td>
<td>67(0.238)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs7412</td>
<td>Sample set</td>
<td>Genotype (n, freq)</td>
<td>Allele T (n, freq)</td>
<td>OR[95% CI]</td>
<td>p*</td>
<td>p#</td>
<td>OR[95% CI]^</td>
<td>p^</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>280(0.856)</td>
<td>44(0.135)</td>
<td>3(0.009)</td>
<td>50(0.076)</td>
<td>0.90[0.63-1.30]</td>
<td>0.573</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>537(0.842)</td>
<td>95(0.149)</td>
<td>6(0.009)</td>
<td>107(0.084)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Māori</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>129(0.843)</td>
<td>23(0.150)</td>
<td>1(0.007)</td>
<td>25(0.082)</td>
<td>0.80[0.50-1.29]</td>
<td>0.363</td>
<td>0.427</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>276(0.812)</td>
<td>60(0.176)</td>
<td>4(0.012)</td>
<td>68(0.100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eastern Polynesian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>178(0.856)</td>
<td>29(0.139)</td>
<td>1(0.005)</td>
<td>31(0.075)</td>
<td>0.79[0.50-1.23]</td>
<td>0.288</td>
<td>0.322</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>283(0.823)</td>
<td>58(0.169)</td>
<td>3(0.009)</td>
<td>64(0.093)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Western Polynesian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>199(0.796)</td>
<td>50(0.200)</td>
<td>1(0.004)</td>
<td>52(0.104)</td>
<td>0.80[0.51-1.26]</td>
<td>0.332</td>
<td>0.317</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>108(0.761)</td>
<td>32(0.225)</td>
<td>2(0.014)</td>
<td>36(0.127)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NB: p values presented are allelic p values. *Raw p value. ^p value after adjustment for Caucasian ancestry in the Māori, Eastern Polynesian and Western Polynesian sample sets. *Results after multiple logistic regression, where covariates adjusted for were age, sex, waist circumference and the presence of type II diabetes mellitus, hyperlipidemia and heart problems. Only the Aotearoa controls were used for logistic regression analysis, as there was limited demographic information in the New Zealand Caucasian, Māori and Pacific controls (see section 2.4.5). All sample sets were in Hardy-Weinberg equilibrium (p > 0.05). OR = odds ratio. CI = confidence interval.
Table 19. Minor allele frequencies from various sources for single nucleotide polymorphisms in APOE and LPA.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>rs429358</td>
<td>0.092</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.064 - 0.227</td>
</tr>
<tr>
<td>rs7412</td>
<td>0.087</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.000 - 0.130</td>
</tr>
<tr>
<td>rs3798220</td>
<td>0.000</td>
<td>0.025</td>
<td>0.02</td>
<td>0.02</td>
<td>-</td>
</tr>
<tr>
<td>rs10455872</td>
<td>0.083</td>
<td>-</td>
<td>0.07</td>
<td>0.08</td>
<td>-</td>
</tr>
</tbody>
</table>

*CEU population (Caucasian) data taken from 1000 genomes as of 28/2/2011, accessed via http://asia.ensembl.org/index.html. #Caucasian populations. ^Multi-racial populations, including American Indian, Caucasian, Chinese and Japanese populations. SNP = single nucleotide polymorphism.

For rs429358, the power to detect for association with gout at an OR of 1.2 was very low, ranging from 19.6% to 28.2% across sample sets (Table 20). However, at an OR of 1.6 or above, the power to detect for association increased to above 82.6% in all sample sets (Table 20). For the SNP rs7412, the power to detect for association with gout was low for the Caucasian sample set at an OR of 1.2 (18.4%), however increased to 84.1% at an OR of 1.6, and above 99% for OR of 2.0 and over (Table 20). For the Māori, EP and WP sample sets, the power to detect for association between rs7412 and gout remained relatively low at an OR of 1.6 (approximately 60%), but increased to above 90% at an OR of 2.0 and above (Table 20).

Logistic regression analysis was also conducted in allelic analysis (see section 2.4.5). In the Māori sample set, the minor allele C of the APOE SNP rs429358 achieved a significantly protective effect towards gout ($p = 0.027$, OR [95% CI] = 0.57 [0.35 - 0.94]; Table 18). Otherwise, no other sample set reached significance with rs429358 or rs7412 (Table 18).

Imputation for generating genotype data for FHS and ARIC datasets was attempted for the SNPs in APOE. However, data generated had frequencies that differed to expected frequencies. Frequencies were expected to be similar to the Caucasian sample set, as the FHS and ARIC subjects used in analysis were Caucasian in ethnicity. The inconsistent imputation results in APOE lead to the investigation of the LD between the SNPs of interest and the surrounding SNPs in the APOE gene. Unfortunately, a LD plot could not be generated, as data from only three SNPs (rs405509, rs769450 and rs769451), and not rs429358 or rs7412, could be extracted from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/).
Table 20. Power to detect for association with gout for genotyped single nucleotide polymorphisms.

<table>
<thead>
<tr>
<th>SNP</th>
<th>OR</th>
<th>Power at the significance level of 0.05 (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
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<tr>
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<td></td>
<td>Caucasian</td>
<td>Māori</td>
<td>EP</td>
<td>WP</td>
<td>FHS</td>
<td>ARIC</td>
</tr>
<tr>
<td>rs479358</td>
<td>1.2</td>
<td>28.2</td>
<td>22.9</td>
<td>27.3</td>
<td>19.6</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>1.6</td>
<td>96.6</td>
<td>90.1</td>
<td>94.9</td>
<td>82.6</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>2.0</td>
<td>100.0</td>
<td>99.8</td>
<td>100.0</td>
<td>99.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs7412</td>
<td>1.2</td>
<td>18.4</td>
<td>12.1</td>
<td>13.7</td>
<td>14.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>84.1</td>
<td>59.9</td>
<td>67.1</td>
<td>65.7</td>
<td>-</td>
<td>-</td>
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<td></td>
<td>2.0</td>
<td>99.6</td>
<td>93.1</td>
<td>96.0</td>
<td>94.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>100.0</td>
<td>99.8</td>
<td>99.9</td>
<td>99.8</td>
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<td>-</td>
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<tr>
<td>rs3798220</td>
<td>1.2</td>
<td>7.4</td>
<td>5.4</td>
<td>5.9</td>
<td>8.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>31.2</td>
<td>17.8</td>
<td>20.9</td>
<td>35.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>64.6</td>
<td>38.4</td>
<td>44.5</td>
<td>66.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>90.9</td>
<td>66.1</td>
<td>72.8</td>
<td>90.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs10455872</td>
<td>1.2</td>
<td>15.5</td>
<td>6.1</td>
<td>7.0</td>
<td>5.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>75.5</td>
<td>22.5</td>
<td>28.3</td>
<td>16.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>98.5</td>
<td>48.5</td>
<td>58.9</td>
<td>31.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>100.0</td>
<td>77.8</td>
<td>86.2</td>
<td>52.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs1333049</td>
<td>1.26</td>
<td>67.1</td>
<td>36.2</td>
<td>42.3</td>
<td>33.4</td>
<td>26.6</td>
<td>51.3</td>
</tr>
</tbody>
</table>

NB: for the CDKN2BAS single nucleotide polymorphism (SNP) rs1333049, power was calculated at an odds ratio (OR) set to 1.26. This was chosen for the aim to replicate results from Wang et al. (2011), where association was found between the C allele of rs1333049 and increased gout risk (p = 0.01, OR [95% confidence interval] = 1.26 [1.06 - 1.54]). For LPA and APOE SNPs (rs3798220, rs10455872, rs479358 and rs7412), a range of power was shown for arbitrarily chosen OR, as no known studies have investigated the association between these SNPs and gout. EP = Eastern Polynesian. WP = Western Polynesian. FHS = Framingham Heart Study. ARIC = Atherosclerosis Risk in Communities.

3.3. Lipoprotein(a) results

3.3.1. Lipoprotein(a) western blots on serum samples

Lp(a) degradation in serum samples was investigated via western blots. Blots showed minor degradation in samples as seen by multiple faint bands below one or two main intense bands (Figure 11). The apo(a) positive control showed faintly in the blots, due to relatively lower concentrations of Lp(a) (approximately 15 nmol/L), even after being loaded undiluted onto the gels.
3.3.2. Analyses of lipoprotein(a) levels

The values for the Lp(a) quality controls are presented in Table 12. Across assays, L1, L2, L3 and L4 obtained from the Northwest Lipid Research Labs (Marcovina et al., 1995) had mean Lp(a) concentrations of 7.2 nmol/L, 21.4 nmol/L, 38.8 nmol/L and 57.8 nmol/L (with SD of 1.2 nmol/L, 2.3 nmol/L, 5.4 nmol/L and 7.2 nmol/L), respectively, and an inter-assay coefficient of variation ranging from 0.107 to 0.160 (Table 12). The mean intra-assay coefficient of variation for Lp(a) measurements was 0.046.

The distribution of Lp(a) levels stratified by ethnicity and gout affliction can be seen in Figure 12. Levels were highly skewed towards lower levels in all groups and ranged from zero to 650.5 nmol/L (Figure 12). Median Lp(a) levels were around 13 nmol/L for all groups, except Caucasian gout cases where the median was higher at 20.4 nmol/L (Figure 12). There were no significant differences in the Lp(a) level between EP and WP cases \( (p = 0.8843) \) or controls \( (p = 0.3621; \) Table 21), therefore an all Māori and Pacific group was formed for further analyses to increase sample size, including individuals of EP, WP or mixed Polynesian ancestry.

When comparing Lp(a) levels between ethnicities, there were no significant differences between Māori/Pacific and Caucasian gout controls \( (p = 0.2367) \), but a significant difference was borderline between Māori/Pacific and Caucasian gout cases \( (t \text{ test } p = 0.0893, \text{ MW } p = 0.0460; \) Table 21). When cases and controls were combined into one group, Caucasian subjects were
found to have a higher median Lp(a) level (18.7 nmol/L) than Māori and Pacific subjects (12.6 nmol/L, \( p = 0.0326 \); Table 21).

Lp(a) levels did not differ between gout cases or controls in Caucasian (\( p = 0.3593 \)) or Māori and Pacific (\( p = 0.6231 \)) sample sets (Table 21). As the presence of heart problems (which was self-reported and unspecified) may be a confounder for high Lp(a) levels in the case and control sample sets, this factor was investigated in subjects with high Lp(a) levels. Two of the six Māori and Pacific case outliers (Lp(a) values above the 97.5th percentile; Figure 12) reported to have heart problems, as did all three Caucasian case outliers. No outliers in the Māori and Pacific (\( n = seven \)) or Caucasian (\( n = three \)) control groups (Figure 12) reported to have heart problems. The percentage for heart problems in all sample sets were 13\% (\( n = 33 \) out of 256) in Māori and Pacific cases, 2.3\% (\( n = seven \) out of 302) in Māori and Pacific controls, 29\% (\( n = 30 \) out of 139) in Caucasian cases and 2.3\% (\( n = three \) out of 133) in Caucasian controls.

Lp(a) levels were further compared between subjects with or without self-reported and unspecified heart problems, irrespective of gout status (Figure 13). In Caucasians, there were no significant differences between subjects with (\( n = 43 \)) or without (\( n = 229 \)) heart problems (median Lp(a) levels were 25.2 nmol/L and 17.3 nmol/L, respectively, \( p = 0.1419 \); Figure 13, Table 21). In contrast, Māori and Pacific subjects with heart problems (\( n = 40 \)) had significantly higher Lp(a) levels than Māori and Pacific subjects without heart problems (\( n = 517 \), median Lp(a) levels were 22.8 nmol/L and 11.4 nmol/L, respectively, \( p = 0.0091 \); Figure 13, Table 21).

Lp(a) levels were not found to correlate with TG levels for any gout case or control sample set (Pearson’s correlation coefficient ranged from -0.0065 to 0.0085, \( p \) ranged from 0.4293 to 0.9211; Table 8).

Lp(a) levels were further investigated when stratified by alleles of SNPs in LPA (\( rs3798220 \) and \( rs10455872 \)) and APOE (\( rs429358 \) and \( rs7412 \)). Specifically, gout cases and controls were subgrouped into major homozygotes, or genotypes containing at least one minor allele. For LPA, this was done individually for each SNP, and with both SNPs combined. For APOE, subgroups of APOE3 homozygotes, subgroups of APOE2 carriers, and subgroups of APOE4 carriers, were formed. Distributions can be found in Figure 14 and Figure 15.

Very few subjects were found to be carriers of the minor alleles of the two LPA SNPs (Figure 14, Figure 15). Carrier numbers for gout case or control groups ranged from three to 21
for rs3798220, seven to 19 for rs10455872, and 20 to 28 when both SNPs were combined (Figure 14, Figure 15).

For the SNP rs10455872 in Caucasian gout cases and controls, significant differences in Lp(a) levels ($p = 2.71 \times 10^{-43}$ and $8.45 \times 10^{-6}$, respectively) were found between major homozygotes and the group with at least one minor allele (Table 21), where minor allele carriers had a higher medium Lp(a) level (193.5 nmol/L in heterozygote cases, 347.6 nmol/L in minor homozygote cases, 113.7 nmol/L in heterozygote controls) than major homozygotes (14.1 nmol/L in cases and 11.5 nmol/L in controls; Figure 14). This significant association was also true for the SNP rs3798220 for Caucasian cases (the median Lp(a) level was 274.4 nmol/L for heterozygotes and 20.3 nmol/L for major homozygotes, $p = 0.0117$; Figure 14, Table 21). However, significance was not reached in Caucasian controls (the median Lp(a) level was 204.4 nmol/L in heterozygotes and 13.1 nmol/L in major homozygotes, t test $p = 0.0864$, MW $p = 0.0752$; Figure 14, Table 21). The large difference in the median Lp(a) value was due to very low numbers (n = three) in the control heterozygote group.

For the Māori and Pacific gout cases and controls, the SNP rs10455872 reached significant differences in the Lp(a) level between major homozygotes (11.5 nmol/L in cases and 12.4 nmol/L in controls) and the group with at least one minor allele (312.5 nmol/L in cases and 203.6 nmol/L in controls, $p = 1.70 \times 10^{-6}$ for cases and $4.05 \times 10^{-6}$ for controls; Figure 15, Table 21). There were no significant differences between major homozygotes and carriers of at least one minor allele for the SNP rs3798220 in Māori and Pacific cases ($p = 0.9962$) or controls ($p = 0.6537$; Table 21), where the median Lp(a) level ranged from 9.7 nmol/L to 31.8 nmol/L (Figure 15).

For APOE SNPs, no significant differences in Lp(a) levels were found between individuals containing at least one APOE2 or one APOE4 allele versus APOE3 homozygotes in any of the sample sets ($p$ ranged from 0.1549 to 0.9754; Table 21).
Figure 12. Lipoprotein(a) distribution in gout case and control sample sets for different ethnicities. Boxes represent values from the 25th, to the 50th, to the 75th percentiles. Whiskers represent values from the 2.5th to the 97.5th percentiles. Dots represent values greater than the 97.5th percentile. On the x axis, the numbers in brackets indicate median lipoprotein(a) values (nmol/L). EP = Eastern Polynesian. WP = Western Polynesian.

Figure 13. Lipoprotein(a) distribution in self-reported heart problem case and control sample sets for different ethnicities. Cases defined here were combined gout cases and controls reported with unspecified heart problems, whereas controls defined here were combined gout cases and controls reported without heart problems. Boxes represent values from the 25th, to the 50th, to the 75th percentiles. Whiskers represent values from the 2.5th to the 97.5th percentiles. Dots represent values greater than the 97.5th percentile. On the x axis, the numbers in brackets indicate median lipoprotein(a) values (nmol/L).
Table 21. Lipoprotein(a) level analyses in different ethnicities, case control groups and genotypes.

<table>
<thead>
<tr>
<th></th>
<th>Gout affliction</th>
<th>Mann-Whitney $p$</th>
<th>t test $p$*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethnicity comparisons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian versus all Māori and Pacific</td>
<td>Cases</td>
<td>0.0460</td>
<td>0.0893</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>0.2740</td>
<td>0.2367</td>
</tr>
<tr>
<td></td>
<td>Combined cases and controls</td>
<td>0.0218</td>
<td>0.0326</td>
</tr>
<tr>
<td>Eastern Polynesian versus Western Polynesian</td>
<td>Cases</td>
<td>0.9404</td>
<td>0.8843</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>0.3785</td>
<td>0.3621</td>
</tr>
<tr>
<td><strong>Gout affliction comparisons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian cases versus controls</td>
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<td>0.2948</td>
<td>0.3593</td>
</tr>
<tr>
<td>Eastern Polynesian cases versus controls</td>
<td></td>
<td>0.7033</td>
<td>0.9274</td>
</tr>
<tr>
<td>Western Polynesian cases versus controls</td>
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<td>0.7000</td>
<td>0.5792</td>
</tr>
<tr>
<td>All Māori and Pacific cases versus controls</td>
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<td>0.7923</td>
<td>0.6231</td>
</tr>
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<td><strong>Self-reported heart problem affliction comparisons</strong>*</td>
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<td></td>
<td></td>
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<td>Caucasian cases versus controls</td>
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<td>0.1419</td>
</tr>
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<td>All Māori and Pacific cases versus controls</td>
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<td>Cases</td>
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<td>0.0117</td>
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<td></td>
<td>Controls</td>
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<td>All Māori and Pacific</td>
<td>Cases</td>
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<td>0.9962</td>
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<td></td>
<td>Controls</td>
<td>0.7122</td>
<td>0.6537</td>
</tr>
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<td><strong>rs1045587 genotype comparisons</strong>#</td>
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<td></td>
<td></td>
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<td>Caucasian</td>
<td>Cases</td>
<td>3.26 × 10$^{-9}$</td>
<td>2.71 × 10$^{-43}$</td>
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<td></td>
<td>Controls</td>
<td>2.68 × 10$^{-5}$</td>
<td>8.45 × 10$^{-6}$</td>
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<td><strong>Combined rs3798220 and rs1045587 genotype comparisons</strong>#</td>
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<td></td>
<td></td>
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<td>Caucasian</td>
<td>Cases</td>
<td>1.20 × 10$^{-10}$</td>
<td>6.53 × 10$^{-12}$</td>
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<tr>
<td></td>
<td>Controls</td>
<td>2.88 × 10$^{-6}$</td>
<td>7.43 × 10$^{-4}$</td>
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<td>All Māori and Pacific</td>
<td>Cases</td>
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<td>Controls</td>
<td>0.0139</td>
<td>0.0049</td>
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<td><strong>APOE3 homozygotes versus APOE2 carriers</strong>^</td>
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<td></td>
<td></td>
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<tr>
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<td>Cases</td>
<td>0.7020</td>
<td>0.5251</td>
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<td>Controls</td>
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<td>All Māori and Pacific</td>
<td>Cases</td>
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<td>0.2473</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
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<td><strong>APOE3 homozygotes versus APOE4 carriers</strong>^</td>
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<td></td>
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<td>Cases</td>
<td>0.5970</td>
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<td>Controls</td>
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<tr>
<td>All Māori and Pacific</td>
<td>Cases</td>
<td>0.2191</td>
<td>0.1931</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>0.9754</td>
<td>0.9749</td>
</tr>
</tbody>
</table>

*Cases defined here were combined gout cases and controls reported with heart problems, whereas controls defined here were combined gout cases and controls reported without heart problems. #Comparing major homozygotes with subjects carrying at least one minor allele. ^Carriers include APOE2 and APOE4 compound heterozygotes.
Figure 14. Caucasian lipoprotein(a) levels stratified by genotypes of LPA and APOE single nucleotide polymorphisms. Left = gout cases. n from left to right = 132, 3, 116, 18, 1, 22, 18, 38 and 83. Right = gout controls. n from left to right = 116, 5, 106, 15, 20, 24, 36 and 66. Boxes represent values from the 25th, to the 50th, to the 75th percentiles. Whiskers represent values from the 5th to the 95th percentiles. Dots represent values less than the 5th percentile, or greater than the 95th percentile. On the x axis, the numbers in brackets indicate median lipoprotein(a) values (nmol/L). Maj homo = major homozygote. Hetero = heterozygote. Min homo = minor homozygote. Min carrier = minor allele carrier. Homo = homozygote.
Figure 15. Māori and Pacific lipoprotein(a) levels stratified by genotypes of LPA and APOE single nucleotide polymorphisms. Left = gout cases. n from left to right = 210, 15, 219, 7, 22, 30, 114 and 94. Right = gout controls. n from left to right = 214, 20, 1, 227, 7, 46, 118 and 86. Boxes represent values from the 25\textsuperscript{th} to the 50\textsuperscript{th}, to the 75\textsuperscript{th} percentiles. Whiskers represent values from the 5\textsuperscript{th} to the 95\textsuperscript{th} percentiles. Dots represent values less than the 5\textsuperscript{th} percentile, or greater than the 95\textsuperscript{th} percentile. On the x axis, the numbers in brackets indicate median lipoprotein(a) values (nmol/L). Maj homo = major homozygote. Hetero = heterozygote. Min carrier = minor allele carrier. Homo = homozygote. Min homo = minor homozygote.
3.3.3. Genotyping results for LPA single nucleotide polymorphisms

For LPA SNPs, no significant associations between gout cases and controls were found in any of the sample sets after the stratification analysis for Caucasian ancestry in the Māori, EP and WP populations, or the logistic regression analysis in all populations (all \( p > 0.203 \); Table 22; see section 2.4.5 for the details on logistic regression analysis). In all sample sets except for the SNP rs10455872 in WP controls (\( p = 3.89 \times 10^{-9} \)), Hardy-Weinberg equilibrium was achieved (\( p > 0.05 \)).

For the SNP rs3798220, there was low power to detect for association with gout in the Māori and EP sample set, with power to be less than 73% even for an OR of up to 2.5 (Table 20). The power to detect for association in the Caucasian and WP sample set was similar to each other, and ranged from under 10% at an OR of 1.2 to 90% at an OR of 2.5 (Table 20). For the SNP rs10455872, there was high power (over 98%) to detect for association with gout in the Caucasian sample set at an OR of 2.0 and above, but lower power (31.6% to 86.2%) for the Māori, EP and WP sample sets (Table 20). Generally, there was low power (5.4% to 75.5%) to detect for association with gout at OR 1.2 and 1.6 for all sample sets (Table 20).

Imputation for generating genotype data for FHS and ARIC datasets was attempted for the SNPs rs3798220 and rs10455872 in LPA. Like the APOE SNPs rs429358 and rs7412 (see section 3.2.3), the imputation data generated from LPA SNPs had frequencies that differed to the frequencies of the Caucasian sample set. The LD between the two SNPs with surrounding SNPs used during the imputation was very low (Figure 16), hence the unreliable imputation data was attributed to the low LD. The current project revealed low minor allele frequencies for rs3798220 and rs10455872 in all sample sets (Table 22), and this low frequency was supported by many other sources (Table 19).

3.4. CDKN2BAS genotyping results

For the SNP rs1333049 of CDKN2BAS, no significant associations were found between gout cases and controls in all sample sets, even after stratification analysis in the Māori, EP and WP groups, and logistic regression analysis in all groups (Table 23; see section 2.4.5 for the details on logistic regression analysis). Additionally, the non-significant association was supported by FHS and ARIC results (Table 23). All sample sets except for WP controls (\( p = \ldots \))
0.0409) were in Hardy-Weinberg equilibrium ($p > 0.05$). The power to detect for association with gout at an OR of 1.26 ranged from 26.6% to 67.1% (Table 20).

Table 22. Association analyses with gout for single nucleotide polymorphisms in \textit{LPA}.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Genotype (n, freq)</th>
<th>Allele C (n, freq)</th>
<th>OR[95% CI]</th>
<th>(p^*)</th>
<th>(p^#)</th>
<th>OR[95% CI]^</th>
<th>(p^)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textbf{Caucasian}</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Cases</td>
<td>316(0.966)</td>
<td>11(0.034)</td>
<td>0(0.000)</td>
<td>11(0.017)</td>
<td>0.86[0.42-1.75]</td>
<td>0.670</td>
<td>-</td>
</tr>
<tr>
<td>Controls</td>
<td>614(0.962)</td>
<td>23(0.036)</td>
<td>1(0.002)</td>
<td>25(0.020)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>\textbf{Māori}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>145(0.935)</td>
<td>10(0.065)</td>
<td>0(0.000)</td>
<td>10(0.032)</td>
<td>1.47[0.65-3.31]</td>
<td>0.350</td>
<td>0.442</td>
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<td>Controls</td>
<td>324(0.959)</td>
<td>13(0.038)</td>
<td>1(0.003)</td>
<td>15(0.022)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>\textbf{Eastern Polynesian}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>198(0.947)</td>
<td>11(0.053)</td>
<td>0(0.000)</td>
<td>11(0.026)</td>
<td>1.29[0.58-2.88]</td>
<td>0.522</td>
<td>0.671</td>
</tr>
<tr>
<td>Controls</td>
<td>330(0.962)</td>
<td>12(0.035)</td>
<td>1(0.003)</td>
<td>14(0.020)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>\textbf{Western Polynesian}</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>233(0.928)</td>
<td>17(0.068)</td>
<td>1(0.004)</td>
<td>19(0.038)</td>
<td>0.82[0.40-1.69]</td>
<td>0.589</td>
<td>0.748</td>
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<tr>
<td>Controls</td>
<td>129(0.908)</td>
<td>13(0.092)</td>
<td>0(0.000)</td>
<td>13(0.046)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Sample set          | Genotype (n, freq) | Allele G (n, freq) | OR[95% CI] | \(p^*\) | \(p^\#\) | OR[95% CI]^ | \(p^\) |
|---------------------|--------------------|--------------------|------------|        |        |------------|--------|
| \textbf{Caucasian}  |                    |                    |            |        |        |            |        |
| Cases               | 278(0.855)         | 44(0.135)          | 3(0.009)   | 50(0.077) | 1.18[0.82-1.70] | 0.366 | - | 1.11[0.51-2.42] | 0.788 |
| Controls            | 555(0.870)         | 82(0.129)          | 1(0.002)   | 84(0.066) | -        | -        | -        | -        |
| \textbf{Māori}     |                    |                    |            |        |        |            |        |
| Cases               | 146(0.942)         | 9(0.058)           | 0(0.000)   | 9(0.029)  | 1.01[0.46-2.25] | 0.974 | 0.725 | 0.92[0.19-4.33] | 0.913 |
| Controls            | 320(0.941)         | 20(0.059)          | 0(0.000)   | 20(0.029) | -        | -        | -        | -        |
| \textbf{Eastern Polynesian} |                |                    |            |        |        |            |        |
| Cases               | 199(0.948)         | 11(0.052)          | 0(0.000)   | 11(0.026) | 0.90[0.43-1.90] | 0.784 | 0.806 | 0.88[0.20-3.80] | 0.867 |
| Controls            | 325(0.942)         | 20(0.058)          | 0(0.000)   | 20(0.029) | -        | -        | -        | -        |
| \textbf{Western Polynesian} |                |                    |            |        |        |            |        |
| Cases               | 251(0.996)         | 1(0.004)           | 0(0.000)   | 1(0.002)  | 0.14[0.02-1.24] | 0.039 | 0.203 | -        | -        |
| Controls            | 138(0.979)         | 2(0.014)           | 1(0.007)   | 4(0.014)  | -        | -        | -        | -        |

\textbf{NB.} \(p\) values presented are allelic \(p\) values. *Raw \(p\) value. \# \(p\) value after adjustment for Caucasian ancestry in the Māori, Eastern Polynesian and Western Polynesian sample sets. ^Results after multiple logistic regression, where covariates adjusted for were age, sex, waist circumference, and the presence of type II diabetes mellitus, hyperlipidemia and heart problems. Only the Aotearoa controls were used for logistic regression analysis, as there was limited demographic information in the New Zealand Caucasian, Māori and Pacific controls (see section 2.4.5). In Western Polynesian controls, \textit{rs10455872} was not in Hardy-Weinberg equilibrium \((p = 3.87 \times 10^{-9})\). Otherwise, all other sample sets were in Hardy-Weinberg equilibrium \((p > 0.05)\). \text{OR} = \text{odds ratio}. \text{CI} = \text{confidence interval}.}
Association analysis with self-reported and unspecified heart problems were also investigated for rs1333049, irrespective of gout affliction. No significant associations were found between subjects with and subjects without heart problems in any of the sample sets (Table 24). All sample sets except for Māori cases ($p = 0.0299$) were in Hardy-Weinberg equilibrium ($p > 0.05$). The power to detect for association with heart problems was above 60% at an OR of 1.4 for the Caucasian sample set, 1.6 for the Māori and EP sample set, and 1.8 for the WP sample set (Table 25). The power to detect for association with heart problems reached over 80% at an OR of 1.6 for the Caucasian sample set, 2.0 for the Māori sample set, 1.8 for the EP sample set and 2.2 for the WP sample set (Table 25).
Table 23. Association analyses with gout for the single nucleotide polymorphism in CDKN2BAS.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Genotype (n, freq)</th>
<th>Allele C (n, freq)</th>
<th>OR[95% CI]</th>
<th>p*</th>
<th>p^</th>
<th>OR[95% CI]^</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caucasian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>96(0.294)</td>
<td>168(0.515)</td>
<td>291(0.448)</td>
<td>0.86[0.71-1.04]</td>
<td>0.129</td>
<td>0.79[0.53-1.17]</td>
</tr>
<tr>
<td>Controls</td>
<td>171(0.268)</td>
<td>315(0.495)</td>
<td>151(0.237)</td>
<td>617(0.484)</td>
<td>1.21[0.91-1.62]</td>
<td>0.188</td>
</tr>
<tr>
<td><strong>Māori</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>20(0.130)</td>
<td>57(0.370)</td>
<td>77(0.500)</td>
<td>211(0.685)</td>
<td>1.26[0.97-1.63]</td>
<td>0.085</td>
</tr>
<tr>
<td>Controls</td>
<td>48(0.142)</td>
<td>146(0.432)</td>
<td>144(0.426)</td>
<td>434(0.642)</td>
<td>1.02[0.76-1.38]</td>
<td>0.878</td>
</tr>
<tr>
<td><strong>Eastern Polynesian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>23(0.111)</td>
<td>80(0.385)</td>
<td>105(0.505)</td>
<td>290(0.697)</td>
<td>1.05[0.83-1.31]</td>
<td>0.693</td>
</tr>
<tr>
<td>Controls</td>
<td>49(0.144)</td>
<td>143(0.419)</td>
<td>149(0.437)</td>
<td>441(0.647)</td>
<td>1.02[0.76-1.38]</td>
<td>0.878</td>
</tr>
<tr>
<td><strong>Western Polynesian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>49(0.196)</td>
<td>109(0.436)</td>
<td>92(0.368)</td>
<td>293(0.586)</td>
<td>1.02[0.76-1.38]</td>
<td>0.878</td>
</tr>
<tr>
<td>Controls</td>
<td>18(0.131)</td>
<td>79(0.577)</td>
<td>40(0.292)</td>
<td>159(0.580)</td>
<td>1.02[0.76-1.38]</td>
<td>0.878</td>
</tr>
<tr>
<td><strong>Framingham Heart Study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>18(0.269)</td>
<td>32(0.478)</td>
<td>17(0.254)</td>
<td>66(0.493)</td>
<td>0.92[0.65-1.29]</td>
<td>0.634</td>
</tr>
<tr>
<td>Controls</td>
<td>1127(0.239)</td>
<td>2333(0.495)</td>
<td>1252(0.266)</td>
<td>4837(0.513)</td>
<td>1.05[0.83-1.31]</td>
<td>0.693</td>
</tr>
<tr>
<td><strong>Atherosclerosis Risk In Communities study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>37(0.243)</td>
<td>81(0.533)</td>
<td>34(0.224)</td>
<td>149(0.490)</td>
<td>1.05[0.83-1.31]</td>
<td>0.693</td>
</tr>
<tr>
<td>Controls</td>
<td>1594(0.229)</td>
<td>3481(0.500)</td>
<td>1891(0.271)</td>
<td>6669(0.479)</td>
<td>1.31[0.89-1.90]</td>
<td>0.693</td>
</tr>
</tbody>
</table>

NB; p values presented are allelic p values. *Raw p value. ^p value after adjustment for Caucasian ancestry in the Māori, Eastern Polynesian and Western Polynesian sample sets. ^Results after multiple logistic regression, where covariates adjusted for were age, sex, waist circumference and the presence of type II diabetes mellitus, hyperlipidemia and heart problems. Only the Aotearoa controls were used for logistic regression analysis, as there was limited demographic information in the New Zealand Caucasian, Māori and Pacific controls (see section 2.4.5). The Western Polynesian control group was not in Hardy-Weinberg equilibrium (p = 0.0409). Otherwise, all other sample sets were in Hardy-Weinberg equilibrium (p > 0.05). OR = odds ratio. CI = confidence interval.
Table 24. Association analysis with self-reported heart problems for the single nucleotide polymorphism in CDKN2BAS.

<table>
<thead>
<tr>
<th>Sample set (n)</th>
<th>Genotype (n, freq)</th>
<th>Allele C (n, freq)</th>
<th>Odds ratio [95% confidence interval]</th>
<th>Allelic p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GC</td>
<td>CC</td>
<td></td>
</tr>
<tr>
<td><strong>Caucasian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases (123)</td>
<td>37(0.301)</td>
<td>61(0.496)</td>
<td>25(0.203)</td>
<td>111(0.451)</td>
</tr>
<tr>
<td>Controls (336)</td>
<td>93(0.277)</td>
<td>178(0.530)</td>
<td>65(0.193)</td>
<td>308(0.458)</td>
</tr>
<tr>
<td><strong>Māori</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases (67)</td>
<td>9(0.134)</td>
<td>20(0.299)</td>
<td>38(0.567)</td>
<td>96(0.716)</td>
</tr>
<tr>
<td>Controls (266)</td>
<td>30(0.113)</td>
<td>115(0.432)</td>
<td>121(0.455)</td>
<td>357(0.671)</td>
</tr>
<tr>
<td><strong>Eastern Polynesian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases (80)</td>
<td>8(0.100)</td>
<td>26(0.325)</td>
<td>46(0.575)</td>
<td>118(0.738)</td>
</tr>
<tr>
<td>Controls (315)</td>
<td>34(0.108)</td>
<td>133(0.422)</td>
<td>148(0.470)</td>
<td>429(0.681)</td>
</tr>
<tr>
<td><strong>Western Polynesian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases (37)</td>
<td>6(0.162)</td>
<td>20(0.541)</td>
<td>11(0.297)</td>
<td>42(0.568)</td>
</tr>
<tr>
<td>Controls (307)</td>
<td>58(0.189)</td>
<td>145(0.472)</td>
<td>104(0.339)</td>
<td>353(0.575)</td>
</tr>
</tbody>
</table>

NB: cases defined here were combined gout cases and controls self-reported with unspecified heart problems, whereas controls defined here were combined gout cases and controls self-reported without heart problems. The Māori case sample set was not in Hardy-Weinberg equilibrium (p = 0.0299). Otherwise, all other sample sets were in Hardy Weinberg equilibrium (p > 0.05).

Table 25. Power to detect for association with self-reported heart problems for the CDKN2BAS single nucleotide polymorphism.

<table>
<thead>
<tr>
<th>Single nucleotide polymorphism</th>
<th>Odds ratio</th>
<th>Power at the significance level of 0.05 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Caucasian</td>
</tr>
<tr>
<td><strong>rs1333049</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td></td>
<td>23.0</td>
</tr>
<tr>
<td>1.4</td>
<td></td>
<td>61.6</td>
</tr>
<tr>
<td>1.6</td>
<td></td>
<td>88.1</td>
</tr>
<tr>
<td>1.8</td>
<td></td>
<td>97.4</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td>99.6</td>
</tr>
<tr>
<td>2.2</td>
<td></td>
<td>99.9</td>
</tr>
</tbody>
</table>
4. Discussion

4.1. Lipid, lipoprotein and apolipoprotein analyses

4.1.1. Basic comparison of lipids in multi-ethnic gout cases and controls

The total cholesterol level between gout cases and controls was found to be significantly different only in the Māori/Pacific, and not the Caucasian, sample set. In both groups, total TG was significantly increased in cases. Higher total TG and no difference in total cholesterol have been found in European, North American, and Japanese gout cases when compared with respective control sample sets (Darlington and Scott, 1972; Feldman and Wallace, 1964; Gibson and Grahame, 1974; Moriwaki et al., 1995; Naito and Mackenzie, 1979; Ulreich et al., 1985). In only some studies were both total TG and cholesterol levels increased in gout cases compared to controls (Barlow, 1968; Mielants et al., 1973), although these populations were European, thus not corresponding to the Māori and Pacific ethnicity in the present study. Only one known Polynesian gout case and control study presented lipid values. Chou and Chao (1999) found a significantly higher total TG, but not total cholesterol level, in Taiwanese aboriginal gout cases when compared to Taiwanese aboriginal controls or Han Chinese controls.

In the present study, Caucasian TG levels were not found to be different to Māori and Pacific sample sets. In the literature, there has been mixed results for TG levels between Caucasian and Pacific Island populations, with either significantly increased or decreased TG levels in the Polynesian groups (Gentles et al., 2007; Kottke et al., 1990; McAnulty and Scragg, 1996).

Increased TG levels have also been found to associate with increased UA levels (Lippi et al., 2010; Rathmann et al., 2007) and decreased UA excretion (Tinahones and Collantes, 1995). Although a positive correlation between TG and UA levels was seen in the Caucasian gout cases and controls and in the Māori/Pacific controls, there was no correlation in the Māori and Pacific cases. Several reasons may account for this. Firstly, the mean TG and UA levels in Māori and Pacific cases (TG was 2.50 mmol/L and UA was 0.45 mmol/L) were higher than the three other groups (TG ranged from 1.73 mmol/L to 2.33 mmol/L and UA ranged from 0.29 mmol/L to 0.39 mmol/L). It is possible that at the levels of the Māori and Pacific cases, the positive association
between TG and UA had reached a plateau, therefore no correlation was witnessed in this group. Secondly, a particular polymorphism (rs12510549) in the urate transporter gene SLC2A9 has been found to have a protective effect against gout in the Caucasian, but not the Māori and Pacific sample sets (Hollis-Moffatt et al., 2009). Genetic differences in urate excretion genes such as this may be additionally influenced by unknown factors such as TG. It would be interesting to re-analyze these data using TG as a covariate in multiple logistic regression analyses. It would further be interesting to investigate the renal excretion of urate in Māori/Pacific and Caucasian gout cases and controls, and compare the effect of TG on this. For example, the fractional renal urate excretion could be measured and correlated against plasma TG levels, or compared between differing levels of TG infusion in the blood. Thirdly, other unknown factors may contribute to determining the relationship between TG and UA levels. For example, the LDL receptor related protein 2, encoded by LRP2, binds apo B and apo E (Moestrup and Verroust, 2001). Variations in LRP2 have been shown to associate with serum UA levels in a Japanese GWAS (p = 3.74 × 10^{-8}; Kamatani et al., 2010). It is speculative that this genetic variation influences the resulting protein function or expression levels, and thus affects binding with apo B and apo E on VLDL to influence TG levels. This may then by some unknown mechanism associate with UA levels. As the association of LRP2 and UA levels were not reported in a Caucasian GWAS (Kolz et al., 2009), its hypothetical effect may be specific for some ethnicities, such as the Japanese, and potentially the Māori and Pacific ethnicities. Lastly, it is unknown whether the UA levels were measured before or after any gout treatment in the gout cases. If gout treatment in the Māori and Pacific cases was started before the measurement of UA levels, it may have had a confounding effect by lowering UA levels, and hence abolishing the positive correlation with TG levels. However, Caucasian gout cases had significant positive correlation between the levels of UA and TG. This may be due to the measurement of UA levels in this group before gout treatment. Alternatively, the percentage of treated cases may have been significantly different between the Māori/Pacific and the Caucasian groups, resulting in different correlation analysis results.

No differences were found between Caucasian gout cases and controls for HDL cholesterol values, and the HDL cholesterol level in Caucasian cases was higher than the level in Māori and Pacific cases. In general population studies, lower HDL cholesterol, apo AI or apo AII have been found in Pacific Island people when compared with Caucasians (Gentles et al., 2007;
Kottke \textit{et al.}, 1990; Nestel and Zimmet, 1981). Like the Māori and Pacific sample set studied here, some studies which focused on HDL levels found decreased HDL cholesterol in gout cases when compared to controls (Mielants \textit{et al.}, 1973; Ulreich \textit{et al.}, 1985). Low HDL cholesterol is often associated with increased total TG (Genest Jr \textit{et al.}, 1992; Schaefer \textit{et al.}, 1978), as was also seen in both Māori/Pacific and Caucasian case and control groups. High TG levels may result in increased TG proportions in HDL particles due to increased transfer from other lipoproteins (Patel \textit{et al.}, 2009). High TG HDL is prone to increased processing by hepatic lipase and increased degradation, hence resulting in decreased levels of HDL (Xiao \textit{et al.}, 2008a).

For both Māori/Pacific and Caucasian sample sets, LDL cholesterol was significantly different between gout cases and controls. However, this was in opposite directions - Māori and Pacific cases had higher and Caucasian cases had lower LDL cholesterol levels when compared with the respective control groups. The Taiwanese population in the study by Chou and Chao (1999) also showed increased LDL cholesterol in aboriginal gout cases when compared with aboriginal controls, but not when compared with Han Chinese controls. In the general population for another New Zealand sample set, Māori individuals (n = 1006) but not Pacific Island people (n = 996) were found to have a lower LDL cholesterol level than the combined European and Asian group (n = 2021) after multiple linear regression (Gentles \textit{et al.}, 2007). A smaller sample set further found no differences in LDL cholesterol between European (n = 279) and Pacific Island (n = 231) populations (McAnulty and Scragg, 1996). The results from the current study were likely to be biased as the LDL cholesterol calculation was limited to subjects with a TG level of less than 4.2 mmol/L. To investigate real differences in LDL cholesterol levels between cases and controls, an assay specific for measuring LDL cholesterol (such as the MaxDiscovery™ LDL Cholesterol Assay Kit) would be required for subjects with high TG levels.

The oxidation of LDL is involved with the promotion of atherosclerosis (see section 1.2). Plasma levels of oxidized LDL was found to be increased in gout cases (n = 49) when compared with controls (n = 42; Jiang \textit{et al.}, 2011). Plasma malondialdehyde levels, which indicate lipid peroxidation, was also increased in cases compared to controls (Jiang \textit{et al.}, 2011). Furthermore, the antioxidant molecules superoxide dismutase and paraoxonase-1 in plasma were found to be decreased in cases when compared to controls (Jiang \textit{et al.}, 2011). In another study, the level of oxidized LDL auto-antibodies were increased in gout cases (n = 117) when compared with
controls (n = 38), although LDL diameter size was not significantly different between the two groups (Tsutsumi et al., 2004). Although the LDL cholesterol level was increased in Māori and Pacific gout cases compared to controls, investigating the oxidation status of the LDL particles in this sample set would provide more insight into the degree of atherogenicity in gout.

A limitation for many of the studies compared above was the low number of subjects in the gout case and control groups, many of which were each under 50. This may explain the variability in results, although the general consensus was that gout patients had increased TG levels when compared with controls. Another limitation was the inconsistent or unreported intake of medication which may affect lipid levels. A range of lipid-lowering medications exist, such as bile acid binding resins, fibrates, nicotinic acid and statins (O'Connor et al., 1990). Other medications may increase or decrease lipid levels as a side effect, for example cardiovascular drugs such as diuretics and β-blockers, hormonal drugs such as estrogens and growth hormones, retinoids, immunosuppressive drugs, protease inhibitors, anti-psychotics and anti-convulsants (Mantel-Teeuwisse et al., 2001). Unfortunately, lipid-lowering medication information was unavailable in the Māori/Pacific and Caucasian datasets, therefore could not be added as a confounding variable in analyses. However, inequalities in health care have been reported between Māori and Caucasian populations in New Zealand (Reid and Robson, 2006), which may suggest lower medication prescription rates in the Māori population compared with the Caucasian population. If the prescription of lipid-lowering medications, especially statins and fibrates, was higher in Caucasians, this may explain the lower level of LDL cholesterol in Caucasian gout cases when compared to Māori and Pacific gout cases. A further limitation is that all literature studies compared above measured lipid parameters on fasting samples, whereas the samples in the current project were assumed to be non-fasting. In a cross-sectional study (n = 33391), lipid levels of the non-fasting state (up to eight hours after the last meal) was compared to the fasting state. After adjustment for age, sex and albumin (as a measure for hemodilution from fluid intake), no difference in the total or LDL cholesterol level was found, whereas the HDL cholesterol level was decreased for up to four hours post-prandial (p < 0.05), and the total TG level was increased for up to six hours post-prandial (p < 0.001; Langsted et al., 2008). Similarly, for healthy subjects where lipid levels were followed up for four and eight hours post-prandially (n = 12), total apo B and total cholesterol levels did not differ, whereas HDL and LDL cholesterol levels decreased (p < 0.05) and total TG levels increased (p < 0.05) when compared
to levels from the fasting state (Otokozawa et al., 2009). The increased TG level was likely to be reflected by the increased apo B48 level (p < 0.001; Otokozawa et al., 2009). Therefore, relative to total cholesterol, the TG levels presented in the current study may have been overestimated, and the HDL (and possibly LDL) cholesterol levels underestimated due to a non-fasting status. For more discussion on the non-fasting status, see section 4.1.5.

4.1.2. Māori and Pacific lipid, lipoprotein and apolipoprotein parameters

To investigate the composition of lipoproteins in more detail, FPLC experimentation was conducted in a subgroup of Māori and Pacific gout cases and controls. This is the first known FPLC analysis on lipoproteins to be conducted on a Māori and Pacific population. FPLC analysis revealed an increased TG, but not cholesterol content, in VLDL fractions of gout cases when compared to controls, which caused an increased total TG level. Thus, the VLDL particle was characterized. As only one apo B is found per VLDL particle (Elovson et al., 1988), apo B measurements were used to provide information on the number of VLDL particles. Apo B analysis was conducted using the VLDL apo B to total apo B ratio, where VLDL apo B was measured in FPLC fractions, and total apo B was measured in whole plasma and adjusted for lipid recovery. There was an absence of a significant difference between cases and controls for the VLDL apo B to total apo B ratio in univariate and multivariate analysis. This suggested that there were no increases in the number of VLDL particles in cases compared to controls, and rather, the VLDL particles were TG-enriched to give the increased VLDL TG levels. However, this non-significant result may have been due to the limited number of subjects in this analysis. In support of this, the p values (univariate p = 0.1467, multivariate p = 0.0819) between cases and controls for this ratio were trending low enough to suggest that the result would become significant if the sample size was increased. Furthermore, two other pieces of evidence indicate that there were increased VLDL particle numbers rather than TG-enriched VLDL particles. The VLDL apo B to total apo B ratio showed clear and significant increases across VLDL TG to total TG ratio quartiles. Also, there were no significant differences between cases and controls in the VLDL TG to VLDL apo B ratio, indicating that subjects did not have increased VLDL TG content when scaled to the VLDL apo B concentration. There was therefore good reason to conclude from the current data that for gout cases, the increase in the levels of VLDL TG and total TG was due to an increased number of VLDL particles.
VLDL particles can be subclassed into larger, more TG-rich VLDL\textsubscript{1} particles, and smaller VLDL\textsubscript{2} particles with less TG (Zhao et al., 1995). Interestingly, insulin resistance, the presence of TIIDM and increased liver fat have been positively correlated with the production of VLDL\textsubscript{1} particles (Adiels et al., 2005; Adiels et al., 2006; Gill et al., 2004). Given the association between gout and TIIDM (see section 1.4), it would be interesting to compare the VLDL subclasses between gout cases and controls. VLDL subclasses could be separated using density gradient ultracentrifugation, and concentrations subsequently assayed for.

Unlike results from SCL where only HDL and LDL cholesterol values were reported, FPLC methodology is able to reveal the amount of TG and cholesterol in VLDL, LDL and HDL lipoprotein classes, providing a further depth of knowledge in this area. FPLC has repeatedly been shown to successfully isolate individual lipoprotein classes based on size (Chétiveaux et al., 2002; Collins et al., 2010; Marz et al., 1993). However, when comparing FPLC results with reference methods for determining specific lipoprotein lipid concentrations, outcomes were variable. Some studies showed that cholesterol levels were over or under estimated in some lipoprotein fractions (Kahlon et al., 2001; Marz et al., 1993), whereas other studies showed high concordance between methods (Innis-Whitehouse et al., 1998; Parini et al., 2006). This variability between studies may be explained by inter-laboratory variations in conducting both FPLC and the reference method. In fact, a variety of combinations with ultracentrifugation, tube slicing, and precipitation reference methods have been reported for comparison with FPLC. The non-standardized nature of FPLC supports the use of ratios rather than absolute values as measures in the current project. Despite this, studies have shown that FPLC provides a high reproducibility of results from injections of the same plasma sample (Innis-Whitehouse et al., 1998; Marz et al., 1993; Parini et al., 2006). Furthermore, FPLC methodology has already been implemented in clinical statistical comparisons, for example, for the detection of significant differences in lipid levels between baseline samples and lipid-lowering atorvastatin post-treatment samples (Le et al., 2000).

Unfortunately, FPLC throughput was limited due to the requirement of high maintenance on the machine and column. In the future, Caucasian gout case and control samples should be investigated by FPLC analyses to compare ethnicity effects on lipid, lipoprotein and apolipoprotein compositions. In hindsight, it would have been preferable to measure the apo B content in the LDL fractions from FPLC along with the VLDL fractions. This would have
allowed for more comprehensive apo B analysis, and should be conducted in future samples undergoing this analysis.

4.1.3. Type IV hyperlipoproteinemia dissection

TIVH is characterized by an increased TG level due to increased VLDL TG (Levy and Fredrickson, 1968). As a relatively high rate of this phenotype was discovered in gout cases from FPLC results (see section 3.2), TIVH classification criteria were investigated. Theoretically, to ensure that the increased total TG is only from VLDL, the most preferable diagnostic criterion would involve the use of total, VLDL, LDL and CM TG information. Based on this, FPLC, western blot and TG assay results provided the incorporation of all these factors, where the most preferable criterion involved excluding subjects with an apo B48 band, and the use of total TG, LDL TG to total TG ratio, and either VLDL TG to total TG ratio or VLDL apo B to total apo B ratio. Both criteria gave a significantly increased prevalence of TIVH in gout cases when compared to controls. Other criteria, such as the use of the VLDL TG absolute value in the VLDL component, or the use of the LDL TG absolute value in the LDL component, also yielded a significantly higher prevalence of TIVH in cases than controls, supporting a genuine result of an increased TIVH prevalence in gout patients. In support of this, Naito and Mackenzie (1979) found a significantly ($p < 0.05$) higher prevalence of TIVH in gout cases (43%) when compared with controls (19%) in a United States of America sample set, although ethnicity was not reported. Japanese male gout cases ($n = 196$) were also reported to have a higher prevalence of TIVH (70%) compared to males with primary hyperlipoproteinemia without gout ($n = 877$, 43%, $p < 0.01$; Jiao et al., 1986).

As the FPLC is a laborious process with low throughput, components for a TIVH criterion that can be obtained without the use of the FPLC methodology was also investigated. This excluded the use of the VLDL component and values derived from LDL TG. None of these criteria yielded significant differences in the number of TIVH cases classified between gout cases and controls (Table 16). Additionally, there was high variability in subject classification between criteria 3a, 3b and 3c with the theoretically preferable criteria (involving all of total, VLDL and LDL components, such as criterion 1a), rendering these criteria less than preferable. There is also the limitation of low sample size in this analysis, giving low statistical power. If the
clinical use for criteria 3a, 3b or 3c was to be further considered, it is vital for a larger sample size to undergo FPLC for comparison analysis to establish the feasibility of the criteria.

Due to a lack of precise clinical definition for TIVH, many studies have used a variety of measures for categorizing TIVH cases. These included various combinations of TG, LDL cholesterol, CM, total cholesterol and VLDL cholesterol levels (Efe et al., 2004; Eto et al., 1991; Marcoux et al., 1999; Snideman et al., 1998; Zoppo et al., 1999). This study highlights the importance of specifically using VLDL and LDL TG or apo B measures as a part of the TIVH classification, as a misclassification of subjects may otherwise result. The use of only total and LDL lipid components would not be as sensitive in TIVH detection. In conclusion, without the use of FPLC, the classification of TIVH would be less than satisfactory.

In lipoprotein gel electrophoresis, the stain used was Fat Red 7B (C_{24}H_{21}N_{5}) which stains more specifically for TG. Thus, any increase in the pre-β area was seen as increased VLDL TG. The match rate between FPLC results and lipoprotein gel classification for TIVH was not high. For theoretically preferable criteria involving total, VLDL and LDL TG or apo B values (criteria 1a, 1b, 1c 1d and 2), the match rate was approximately 73% to 78%. Furthermore, the criterion with the highest match rate (approximately 80%) included the use of measurements which did not require FPLC methodology, that is, the use of total TG and LDL cholesterol measurements. This criterion showed no significant differences in the number of subjects classified with TIVH between gout cases and controls. The FPLC methodology was considered superior over lipoprotein gel pattern analysis for the classification criteria of TIVH. The FPLC is able to provide specific lipid details for each lipoprotein class, whereas lipoprotein gel results (Figure 10) were difficult to interpret and exposed to conflicting judgement. Lipoprotein gels were also unable to reveal the presence of CM in most subjects who were positive for the apo B48 band in western blots. Furthermore, the higher affinity for TG staining of Fat Red 7B does not reflect a true quantitation of total lipoprotein, and was not recommended for quantitative analysis according to protocol instructions.

4.1.4. Genetic interest in very low density lipoprotein metabolism

FPLC results indicate an increased TIVH prevalence in gout cases compared to controls. Owing to time limitations, genetic polymorphisms related to TIVH were not investigated. This approach may help to determine whether if TIVH is a causative factor in gout.
The increased number of VLDL particles found in Māori and Pacific gout cases may have resulted from its increased production. Therefore, it is possible that differences exist in genes which give rise to proteins involved with VLDL synthesis. These include the genes of apo B, microsomal TG transfer protein, protein disulfide isomerise, coat protein complexes I and II, Sar 1, ADP-riboosylation factor 1 and phospholipase D (see section 1.1.4.2). It would be interesting to sequence the genes of these proteins in gout case and control sample sets to detect for possible polymorphisms associated with gout. Multiple GWAS have previously shown that SNPs in the apo B gene (APOB) associate with TG levels, including rs7557067, rs1042034 and rs4635554 (Johansen et al., 2010; Kathiresan et al., 2008; Teslovich et al., 2010). The ability of apo B to become sufficiently lipidated on the rough endoplasmic reticulum during and shortly after translation is important for the first step of VLDL synthesis (Olofsson et al., 2000). Therefore, it is possible that these SNPs affect VLDL TG levels by altering the translational efficiency, the ability to become lipidated, the stability of folding, or the susceptibility to degradation of apo B on the endoplasmic reticulum. Functional studies on these SNPs should be conducted to confirm this. In another SNP (rs7575840) of APOB, the minor allele T was associated with increased apo B levels in a Finnish population (n = 7710; Haas et al., 2011). On the other hand, the major allele G positively correlated with the expression of BU630349 (a non-coding RNA) in adipose tissue, where BU630349 was negatively correlated with TG and VLDL levels (Haas et al., 2011). It is possible that BU630349 acts by regulating APOB expression, therefore it would be interesting to research BU630349 expression levels in gout case and control sample sets.

A possible alternative explanation for the increased VLDL TG in gout cases compared to controls may be due an attenuated ability for cases to hydrolyse the particles. Although gout cases were not found to have TG-enriched VLDL, the number of same-sized VLDL may build up (as was found in this study) due to decreased particle catabolism. LPL is involved with hydrolyzing TG in circulating lipoproteins (Pownall and Gotto Jr, 1999). Apo AV is found at a relatively low concentration in the blood on CM, VLDL and HDL particles (O’Brien et al., 2005) and increases the hydrolysis of TG by HSPG-bound LPL (Merkel et al., 2005). Like APOB, the genes for LPL (LPL) and apo AV (APOAV) have been found by multiple GWAS to be strongly associated with increased TG levels (Johansen et al., 2010; Kamatani et al., 2010; Kathiresan et al., 2008; Teslovich et al., 2010).
APOAV is tandemly organized in an approximately 60 kb multi-gene cluster region with APOAI, APOCIII and APOAIV on chromosome 11q23 (Karathanasis, 1985; Pennacchio et al., 2001). Within this cluster, high LD exists between SNPs, where 64% of 49 investigated SNPs were found to possess complete LD in a Caucasian population (Olivier et al., 2004). A mutation studied extensively in APOAV is Ser19Trp (rs3135506). In Europeans, the frequency of the minor allele C of rs3135506 was higher ($p < 0.05$) in various hyperlipoproteinemia patient groups with an increased TG level (including TIVH where frequency was 0.083, n = 48) when compared with controls (frequency = 0.052, n = 242; Hegele et al., 2009). The apo AV protein with Ser19Trp was found to cause conformational differences which decreased the amount of secreted protein from cultured HepG2 cells (Talmud et al., 2005), predicting a lowered apo AV concentration in blood compared to the wild type protein, and hence an increased TG level. For more discussion on Ser19Trp, see section 4.3.2. Another interesting SNP in the multi-gene cluster on 11q23 is rs670 (-75G→A of APOA1). There has been indication of a relationship between the A allele of rs670 with increased gout risk in a small Spanish sample set (n = 68 cases and 165 controls; Cardona et al., 2005). However, association studies between this SNP and TG levels or cardiovascular disease have been mixed, with some studies reporting association (Chhabra et al., 2005; Mar et al., 2004) and some not (Chien et al., 2008; Xiao et al., 2008b). Further large scale replication studies are required to conclude the influence of this SNP with gout, TG levels and cardiovascular disease.

LPL has repeatedly been found to associate with TG levels. For example, one rare variant (Gly188Glu, rs118204057) and three common variants (Asp9Asn, rs1801177; Ser447Ter, rs328; Asn291Ser, rs268) were investigated in a meta-analysis of 29 family, case control, cross sectional and case referent studies totalling 20930 Caucasian subjects (Wittrup et al., 1999). The carriers of all variants except Ser447Ter were associated with increased TG and decreased HDL cholesterol levels, and none caused differences in the total cholesterol level (Wittrup et al., 1999). On the other hand, there were fewer Ser447Ter carriers in subjects with hypertriglycerideridemia when compared to control or TIIIH subjects (Evans et al., 2010).

4.1.5. The role of triglycerides in cardiovascular risk and links to gout

It is to be noted that for the current project, the fasting status during blood collection was not specified, and due to the nature of the collection process, most samples were assumed to be
non-fasting. In a female sample set, the non-fasting \( n = 6347 \) TG level was a better predictor of cardiovascular diseases than the fasting \( n = 19983 \) TG level, even after adjustment for other lipids, whereas the non-fasting total or LDL cholesterol level did not predict cardiovascular diseases (Mora et al., 2008). In the Copenhagen City Heart Study of Danish subjects, the non-fasting TG but not cholesterol level was found to increase risk for ischemic stroke in both men \( n = 6372 \) and women \( n = 7579 \); Varbo et al., 2011b). In Norwegian women \( n = 24535 \), increasing non-fasting TG levels correlated with an increased risk for death from coronary heart disease, other cardiovascular diseases, and non-cardiovascular related causes (Stensvold et al., 1993). However, this effect was not seen in Norwegian men \( n = 25058 \); Stensvold et al., 1993). The non-fasting TG level was also not found to associate with ischemic heart disease in British men \( n = 5675 \); Pocock et al., 1989). The mixed results indicate that non-fasting TG levels require further investigation for association towards specific cardiovascular diseases, stratified by sex, and adjusted for independence from other lipid factors. This information would be highly beneficial for understanding human health, as most individuals are in a postprandial status for the majority of an average day. The fasting status occurs mostly only overnight during sleep. The study of fasting TG levels in relation to human health may therefore reflect artificial information which is non-applicable to everyday life. Furthermore, as atherosclerosis is more likely to occur due to an excess of lipids in the blood causing its deposition in the intima of arteries, the postprandial state is more likely to facilitate this event.

Increased TG has been implicated in cardiovascular function. An \textit{in vitro} study found that hypertriglyceridemic serum, particularly postprandial serum, induced the expression of proteins involved with endothelial dysfunction, such as lymphocyte function associated antigen-1, platelet/endothelial cell adhesion molecule-1, intercellular adhesion molecule-1 and vascular cell adhesion protein-1 (Norata et al., 2007). Furthermore, TG-rich VLDL and VLDL remnant particles were found to be susceptible to oxidation, and the oxidized particles were found to cause the accumulation of cholesterol esters in macrophage cells (Whitman et al., 1998). These results suggest mechanisms where TG could influence atherosclerosis.

Due to the association of TIVH with gout, hypertriglyceridemia may be a mechanism in which gout links to cardiovascular disease (see section 1.4). The current project has revealed differences in the lipid composition of VLDL particles between gout cases and controls. Extended study on the characteristics of the lipoprotein particles in cases and controls would
provide further insight into links between gout and cardiovascular disease. For example, investigations could be focused on looking at the atherogenic properties of VLDL in gout cases and controls, such as oxidation susceptibility, ability to induce protein expression, ability to induce cholesterol accumulation in macrophage cells, the amount of particles found in atherosclerotic plaques, or the influence of the particles in different animal models. Results of these investigations could indicate possible cause or effect mechanisms in which gout is biochemically related to cardiovascular disease.

Monosodium urate crystals have been found to bind apolipoproteins, including apo A1, apo B and apo E (Terkeltaub et al., 1984). Interestingly, when crystals were exposed to VLDL, LDL and IDL, but not HDL, neutrophil oxidative metabolism of the crystals was suppressed in vitro (Terkeltaub et al., 1984). In vivo, after an acute inflammatory gout attack in knee or elbow synovial fluid, apo B content was increased on purified monosodium urate crystals (Ortiz-Bravo et al., 1993). Little or no amount of apo B was found on crystals during inflammation (Ortiz-Bravo et al., 1993). This result was similar in a rat model of the synovium, where an air pouch was created subcutaneously in the rat (Ortiz-Bravo et al., 1993). A separate group of researchers recently found HDL to be suppressive of monocyte chemoattractant protein-1 mRNA transcription and protein release from intracellular stores of fibroblast-like synoviocytes (Scanu et al., 2010), corresponding to the anti-inflammatory property of HDL (Tsompanidi et al., 2010).

Although the concentration of lipoproteins in synovial fluid is only a fraction of that in plasma, patients with rheumatoid arthritis were found to have greater synovial membrane permeability for lipids compared to synovial membranes in the normal state (Prete et al., 1993). It would be interesting to investigate the permeability of synovial membrane for lipoproteins or apolipoproteins in gout patients. Example procedures to compare gout cases and controls include the use of histological techniques to characterise the synovial membrane, or to investigate the lipoprotein and apolipoprotein composition of synovial fluid. With this, inferences can be drawn on whether lipoproteins may be influencing the mechanisms of a gouty inflammatory attack. More importantly, plasma levels of lipoproteins and apolipoproteins (namely, higher levels of total plasma apo B and lower levels of HDL cholesterol in gout cases when compared with controls) can be compared with this.
4.2. Lipoprotein(a) levels in the Māori/Pacific and Caucasian populations

4.2.1. The lack of association between lipoprotein(a) levels and gout

No significant differences were found in Lp(a) levels between gout cases and controls in Caucasian or Māori and Pacific ethnicities. Only one other known group of investigators had studied Lp(a) levels in gout case and control sample sets. Japanese men with gout were shown to have increased Lp(a) levels when compared to controls, as well as increased total TG and decreased HDL cholesterol levels, but no differences in total cholesterol levels (Takahashi et al., 1995; Tsutsumi et al., 1998). Unfortunately, Takahashi et al. (1995) and Tsutsumi et al. (1998) did not conduct correlation analysis between Lp(a) and TG levels. An inverse relationship has been found between Lp(a) and TG levels in a Korean population of subjects visiting a medical centre (n = 1189; Chun et al., 2001) and several European populations of subjects recruited from lipid clinics (n ranged from 1009 to 1562; Ritter et al., 1997; Walek et al., 1995; Werba et al., 1993). These results are in contrast to the lack of correlation found between Lp(a) and TG levels in the current study’s Māori/Pacific and Caucasian gout case and control groups. The discrepancy may be due to the different clinical background between the populations (gout case and control sample sets compared with populations recruited from medical centres or lipid clinics). Indeed, in a German study with 1237 individuals of the general population, no relationship was found between Lp(a) and TG levels (Heinrich et al., 1991). In contrast, a smaller scaled study of 466 randomly selected Caucasian men found an inverse relationship between Lp(a) and TG levels (Klausen et al., 1996).

High Lp(a) levels have been associated with increased risk for cardiovascular disease. A meta-analysis of 36 studies including 126634 individuals showed that Lp(a) levels associated with increased risk for non-fatal MI, coronary death and ischemic stroke, even after multivariate adjustment for confounders (Erqou et al., 2009). No associations were found between Lp(a) levels and non-cardiovascular deaths, such as unclassified stroke, hemorrhagic stroke, and cancer (Erqou et al., 2009). Other studies confirmed the associative result between Lp(a) and increased risk for MI (Kamstrup et al., 2009), the degree of CAD (Beneš et al., 2002) and other forms of vascular diseases (Jones et al., 2007). These results were supported by the higher Lp(a) levels found in Māori and Pacific subjects self-reported to have unspecified heart problems, when
compared to the Māori and Pacific subjects who did not (the median Lp(a) level differed by 11.4 nmol/L). Although no differences were found between Caucasian subjects with and without heart problems, there was a trend towards significance (the Lp(a) median level differed by 7.9 nmol/L, $p < 0.15$). The association may have been reached in Caucasian subjects if the sample size was increased. However, caution must be placed on results based on sample sets where the case criterion was self-reported and non-specific.

Animal studies have shown Lp(a) to accumulate in the arterial intima, more so in atherosclerotic lesions when compared to non-lesioned intima, and to be trapped with greater affinity than LDL to proteoglycans and fibrin (Nielsen, 1999). Also, the degradation of trapped Lp(a) was slower than LDL (Nielsen, 1999). Interestingly, oxidized phospholipids were found to be preferentially carried by Lp(a) compared to LDL (Bergmark et al., 2008). In a human autopsy study, it was the oxidized version of Lp(a) that was located at the calcified lining of coronary arteries with MI (Morishita et al., 2009). This may indicate a means analogous to LDL in which Lp(a) increases cardiovascular risk, where the pathological impact comes upon the oxidation of the particle at the atherosclerotic site.

Gout is linked with cardiovascular disease through related factors such as increased insulin resistance (Takahashi et al., 2001), increased serum UA levels (see section 1.4) and hypertriglyceridemia (see sections 4.1.1 and 4.1.2). It should be noted that most of these factors are an integral part of the metabolic syndrome, which is also associated with gout (Hernández-Cuevas et al., 2009; Rho et al., 2005). Most definitions for the metabolic syndrome, such as criteria from the International Diabetes Federation, World Health Organization and the United States National Cholesterol Education Program, incorporate factors such as blood pressure, central obesity measures, TG levels and glucose levels. The Lp(a) level is not involved with metabolic syndrome diagnosis. As current results showed no associations between Lp(a) levels and gout, this indicates that Lp(a) does not additionally contribute to the relationship between gout and cardiovascular disease, at least not so in Caucasian and Māori/Pacific populations. Despite this, it is interesting to note that a high proportion of gout cases with high Lp(a) levels (greater than the 97.5th percentile) were reported to have unspecified heart problems (two out of six Māori/Pacific cases and all three Caucasian cases), but no controls (Māori and Pacific $n = 7$, Caucasian $n = 3$) were reported to have heart problems. Sample size needs to be increased for more appropriate analysis on this matter.
4.2.2. Lipoprotein(a) levels in different ethnicities

Between Caucasian and Māori/Pacific populations, no significant differences in the Lp(a) level were found in gout control subjects. However, Caucasian gout cases had a higher median Lp(a) level than Māori and Pacific gout cases, resulting in an overall significantly higher Lp(a) median level in all Caucasian subjects when compared with all Māori and Pacific subjects. The Lp(a) level distribution in both populations was very similar, with data skewed towards lower levels. Only one other known study has reported Lp(a) level data on a Polynesian sample set. Kamboh et al. (2000) found Lp(a) median levels to be significantly lower in Samoan individuals recruited from Western Samoa (2.7 mg/dl, n = 560) and the United States of America (2.1 mg/dl, n = 361) when compared with other ethnicities including Caucasians (3.8 mg/dl, n = 456), Hispanics (5.0 mg/dl, n = 262), African Americans (17.9 mg/dl, n = 217) and Africans from Nigeria (20.8 mg/dl, n = 786). Due to the variable size polymorphism of the apo(a) protein of Lp(a), there is no conversion factor for translating Lp(a) values from mg/dl to nmol/L, therefore direct values of Lp(a) cannot be compared between the current study and the study by Kamboh et al. (2000). Nevertheless, the relative Lp(a) levels between ethnicities were consistent.

The Lp(a) level of gout control subjects can be compared with other studies which used nmol/L as the unit of measurement for Lp(a). The Lp(a) median level for Caucasian controls (14 nmol/L) was slightly lower, if not approximately equal, to Caucasian controls of other studies. Jones et al. (2007) found a median value of 17 nmol/L in 230 controls for vascular disease from New Zealand, whereas Ronald et al. (2011) found a median level of 23 nmol/L for 770 controls for carotid artery stenosis in the United States of America.

4.2.3. Lipoprotein(a) levels and genotypes of lipoprotein related genes

The minor allele G of the LPA SNP rs10455872 showed significant positive associations with Lp(a) levels in Caucasian and Māori/Pacific ethnicities. This was in support of other studies showing increased Lp(a) levels in allele G carriers (p ranged from $3.6 \times 10^{-166}$ to $4.2 \times 10^{-23}$; Clarke et al., 2009; Lanktree et al., 2010; Ronald et al., 2010). Lanktree et al. (2010) further showed that along with 11 other SNPs within 100 kb of the LPA locus, rs10455872 explained 30.1% of the variation in Lp(a) levels in a Caucasian population (n = 272). Interestingly, the minor allele of this SNP was not detected in South Asians (descendants of individuals from India, Pakistan, Sri Lanka or Bangladesh, n = 330) or East Asians (descendants of individuals
from China, Taiwan or Hong Kong, n = 304; Lanktree et al., 2010). In the current study, the frequency of the minor allele of rs10455872 was noticeably low in the WP sample set, in which it did not exceed 1.4% (Table 22).

In comparison, the minor and risk allele C for the LPA SNP rs3798220 showed significant association with higher Lp(a) levels in Caucasian gout cases only. In Caucasian gout controls, the result was tending towards significance ($p = 0.0864$). It is likely that the borderline significance resulted from a low power for detection due to the small carrier group size (n = five). In fact, only three carriers had Lp(a) levels greater than the 75th percentile of the Caucasian control group value. In Caucasian studies, carriers of the C allele of rs3798220 compared to non-carriers were found to have higher Lp(a) levels ($p < 0.0001$; Arai et al., 2010; Clarke et al., 2009; Ronald et al., 2010) and an increased amount of oxidized phospholipids on apo B100-containing lipoproteins (Arai et al., 2010). No signs of association or tentative association between rs3798220 alleles and the Lp(a) level were witnessed in Māori and Pacific gout cases or controls. This implied that the rs3798220 influence on the Lp(a) level was limited to certain ethnicities, and in this case, the Caucasian ethnicity. Nevertheless, Lp(a) measurement in larger Caucasian and Māori/Pacific sample sets would be desired for greater statistical power in analysis.

There were no significant differences between the three main APOE genotypes in relation to Lp(a) levels. Apo E protein or genotypic isoforms have not been found to influence Caucasian Lp(a) levels, including Danish, Dutch, French, German, North American or Spanish populations (Anuurad et al., 2007; Bach-Ngohou et al., 2001; Boomsma et al., 2000; Klausen et al., 1996; Muros and Rodríguez-Ferrer, 1996; Ritter et al., 1997). This study’s Caucasian population results were in line with other Caucasian results in that APOE genotypes were not correlated to Lp(a) levels. However, in other populations, the evidence for an effect of the apo E or APOE isoform influence on Lp(a) levels have been mixed. Korean carriers of APOE2 and APOE4 isoforms showed lower Lp(a) levels compared to subjects homozygous for the APOE3 isoform (Chun et al., 2001). In contrast, Japanese subjects with the apo E2 isoform did not differ in Lp(a) levels from subjects with the apo E3 isoform (Sanada et al., 1998). However, subjects with the apo E4 isoform had significantly higher Lp(a) levels than subjects with apo E2 and apo E3 isoforms (Sanada et al., 1998). In African descendants, APOE2 carriers showed significantly lower, and APOE4 carriers showed significantly higher Lp(a) levels than APOE3 homozygotes (Anuurad et al., 2007). These results may implicate an ethnic effect on the influence of apo E
isoforms on Lp(a) levels. The present study showed that the Māori and Pacific ethnicity had no interaction effects with APOE isoforms for the influence on Lp(a) levels.

Lp(a) has been distinguished by the presence or absence of apo E on the particle (Bard et al., 1992). In fasting plasma, apo E bound Lp(a) was shown to have increased lipid levels (especially TG) and apo CIII particles (Bard et al., 1992). These apo E bound Lp(a) were able to interact with the LDL receptor better than LDL or non-apo E bound Lp(a) (Bard et al., 1992). In postprandial subjects, TG-rich Lp(a) was isolated and found to compositionally resemble VLDL particles more so than LDL particles (Scanu et al., 1994). Apo E was detected to covalently bind this particle (Scanu et al., 1994). Other investigations showed that certain types of Lp(a), namely “buoyant” Lp(a) which appeared in the VLDL and VLDL remnant density range, were metabolized faster in control subjects compared to an apo E deficient subject (Ikewaki et al., 2004). Therefore, apo E influences on Lp(a) levels may result from disparities in circulating levels of apo E-containing Lp(a), or the number of apo E molecules per Lp(a) particle. In fact, an in vitro study found thatapo E-enriched Lp(a) was better able to bind heparin sulfate than normal Lp(a) (van Barlingen et al., 1997). However, in vivo, after liberating HSPG of LPL with heparin injections in 39 individuals, the increased catabolism of Lp(a) was not demonstrated (van Barlingen et al., 1997). This indicates that the metabolism of Lp(a) in human is complex, where apo E may differ in accessibility to the Lp(a) particle between individuals or ethnicities, or catabolism pathways excluding HSPG and LPL exist for Lp(a). It would be interesting to investigate for differential effects of different apo E isoforms on Lp(a) metabolism. For example, as a starting point, an ELISA could be developed to determine the concentration of apo E and non-apo E bound Lp(a) in subjects with different apo E isoforms.

4.2.4. Limitations of lipoprotein(a) measurements: sample storage

In the current study, some of the serum samples used in the Lp(a) ELISA had been stored at -20°C for up to 1.5 years. The antibodies used in the Lp(a) ELISA bind the kringle IV type 2 and type 8 motifs of apo(a). Theoretically, the ELISA would still be able to accurately reflect true Lp(a) levels as long as degradation did not occur between these two motifs of apo(a). The degree of degradation of selected serum samples in western blot analysis was minor, suggesting sample preservation. Still, the issue of Lp(a) degradation cannot be completely disregarded.
Studies investigating the storage of samples for Lp(a) assays have produced varied results. For example, Sgoutas and Tuten (1992) found that Lp(a) levels decreased after one freeze-thaw cycle if serum was stored at -20°C, but two freeze-thaw cycles were required to decrease Lp(a) levels if serum was stored at -70°C. Kronenberg et al. (1994) found that Lp(a) levels measured from plasma frozen at -20°C or -80°C for one month were lower than fresh plasma measurements, but from then on, will remain significantly unchanged for up to 12 months of storage. After six months of sample storage at -20°C and -70°C, Evans et al. (1996) found that Lp(a) levels decreased more at -20°C when compared to -70°C, whereas Craig et al. (1992) found no differences between the two temperatures. Furthermore, measurements of low molecular weight Lp(a) decreased in level over time significantly more when compared to measurements of high molecular weight Lp(a) (Kronenberg et al., 1996). Part of the variability of the results in these studies on Lp(a) measurements over time and storage temperatures could be due to the use of different Lp(a) assay protocols.

The collection of blood samples can be highly intermittent, rendering the conductance of Lp(a) assays on fresh samples inconvenient. Therefore, to be able to measure Lp(a) from frozen samples would be preferred. Specifically for the current project’s Lp(a) assay protocol, it would be preferable to have investigated Lp(a) level changes over time and storage temperatures. However, this was an unrealistic goal for the current project as more time and work would be required. The general trend for optimal Lp(a) integrity is for samples to be stored at a minimal period of time and at the coldest temperature possible, that is, -80°C. Therefore, future and incoming samples should immediately be stored at -80°C. Furthermore, samples undergoing Lp(a) assays should have storage times as similar to each other as possible, and should be conducted as close as possible to the sample collection date. Lastly, more than one freeze-thaw cycle should be avoided. These factors would keep inter-sample variability resulting from laboratory handling to a minimum.

4.3. Genetics between gout and cardiovascular loci

4.3.1. Hardy-Weinberg equilibrium

In two out of five of the genotyped SNPs in association to gout (rs10455872 and rs1333049), the WP control sample set did not achieve Hardy-Weinberg equilibrium ($p < 0.05$).
Also, the Māori cases of self-reported heart problems did not reach Hardy-Weinberg equilibrium for the SNP rs1333049 ($p < 0.05$). These results may not be meaningful due to the small sample sizes of the groups (n = 143 and 67, respectively). Otherwise, Hardy-Weinberg disequilibrium may indicate poor quality of genotype assignment to samples (Hosking et al., 2004). However, the Taqman® genotype scatter graphs consisted of good quality genotyping for these groups (Appendix B.II). Non-specific primers towards the DNA region of interest during polymerase chain reaction may also contribute to Hardy-Weinberg disequilibrium (Hosking et al., 2004). Unfortunately, primer sequences were unavailable, therefore could not be checked for specificity. However, if primers for one SNP were non-specific, it would be expected that for that SNP, all sample sets would deviate from Hardy-Weinberg equilibrium. This was not the case for the current study. Hosking et al. (2004) reported that 3.19% of 313 SNPs were out of Hardy-Weinberg equilibrium with no apparent quality deviations. Therefore, it is possible that the WP gout controls for rs10455872 and rs1333049 and the Māori heart problem cases for rs1333049 did not meet Hardy-Weinberg equilibrium out of chance. Alternatively, these groups may have represented a heterogeneous non-random sample resulting from mutations, migrations or selective breeding (Crow and Dove, 1988). No population, especially case groups, is expected to be completely random, therefore the deviation from Hardy-Weinberg equilibrium was not a serious concern.

4.3.2. Gout versus APOE - involved with lipid metabolism and disorders

For the APOE SNP rs429358, the statistical power to detect association with gout at an OR of 1.6 or above was strong (greater than 80%) in all sample sets, but weak (19.6% to 28.2%) at an OR of 1.2. No significant associations with gout were found between cases and controls in any of the ethnicities after adjustment for Caucasian admixture. After logistic regression accounting for covariates, only the Māori sample set showed a significant protective association of the APOE4 allele with gout. This significance may have been a spurious false positive, or a false positive due to the decreased sample size (154 cases and 187 controls) available for logistic regression analysis. To establish a genuine association, analysis needs to be repeated on an increased sample size. Otherwise, from these results, the APOE4 allele was not likely to pose an association with the gout phenotype. This lack of association was supported by the lack of
association between apo E phenotypes with gout in a Japanese population (n = 221 cases and 141 controls; Moriwaki et al., 1995).

For the APOE SNP rs7412, no significant differences were found in any of the gout case and control sample sets, even after adjustment for ethnicity admixture in Māori, EP and WP sample sets, or for other covariates in all sample sets. This indicated that there was not a significantly increased frequency of the APOE2 allele in cases. There was sufficient statistical power (greater than 90%) in all sample sets to detect a strong association (OR greater than 2.0) between gout and rs7412, although there was low power (less than 20%) to detect weaker associations (OR at 1.2). APOE2 predisposes towards TIIIH, and environmental factors are required to induce the disease (Mahley and Rall Jr, 2000). The lack of increased APOE2 allele frequency in cases supports the FPLC results where no increase in total or VLDL cholesterol was found in cases when compared to controls. These results indicate that gout cases do not have a higher prevalence or predisposition to TIIIH. Similarly, Jiao et al. (1986) and Naito and Mackenzie (1979) did not find an increased TIIIH prevalence in gout cases compared to controls.

Past studies with results on the APOE isoform and its relationship with serum UA levels have been limited and mixed. In a Brazilian population (n = 1493), APOE4 carriers had higher UA levels than APOE2 carriers, but not APOE3 homozygotes (Alvim et al., 2010). In Caucasian subjects (n = 290), APOE2 carriers had higher serum UA levels than homozygote APOE3 subjects or APOE4 carriers (Liberopoulos et al., 2005). Interestingly, the apo E2 phenotype was shown to decrease UA excretion, but only in gout cases (n = 68) and not in controls (n = 50; Cardona et al., 2003). In contrast, the current study showed that Māori and Pacific gout cases who were APOE4 carriers had significantly higher serum UA levels than APOE3 homozygotes. Otherwise, there was a lack of association in serum UA levels between APOE genotypes. The results found by Liberopoulos et al. (2005), Cardona et al. (2003) and the current study for the association between apo E or APOE isoforms and gout may be erroneous outcomes due to the small sample sizes. Nevertheless, there are reported biochemical associations between apo E and UA. Low concentrations of apo E has been found in synovial fluid and was shown to bind urate and indirectly affect neutrophil action (Terkeltaub et al., 1991), possibly affecting gout inflammation. However, any effects are predicted to be weak due to the low concentrations of apo E in synovial fluid. To determine if apo E isoforms genuinely express differential effects in
gout cases and controls, the isoforms, concentrations and actions of apo E in the synovial fluid should be investigated in gout case and control sample sets.

Interestingly, the homozygote apo E2 phenotype has been associated with the Ser19Trp (rs3135506) polymorphism in APOAV, which is associated with increased TG levels (see section 4.1.4). Six out of seven of the homozygote apo E2 hypertriglyceridemic patients were found to be heterozygotes for the Ser19Trp mutation in a German study (Schaefer et al., 2004). Furthermore, a Spanish study showed that a larger percentage of TIIIH patients (33%) had a higher prevalence for the Ser19Trp mutation than homozygote APOE2 controls (8.9%, \( p = 0.005 \), OR [95% CI] = 6.7 [1.7 - 26.4]; Martin-Campos et al., 2006). This implicated that Ser19Trp may increase the chances of abnormal lipid levels in APOE2 subjects. In the current study, the presence of Ser19Trp was not investigated. Although general population studies found no differences in TG levels across APOE isoforms (Alvim et al., 2010; Burman et al., 2009), the current study showed that APOE4 carriers had higher TG levels than APOE3 homozygotes in combined (Māori/Pacific and Caucasian) gout cases and controls and APOE2 carriers in combined controls. Regardless of the contrast in association between the studies, gout is related to hypertriglyceridemia (see sections 4.1.1 and 4.1.2), therefore apo E isoform influence on gout may be via small effects from lipid-related mechanisms.

4.3.3. Gout versus LPA - a genetic determinant of lipoprotein(a) parameters

Neither genotyped SNPs in LPA (rs3798220 and rs10455872) showed association with gout in any ethnic sample set after adjustment for covariates, or adjustment for Caucasian admixture in the Māori, EP and WP sample sets. This may have been due to low statistical power to detect association in the sample sets, especially for the Māori and Pacific sample sets where power ranged from 5.4% to 66.9% to detect for association at an OR of 2.0 or below. The low minor allele frequencies in these two SNPs would have contributed to the low power to detect for association. Additionally, the high variability of Lp(a) levels in any given population (Nordestgaard et al., 2010) would contribute to the difficulty in achieving power to detect for association in SNPs which influence Lp(a) levels.

The lack of association between gout and the investigated LPA SNPs corresponded with the lack of significant differences found in Lp(a) levels between gout cases and controls (see section 4.2.1). Both rs3798220 and rs10455872 had been associated with Lp(a) levels and risk
for cardiovascular diseases (Clarke et al., 2009; Luke et al., 2007; Ronald et al., 2011; Shiffman et al., 2010). The lack of association between these two SNPs and gout supports a genuine lack of association between the Lp(a) factor and gout.

4.3.4. Gout versus CDKN2BAS - the 9p21 locus

For the SNP rs1333049 of CDKN2BAS, a recent study reported the association of the risk allele C with gout in a Han Chinese population (n = 461 cases and 439 controls, \( p = 0.01 \), OR [95% CI] = 1.26 [1.06 - 1.54]; Wang et al., 2011). This prompted the study of this SNP in the current project’s gout case and control sample sets. Unlike the Han Chinese population, there were no significant differences in the allele frequencies of rs1333049 between gout cases and controls in all sample sets, even after adjustment for various covariates or Caucasian ethnicity in the Māori, EP and WP sample sets. Thus, the current study indicates that gout was not an additional associative trait with rs1333049 for Māori, Pacific or Caucasian populations. This may have been due to low statistical power for the replication of association in the investigated sample sets (minimum power was 26.6% in the FHS, and maximum power was 67.1% in the Caucasian sample set). To robustly demonstrate a lack of association between rs1333049 and gout, sample sizes need to be increased.

There were also no significant differences in the allele frequencies of rs1333049 between subjects with and without self-reported heart problems. However, this may be due to two major limitations. The first limitation was the lack of clinical criteria and specificity used to define heart problems. The second limitation was the small sample size. Although there was sufficient statistical power (mostly over 80%) to detect for a strong association (OR above 2.0) between rs1333049 and heart problems, power was low (below 60%) for detecting a weak association (OR generally below 1.6) between the two factors.

The C allele of rs1333049 has repeatedly been associated with various forms of cardiovascular disease in many ethnicities. Coronary diseases have been associated with the C allele in British, Belgian (Buysschaert et al., 2010), Irish (Meng et al., 2008), Japanese and Korean populations (Hinohara et al., 2008). Furthermore, the severity of CAD, as measured by the number of vessels affected by greater than 50% stenosis, was also dose-dependently affected by the allele (Dandona et al., 2010). The C allele has also been associated with an increased risk for MI in German (Samani et al., 2007; Scheffold et al., 2011), Italian (Emanuele et al., 2010)
and Pakistani (Saleheen et al., 2010) populations, with pronounced risk found in individuals with a family history of MI (Scheffold et al., 2011). Ischemic stroke has been associated with the C allele in Swedish populations (Smith et al., 2009). Other vascular disorders positively associated with the C allele include abdominal aortic aneurysm (Biros et al., 2010, Bown et al., 2008) and vascular dementia (Emanuele et al., 2011). Few studies have investigated the effects of rs1333049 on cardiovascular related functions. When compared with GG homozygotes, CC homozygotes were found to have a lower vasodilation response to acetylcholine or glycerol trinitrite administration, indicating vascular dysfunction (Aschauer et al., 2010). However, blood endothelial progenitor cell concentration and the concentrations of other factors involved with vascular repair were not significantly different across rs1333049 genotypes (Ye et al., 2010). More functional studies are required to elucidate how rs1333049 influences increased cardiovascular risk.

4.3.5. Additional genotyping directions

No associations with gout were found between any of the investigated SNPs in the current study. However, due to the association between TG levels in both gout and cardiovascular disease (see section 4.1), it would be interesting to genotype SNPs associated with hypertriglyceridemia in gout case and control sample sets. Some SNPs in APOB, LPL and APOAV have already been discussed (see section 4.1.4). However, many other loci are also associated with hypertriglyceridemia.

Other loci repeatedly found to be associated with high TG levels in GWAS include GCKR, TRIB1, MLXIPL, APOE and ANGPTL3 (Johansen et al., 2010; Kamatani et al., 2010; Kathiresan et al., 2008; Teslovich et al., 2010). Besides GCKR, most of these loci show weaker effects on TG levels ($p$ ranged from 0.002 to $6 \times 10^{-58}$) in comparison to LPL and APOAV ($p$ ranged from $2.0 \times 10^{-7}$ to $2 \times 10^{-115}$; Johansen et al., 2010; Kamatani et al., 2010; Kathiresan et al., 2008; Teslovich et al., 2010). Despite this, findings were robust and replicable. GCKR encodes a regulatory protein that allosterically inhibits glucokinase, a protein that phosphorylates glucose to glucose-6-phosphate (Agius, 2008). TRIB1 encodes a protein that regulates the mitogen-activated protein kinase signalling pathway (Kiss-Toth et al., 2004). MLXIPL mRNA expression is induced by cell proliferation, and the resultant protein (the carbohydrate response element binding protein) is involved with the transcriptional regulation of cell growth (Cairo et
ANGPTL3 encodes a protein which is implicated in endothelial cell adhesion and migration, and angiogenesis (Camenisch et al., 2002). ANGPTL3 expression was found to increase VLDL TG levels in mice by inhibiting LPL activity (Shimizugawa et al., 2002).

The lead SNP of GCKR (rs1260326) associated with higher TG levels in GWAS (Johansen et al., 2010; Kamatani et al., 2010; Kathiresan et al., 2008; Teslovich et al., 2010) has been associated with increased serum UA levels in a large meta-analysis study conducted by Kolz et al. (2009). The lead SNP of GCKR associated with the increased serum UA levels (rs780094) in the Kolz et al. (2009) study was also associated with increased TG levels in Caucasian and African descendant participants of the ARIC study (Bi et al., 2010) as well as a large multi-cohort study including African Americans, Hispanics, Chinese, Malays, Indians, Singaporeans and Caucasians from Europe and the United States of America, (Orho-Melander et al., 2008). These two GCKR SNPs pose high LD with each other in a Danish population (r² = 0.91; Varbo et al., 2011a). Interestingly, in the Prevention of Renal and Vascular Endstage Disease study from the Netherlands (n = 7795), rs780094 was found to associate with UA levels, but the association was abolished after adjusting for TG levels (van der Harst et al., 2010). Furthermore, rs780094 was associated with TG levels, even after adjustment for UA levels (van der Harst et al., 2010). This suggested that the effect of GCKR on serum UA levels is indirect, and may result from a downstream effect via influence from TG.

The GCKR SNPs along with TRIB1 lead SNP rs2954029 found in GWAS for higher TG levels (Johansen et al., 2010; Kathiresan et al., 2008; Teslovich et al., 2010) showed further associations not just with TG levels in three large Danish populations, but also with levels of remnant cholesterol, apo B and HDL (Varbo et al., 2011a). The TRIB1 variant showed additional associations with LDL cholesterol levels and the risk for MI and ischemic heart disease (Varbo et al., 2011a).

Most genetic studies on polymorphisms related to hypertriglyceridemia were not limited to the TIVH phenotype; most polymorphisms associated with hypertriglyceridemia apply to many types of hyperlipoproteinemia and dyslipidemia, such as type I or type V hyperlipoproteinemia (Schaap et al., 2006; Wang et al., 2007). This is due to a large crossover of the hypertriglyceridemic trait in the various disorders, with a large spectrum of environmental factors and genetic polymorphisms affecting the clinical outcome. Isolated cases of genetic polymorphisms have been detected to induce extreme cases of hypertriglyceridemia, including
mutations in \textit{APOCII}, \textit{LPL} and \textit{APOAV} (Chen et al., 2007; Kuniyoshi et al., 1999; Okubo et al., 1997; Oliva et al., 2005; Yu et al., 2006). However, these mutations are scarce and unlikely to have significant influence on a population basis.

Due to the association found in the current study between gout or serum UA levels and hypertriglyceridemia (see section 4.1.1 and 4.1.2), there is justification for all SNPs influencing TG levels to be genotyped for association analysis with gout or serum UA levels, including the SNPs mentioned in section 4.1.4. Research in this direction may reveal novel genetic factors in the complex disease gout, and hence add understanding, and ultimately better therapy, to the multi-factorial disease.

\textbf{4.4. Summary}

Gout and cardiovascular disease are complex, multivariable, and associated with each other as well as many other factors and disorders. Many components of the metabolic syndrome, such as insulin resistance, hypertriglyceridemia and hyperuricemia are associated with both gout and cardiovascular disease. Results from the current study demonstrate the association between hypertriglyceridemia and gout in Caucasian, Māori and Pacific ethnicities, and show a preliminary lack of association between Lp(a) levels and gout. Furthermore, no genetic links between gout and cardiovascular disease were found in the genes \textit{APOE}, \textit{LPA} or \textit{CDKN2BAS} for those SNPs tested. This study highlights promising directions for consolidating links between gout and cardiovascular disease in the area of metabolic syndrome associated factors. In particular, TG analysis on a wider sample set should be conducted, including investigations into TG level-associated genetics. These will be stepping stones for gaining better knowledge and understanding for the complex links between gout and cardiovascular disease.
5. References


Beneš, P., Mužik, J., Benedík, J., Znojil, V., Vácha, J. (2002). The relationship among apolipoprotein(a) polymorphisms, the low density lipoprotein receptor-related protein, and the very low density lipoprotein receptor genes, and plasma lipoprotein(a) concentration in the Czech population. Human Biology 74, 129-136.


Kamstrup, P.R., Tybjaerg-Hansen, A., Steffensen, R., and Nordestgaard, B.G. (2009). Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. Journal of the American Medical Association 301, 2331-2339.


of apolipoprotein A-I are important for lecithin:cholesterol acyltransferase activation and the maturation of high density lipoprotein in vivo. Journal of Biological Chemistry 276, 48716-48724.


6. Appendices

Appendix A: Recipes for solutions

Appendix A.I. Fast protein liquid chromatography

*Fast protein liquid chromatography-phosphate buffer solution*
34.2 mL Na₂HPO₄ (1 mol/L)
15.8 mL NaH₂PO₄ (1 mol/L)
8.74 g NaCl
Made up to 1L with MilliQ® water

Appendix A.II. Enzyme-linked immunosorbant assay

*Antibody coating buffer*
100 mmol/L Na₂CO₃
100 mmol/L NaHCO₃
Mixed to a pH of 9.6

*Phosphate buffer solution*
8 g NaCl
0.2 g KCl
1.15 g Na₃HPO₄
0.2 g KNa₂PO₄
Made up to 1 L with MilliQ® water
Mixed to a pH of 7.3

*Blocking buffer*
1.5 g bovine serum albumin
30 mL phosphate buffer solution
Filtered

*Wash buffer*
0.5 mL Tween-20
1 L phosphate buffer solution

*Plasma dilution buffer*
0.0396 g bovine serum albumin
40 mL wash buffer
Filtered

*Citrate buffer*
3.65 g Na₃HPO₄
2.33 g citric acid
Made up to 500 mL with MilliQ® water

*Substrate solution*
22 mL citrate buffer
20 µL H₂O₂ (30%; substrate for the detection reaction)
10 g tablet of O-phenylenediamide dihydrochloride (chromagen for the detection reaction)
Appendix A.III. Western blot

**Resolving gel buffer**
140 mL Tris/hydrochloride (1.5 mol/L, pH 8.8)
5.6 mL sodium dodecyl sulfate (10%)
340 mL MilliQ® water

**Resolving gel solution**
6.5 mL resolving gel buffer
1 mL acrylamide (30%)
25 µL ammonium persulfate (20%)
25 µL tetramethylethylenediamine
Makes one 4% gel

**Stacking gel buffer**
62.5 mL Tris/hydrochloride (0.5 mol/L; pH 6.8)
2.5 mL sodium dodecyl sulfate (10%)
156 mL MilliQ® water

**Stacking gel solution**
2.21 mL stacking gel buffer
0.25 mL acrylamide (30%)
10 µL ammonium persulfate (20%)
10 µL tetramethylethylenediamine
Makes one 3% gel

**Reducing sample buffer**
3 mL glycerol
3.75 mL Tris/hydrochloride (0.5 mol/L, pH 6.8)
1 g sodium dodecyl sulfate
1 g dithiothreitol
Trace amount of bromophenol blue
Made up to 10 mL with MilliQ® water

**Non-reducing sample buffer**
3 mL glycerol
3.75 mL Tris/hydrochloride (0.5 mol/L, pH 6.8)
0.7 g sodium dodecyl sulfate
Trace amount of bromophenol blue
Made up to 10 mL with MilliQ® water

**Transfer buffer**
18.19 g Tris
86.52 g glycine
600 mL methanol
Made up to 6 L with MilliQ® water

**Blotto**
187 g NaCl
242.3 g Tris
11.8 g CaCl₂
Made up to 4 L with MilliQ® water
Adjusted to a pH of 8.0 with hydrochloride

**Blocking solution**
50 mL blotto
25 g trim milk power (Pams)
Made up to 500 mL with MilliQ® water

**Tris-buffered saline**
1.2 g Tris
4.0 g NaCl
0.5 mL Tween-20
Made up to 500 mL with MilliQ® water
Adjusted to a pH of 7.6 with hydrochloride
**Electrochemiluminescence reagent A**
1 mL luminol (chromagen for the detection reaction)  
60 µL p-coumaric acid  
17 mL Tris (1.5 mol/L, pH 8.6)  
Made up to 250 mL with MilliQ® water

**Electrochemiluminescence reagent B**
17 mL Tris (1.5 mol/L, pH 8.6)  
60 µL H₂O₂ (30%; substrate for the detection reaction)  
Made up to 250 mL with MilliQ® water

**Appendix A.IV. DNA genotyping**

**Guanidine hydrochloride reagents**
3.5 mL guanidine hydrochloride (6.0 mol/L)  
250 µL ammonium acetate (7.5 mol/L)  
50 µL proteinase K (10 mg/mL)  
250 µL sodium lauroyl sarcosinate (20%)

**Tris-ethylenediaminetetraacetic acid buffer**
1 mL Tris (1.0 mol/L, pH 7)  
20 µL ethylenediaminetetraacetic acid (0.5 mol/L, pH 8)  
Made up to 100 mL with MilliQ® water

**Appendix B: Figures**

**Appendix B.I. Lipoprotein separation traces**

*Representative example of a chromatogram from fast protein liquid chromatography*

![Chromatogram](image)

NB: chromatogram was of a gout control plasma sample. Fraction numbers are indicated at the top. Buffer volume is indicated at the bottom. Blue line indicates the ultraviolet trace at 280 nm (left axis). Red line indicates conductance (mS/cm), which signifies the ion content of the system (right axis). Note that proteins aside from lipoprotein proteins can also be detected.
**Representative example of lipoprotein triglyceride and cholesterol traces**

NB: trace was from a gout case sample. Lipoproteins in plasma samples were separated (as indicated) by fast protein liquid chromatography and collected into 0.5 mL fractions. Each fraction was assayed for triglyceride (TG) and cholesterol concentration. See sections 2.3.1 and 2.3.2 for details. VLDL = very low density lipoprotein. LDL = low density lipoprotein. HDL = high density lipoprotein.

**Appendix B.II. Taqman® genotype scatter graphs**

**Representative example of a genotype graph for rs429358 of APOE**

NB: data was from a subset of the Caucasian gout control sample set. VIC and FAM were the fluorescent dyes attached to the genotype specific probes. Notice the three distinct genotype clusters.
Representative example of a genotype graph for rs7412 of APOE

NB: data was from a subset of the Caucasian gout case sample set. VIC and FAM were the fluorescent dyes attached to the genotype specific probes. Notice the three distinct genotype clusters.

Representative example of a genotype graph for rs3798220 of LPA

NB: data was from a subset of the Māori and Pacific gout case sample set. VIC and FAM were the fluorescent dyes attached to the genotype specific probes. Notice the two distinct genotype clusters. The CC genotype was not found in this subset.
Representative example of a genotype graph for rs10455872 of LPA

NB: data was from a subset of the Māori and Pacific gout control sample set. VIC and FAM were the fluorescent dyes attached to the genotype specific probes. Notice the two distinct genotype clusters. The GG genotype was not found in this subset.

Representative example of a genotype graph for rs1333049 of CDKN2BAS

NB: data was from a subset of the Māori and Pacific case sample set. VIC and FAM were the fluorescent dyes attached to the genotype specific probes. Notice the three distinct genotype clusters.
Appendix C: Other

Standard curve details for various assays

<table>
<thead>
<tr>
<th>Standard curve point</th>
<th>Concentration</th>
<th>Cholesterol assay (mmol/L)*</th>
<th>Lp(a) ELISA (nmol/L)#</th>
<th>Apo B ELISA (nmol/L)*</th>
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*Precipath L (Cobas®, Roche/Hitachi) was used as the reference material for the standard curve values. #A calibrator made from human plasma (from the Northwest Lipid Research Labs) was used as the reference material for the standard curve values. All standard curves had $r^2$ greater than 0.96. TG = triglyceride. Lp(a) = lipoprotein(a). ELISA = enzyme-linked immunosorbant assay. Apo B = apolipoprotein B.

Unlinked genomic markers used for Structure and STRAT analysis

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<th>Associated gene</th>
<th>Marker</th>
<th>Associated gene</th>
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<td>rs2059606</td>
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<td>GFPT2</td>
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*Marker was genotyped by the restriction fragment length polymorphism method. All other markers were genotyped by the Taqman® single nucleotide polymorphism genotyping method. See Phipps-Green *et al.* (2010) for details.

*Marker was not used for the stratification of Eastern Polynesian or Western Polynesian sample sets due to allele frequencies not meeting the required criteria (see section 2.4.3).