

**ASSOCIATION OF HEPATIC LIPASE AND ENDOTHELIAL LIPASE
POLYMORPHISMS WITH VARIATION IN NMR LIPOPROTEIN SUBCLASSES IN
CAUCASIAN, AFRICAN-AMERICAN AND AFRICAN-CARIBBEAN OLDER MEN**

by

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University of Pittsburgh, 2004

Despite higher prevalence of risk factors for coronary heart disease, men of African origin have less coronary atherosclerosis, as measured by coronary calcification, than Caucasians. In part, this is thought to be due to the less atherogenic lipoprotein profile observed in men of African origin, characterized by lower levels of triglycerides and higher levels of HDL-C. The aim of the present study was to investigate the genetic contribution of two candidate genes, endothelial lipase (LIPG) and hepatic lipase (LIPC), to the ethnic variation in nuclear magnetic resonance (NMR) measured lipoproteins in 600 Caucasian, 100 African-American and 205 Tobago African-Caribbean men, older than 65 years. First, using a set of six ancestry informative markers, we estimated high African genetic contribution in the Tobago population (94%). A more favorable lipoprotein profile was observed in men of African origin compared to Caucasians. The frequency of the LIPG 584T allele in Tobago men (0.06) was five times less common than in Caucasians (0.29) and two times less common than in African-Americans (0.14). In African-Caribbeans, 584T allele was associated with lower small HDL and a greater HDL size, whereas in Caucasians and African-Americans, no significant association was found. Although, the LIPG 584T allele is protective in African-Caribbean men, its frequency is too low

to explain the more favorable lipoprotein profile observed in these men. In contrast, the frequency of the LIPC -514T allele (0.57) was somewhat higher than the frequency in African-Americans (0.49), and three times as high as the frequency in Caucasians (0.20). 514C>T interacted with ethnicity to affect the levels of HDL-C, large HDL and HDL and LDL size. Carriers of 514T allele in both populations of African origin, but not in Caucasians, had elevated large HDL and greater HDL size. The higher frequency of the LIPC -514T allele in men of African origin significantly contributes to the more favorable distribution of HDL subclasses compared with Caucasians. Our findings have important public health relevance as they increase our understanding of Black-White differences in lipoprotein distributions, and are likely to increase our understanding of the underlying causes behind the ethnic differences in susceptibility to atherosclerosis.

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Iva Miljković-Gačić

1. INTRODUCTION

At present, elimination of health disparities among ethnic groups is one the most challenging and important public health priorities. The extent to which genetic and environmental factors account for ethnic differences in complex syndromes and diseases, such as cardiovascular disease, is still unknown. African populations are greatly underrepresented in the study of genetics of complex chronic diseases. Therefore, the Tobago population is a valuable resource for studying the genetics of any syndromes or diseases that show significant differences in prevalence between Caucasians of European descent and populations of African descent. Based on historical information, the African-Caribbean population of Tobago appears to be a stable population of descendents from West Africa, and men from this island are believed to share considerable West African ancestry with African-Americans. However, empirical confirmation of low European admixture may help in clarifying the Black-White variation in risk for some chronic diseases, such as coronary heart disease, hypertension, diabetes, or prostate cancer.

Coronary heart disease (CHD) is the leading cause of mortality, and one of the most important causes of physical disability in older individuals. Ethnic differences in CHD burden have been well documented (Harris-Hooker and Sanford 1994; Kuller 2004). However, reported Black-White differences in CHD incidence and mortality are not entirely consistent. Some studies suggest that CHD incidence rates are lower in African-American than in Caucasian men (Keil et al. 1995; Gillum et al. 1997), while other studies report similar rates in men of both ethnicities (Jones et al. 2002). There is some evidence that compared with Caucasian men,

African-American men have higher CHD mortality rates at younger ages and lower mortality rates at older ages (Gillum et al. 1997; Corti et al. 1999; National Heart 2002), whereas some studies report that CHD mortality rates are higher in African-American men of any age (Jones et al. 2002). Despite the higher prevalence of CHD risk factors, including hypertension and diabetes, men of African origin have less evidence of coronary atherosclerosis, as measured by coronary artery calcium (CAC), than Caucasians (Newman et al. 2002; Lee et al. 2003). In part, this is thought to be due to the less atherogenic lipoprotein profile observed in men of African origin, characterized by lower levels of triglycerides (TG) and higher levels of high density lipoprotein cholesterol (HDL-C).

Plasma lipoprotein abnormalities have a central role in the development of atherosclerotic lesions (Daugherty and Schonfeld 1985). The inverse association between HDL-C and the development of atherosclerosis and CHD is well established (Gordon and Rifkind 1989; Badimon et al. 1992; Barter and Rye 1996; Gotto 2001; Assmann and Nofer 2003). In older populations, independent of ethnicity, the HDL-C level is found to be a more specific and powerful predictor of risk for death from CHD than is total cholesterol (TC) or low density lipoprotein cholesterol (LDL-C) (Corti et al. 1995; Li et al. 2004). Lipoprotein cholesterol levels are a function of both particle number and particle size. These characteristics independently contribute to the development of atherosclerosis (Freedman et al. 1998). Elevated small, dense LDL and elevated small HDL lipoprotein subclasses, or decreased large HDL subclasses, have been widely accepted as better predictors of CHD risk than lipid concentrations obtained by the standard assays (total cholesterol, LDL-C and HDL-C) (Freedman et al. 1998; Lamarche et al. 2001; Rosenson et al. 2002). Therefore, because individuals with the same lipid concentrations can have substantially different risks of CHD, dependent on lipoprotein subclasses, the

measurement of lipoprotein subclasses and sizes can significantly improve the assessment of CHD risk. NMR-spectroscopy, used in the present study, has many advantages over traditional methods for measuring lipoprotein subclasses. NMR provides accurate, non-labor intensive and fast lipoprotein profile, by direct quantification of very low density lipoprotein (VLDL), HDL and LDL subclasses simultaneously (Otvos et al. 1992). Additionally, it uses substantially less sample volume than traditional methods.

The underlying reasons for higher level of HDL-C, and favorable distribution of its subclasses and sizes, in men of African descent, remain unclear. Ethnic differences in HDL-C distributions are observed even within relatively homogeneous environments, suggesting that genetic factors may be essential in determining high HDL-C. Several investigators have reported that polymorphisms in candidate genes involved in lipid metabolism affect lipoprotein levels. Still, the extent to which lipoprotein levels are affected by genetic and environmental factors, and the mechanisms by which genetic factors influence ethnic differences in lipoprotein levels and consequently CHD, are yet not fully understood.

It is possible that higher HDL-C is due to, at least in part, lower hepatic lipase (HL) activity that has been observed in African-American men (Vega et al. 1998). HL is an enzyme which has a central role in lipoprotein metabolism by hydrolyzing the phospholipids and TG, and promoting the hepatic lipase uptake of lipoproteins (Bensadoun and Berryman 1996; Perret et al. 2002). Consequently genetic variants in hepatic lipase gene (LIPC) have been shown to be associated with higher levels of HDL-C. The LIPC -514 C>T single nucleotide polymorphism (SNP) has been extensively studied. The frequency of the -514 T allele varies across ethnic groups, ranging 17-27% in Caucasians (Jansen et al. 1997; Zambon et al. 1998; Hokanson et al. 2003; Carr et al. 2004), 44-54% in African-Americans (Vega et al. 1998; Juo et al. 2001; Chen et

al. 2003; Carr et al. 2004), 47% in US Hispanics (Hokanson et al. 2003), 35-44% in Koreans (Hong et al. 2000; Park et al. 2003) and 50% in Japanese (Inazu et al. 2001; Carr et al. 2004). This allele was found to be associated with a more cardio-protective lipoprotein profile, characterized by lower HL activity (Vega et al. 1998; Zambon et al. 1998), increased levels of HDL-C (Guerra et al. 1997; Murtomaki et al. 1997; Inazu et al. 2001), increased large HDL (Zambon et al. 1998; Couture et al. 2000; Juo et al. 2001), and in some studies, increased large LDL particles (Zambon et al. 1998).

Another candidate gene that has a potential impact on HDL metabolism is endothelial lipase gene (LIPG). The endothelial lipase enzyme (EL) has a significant phospholipase A1 activity (Rader and Jaye 2000). One of the most common polymorphisms in this gene, among those identified to date, is 584 C>T (or Thr111Ile). It has been shown that the frequency of the LIPG 584 T allele is differently distributed in African-Americans (10.3%) (deLemos et al. 2002), Japanese (24%) (Yamakawa-Kobayashi et al. 2003), Caucasian controls (31.2%) (deLemos et al. 2002), or Caucasian individuals with high levels of HDL-C (32.6%) (deLemos et al. 2002). In a mouse model, over-expression of EL significantly reduced levels of plasma HDL-C (Jaye et al. 1999), and inhibition of EL increased plasma HDL-C levels (Jin et al. 2003). A relatively small number of reports on the association between LIPG 584C>T and HDL-C or HDL subclasses, have been inconclusive. Some studies reported no association between the 584 T allele and HDL-C (deLemos et al. 2002; Yamakawa-Kobayashi et al. 2003), TC, TG or LDL-C (Yamakawa-Kobayashi et al. 2003). Other studies found a significant association between this allele and HDL-C (Ma et al. 2003) as well as HDL subclasses (Halverstadt et al. 2003), and some found that T allele significantly increased large HDL subclass, but only in women (Paradis et al. 2003).

There is considerable evidence for a genetic contribution to lipoprotein abnormalities and atherosclerosis, but few data are available in populations of African heritage. The present study focused on the genetic contribution to the variation in lipoprotein phenotypes, in older African-Caribbean men on the island of Tobago (The Tobago Health Study), and among older African-American and Caucasian men from the Cardiovascular Health Study (CHS).

Our objective is to identify some of the risk factors responsible for ethnic differences in plasma lipoprotein levels that will more accurately predict risk for coronary atherosclerosis. Our aim is to describe differences in lipoprotein subclass distributions in men of African origin living in different environments, and compare them to Caucasian men. Finally, we plan to determine mechanisms that contribute to the differences in lipoproteins by identifying associated variants in candidate genes.

An increased understanding of the association between the LIPC and the LIPG alleles, and the nuclear magnetic resonance (NMR) measured lipoprotein concentrations and particle sizes in Tobago and CHS men will contribute to our knowledge of the etiology of CHD, and better understanding of the Black-White differences in CHD risk patterns.

2. ESTIMATES OF AFRICAN AND EUROPEAN ANCESTRY IN AFRICAN-CARIBBEAN MEN ON THE ISLAND OF TOBAGO

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2.1. ABSTRACT

Populations of African descent are a valuable resource for studying the genetics of complex diseases that show significant differences in prevalence among major ethnic groups. Prostate cancer risk is high in populations of African descent. Among these populations, risk for prostate cancer is higher among African-Caribbeans on the island of Tobago than among African-Americans. We hypothesize, that compared with African-Americans, the degree of European admixture in the Tobago population is low, resulting in higher burden of high-risk alleles of African origin than in African-Americans, who are more admixed. The Tobago Prostate Survey is a population-based prostate cancer screening study of Tobago males, aged 40 to 79 years. Admixture was estimated in a random sample of 237 men using a set of six autosomal markers that have large allele frequency differences between the major ethnic groups involved in the admixture process, European Caucasians and West Africans. The ancestral proportions of the Tobago population are estimated as $94.03 \pm 0.93\%$ African and $5.97 \pm 0.93\%$ European. Therefore, we conclude that Tobago African-Caribbean men are predominantly of West African ancestry, with minimal European admixture. Further study of this population should deepen our understanding of the contribution of candidate genes of West African origin to the risk of certain chronic diseases.

2.2. INTRODUCTION

An understanding of population admixture furthers the study of genetics of complex diseases or syndromes. Study of ancestry and admixture may explain the variation in risk for some chronic diseases, such as hypertension or prostate cancer, which show significant differences in prevalence across populations (McKeigue 1998). Population admixture perturbs the equilibrium among markers in the admixed population (Pfaff et al. 2001). The extent of this new, admixture-generated disequilibrium depends on the degree of admixture, the parental populations' allele frequencies and the time since the beginning of admixture (Pfaff et al. 2001). Admixture mapping studies are a potentially powerful tool for studying complex diseases that differ significantly in prevalence between ethnic groups.

Despite the fact that Africa plays a central role in human evolution, African populations are greatly underrepresented in the study of human genetic diversity. For the identification of the genetic basis of diseases more common in populations of African descent, the study of genetic diversity in these populations is very important. The sparse data currently available indicates that prostate cancer risk is elevated in men of African descent. Age adjusted incidence rates of prostate cancer are significantly higher in African-American than in European-American men (225/100,000 compared to 149/100,000) (Ries et al. 2000). Very high rates of prostate cancer incidence were also reported in the predominantly African-Caribbean population of Jamaica (304/100,000) (Glover et al. 1998). Moreover, compared with European-American and other populations of African descent, risk for prostate cancer in Tobago African-Caribbean men is elevated (Bunker et al. 2002).

Previous studies have estimated population European admixture rates in populations of African descent living in the US, Great Britain and Jamaica (Parra et al. 1998; Parra et al. 2001;

Shriver et al. 2003). The rates range from 3.5% in Gullah Sea Islanders in South Carolina, 6.8% in Jamaica, 10.2% in Britain, to 22.5 % in New Orleans.

Our main objective in this study was to estimate African and European ancestry in Tobago African-Caribbean men. Based on historical information and the observed differences in prostate cancer incidence, we hypothesized that the degree of European admixture in this population is lower than that observed among US African-Americans. If the European admixture in the Tobago population is in fact low, then this population may carry a higher burden of high-risk prostate cancer alleles of African origin than the more admixed African-American population.

2.3. METHODS

2.3.1. Population History

Tobago was settled over 2,500 years ago by Amerindians moving north from the South American mainland (Douglas 1987). With the arrival of settlers from Europe, diseases of European origin greatly reduced the native population. Official records indicate that Tobago was claimed by the English in the early 1500's and, as was the case for most islands in the Caribbean, it was then fought over by the Dutch, Spanish, English, French and settled by Latvians and buccaneers well into the 18th Century (Douglas 1987). Barbados was the key port of entry for British slave trade to the US, Tobago, and other British-controlled Caribbean islands (Douglas 1987). The first official British records enumerated 268 Whites and 3,110 African slaves on the island in 1770 (Douglas 1987). The slave population grew rapidly. There were 15,470 African slaves on the island in 1819 (Douglas 2003). The British finally occupied Tobago permanently in

1814, after several previous attempts to conquer the island (Douglas 2003). Slavery was officially abolished in 1833 (Douglas 2003). Today, the population of Tobago surpasses 50,000 inhabitants. According to the 1990 census self-reports, the population of Tobago was 92% African descent, 4.5% mixed, 2% East Indian, 0.4% white and 1% other (Tobago 1993).

2.3.2. Study design

The Tobago Prostate Survey (Bunker et al. 2002) is a population-based prostate cancer screening survey of all men on the Caribbean island of Tobago, aged 40 to 79 years (N=5121) (Tobago 1993). Written informed consent was obtained from every participant, using forms and procedures approved by the University of Pittsburgh and the Tobago Ministry of Health and Social Services Institutional Review Boards. Blood samples were obtained from fasting participants. Clots were frozen and later shipped to the University of Pittsburgh for DNA extraction. Admixture was estimated in a random sample of 237 Tobago men who reported 3 or more grandparents of African descent.

2.3.3. Markers used for estimation of population admixture

Earlier reports by Parra et al. (Parra et al. 1998), and Shriver et al. (Shriver et al. 2003) identified a panel of informative autosomal population-specific allele markers, called ancestry informative markers (AIM), used for the estimation of European and African genetic contribution to African-American and European-American populations. The investigators estimated the average frequencies of AIMs in four African and two European parental populations. To estimate the European and African genetic contribution to Tobago population we used six AIMs which

showed allele-frequency differences (δ) exceeding 46% between European and African average allele frequencies (table 2-1) (Shriver et al. 2003).

2.3.4. DNA analysis

DNA isolation from the blood clot from a 15 cc coagulation tube was accomplished by mechanical disruption of the clot, protease K digestion and isolation on a Qiagen column (Qiagen, Inc., Santa Clara, CA). The PCR conditions and primers for genotyping of AIMs are shown in table 2-2. After digestion with the appropriate restriction enzyme at 37°C overnight, electrophoresis was performed on 10% polyacrylamide gel (FY-null) or on 2% agarose gel. Genotypes were assigned by direct comparison to control samples of known genotype, visualized by UV illumination of ethidium bromide stained gels.

2.3.5. Statistical analysis

Allele frequencies were estimated by direct gene counting. Observed numbers of each genotype were compared with those expected for Hardy-Weinberg equilibrium by using the chi-square test. The ADMIX.PAS program (Long 1991), kindly supplied to us by Dr. Jeffrey Long, was used for the estimation of admixture proportions. This program implements a weighted least-squares method which includes the effect of the evolutionary and sampling variance in the admixture estimates (Long 1991). The chi-square is used to detect heterogeneity of admixture estimates from different alleles (Long 1991). In addition to admixture estimates, this program provides the mean squared error (MSE), which represents the proportion of allele-frequencies variation that cannot be explained by the admixture model.

2.4. RESULTS

The distribution of genotypes was not significantly different from the expectation under the assumptions of Hardy-Weinberg equilibrium. The allele frequencies in the Tobago population are similar to those observed in African populations (table 2-1). The ancestral proportions of the Tobago population are estimated as $94.03 \pm 0.93\%$ African and $5.97 \pm 0.93\%$ European. As we hypothesized, African genetic contribution is high ($\sim 94\%$) whereas European genetic contribution ($\sim 6\%$) is low in the Tobago population. The standard error of these estimates is small ($0.0093 \sim 1\%$), as is the MSE (0.0026).

2.5. DISCUSSION

These results support our hypothesis that the Tobago population derives mostly from the African parental population and that European parental population contributes very little to the Tobago population gene pool. Although we have used a relatively small number of AIMs, their selection seems to be appropriate ($SE \sim 1\%$). The proportion of variation in allele-frequencies that could not be explained by admixture model was very low.

FY-null is an allele which is found only in individuals with African ancestry. Among Tobago men, the frequency of this allele was 94.1%. Since the overall estimation of African ancestry after including 6 markers was $\sim 94\%$, it seems that FY-null by itself is a solid indicator of African admixture in this population.

An earlier study reported that the European genetic contribution from males to African-Americans was considerably higher than the female contribution, supporting the possibility of sex-biased gene flow (Parra et al. 2001). Future studies should include mtDNA haplogroups,

which are maternally inherited, and informative Y-chromosome-specific markers transmitted only from father to son. These two methods would allow the evaluation of male and female specific gene flow in this population.

The very recent development of tourism in Tobago has led to some in-migration, but this appears to primarily affect younger age groups. The number of Caucasians enumerated in the census has remained stable since the 1780's. There have not been any significant African immigrations since the early 1800's and the population of African descent has increased steadily (Tobago 1993). We speculate that the admixture occurred slowly and gradually over the past 300 years. Our estimates of a very low European admixture in the Tobago population will allow a more focused search for the prostate cancer candidate genes of West African origin than is possible in most populations outside of West African countries.

Table 2-1 Allele Frequencies of the ancestry informative markers in African and European populations (Shriver et al. 2003) compared to Tobago population

Locus	dbSNP ss # ¹	Location	African Average	European Average	δ ²	Tobago Population
FY-null (C)	4387025	1q23.2	0.999	0.002	0.997	0.941
OCA2 (A)	4387028	15q13.1	0.115	0.746	0.631	0.143
LPL(T)	4387026	8p21.3	0.971	0.492	0.479	0.932
F13B (G)	4387024	1q31.3	0.704	0.063	0.641	0.635
W11153 (G)	4387032	3p12.3	0.785	0.133	0.652	0.753
GNB3 (T)	4387018	12p13.31	0.795	0.332	0.463	0.799

¹ National Center for Biotechnology information (NCBI) public data base of single nucleotide polymorphisms (SNPs) and their unique identifiers (SS#); Available at: <http://www.ncbi.nlm.nih.gov/SNP>

² Allele-frequency differential (δ), the absolute value of the specific allele difference between two populations

Table 2-2 Primer sequences for the population-specific alleles used in the present study

Locus	Alleles	3'Primer	5'Primer	Type	Annealing temp	Reference
FY-null	C/T	GAACCTGATG GCCCTCATT	TCAGCGCCTGT GCTTCCATG	NcoI	56°C	(Bonilla et al. 2004)
OCA2	G/A	CTTTCGTGTGT GCTAACTCC	ACCTCTAGCAT GGTTCTTGGC	HaeIII	52°C	(Lee et al. 1995)
LPL	T/C	GCTTAATTCTC AATTCAATG	CTTTAGACTCTT GTCCAGGT	PvuII	43°C	(Gotoda et al. 1992)
F13B	A/G	CCTGAGTAATG GTTACATCTCTGA	CCCTCCAGTGG TTTTGTACC	NsiI	47°C	(Bonilla et al. 2004)
WI-11153	C/G	TGAAATATGGCTA GTGTGATTGATACA	CTCAAAAATCCAA CAGTCAAGGTCTAC	SnabI	47°C	Present study/(Bonilla et al. 2004)
GNB3	C/T	TGACCCACTTGCC ACCCGTGC	GCAGCAGCCA GGGCTGGC	BseJI	60°C	Present study

PCR reaction mixture included Invitrogen (Carlsbad, CA) 1x PCR reaction buffer, 1.5 mM of magnesium chloride, 200 µM of dNTP (PGC Scientific, Frederick, MD), 0.3 µM of forward primer, 0.3 µM of reverse primer (University of Pittsburgh Sequencing Facility, Pittsburgh, PA), 1 units of Invitrogen Life Tech (Carlsbad, CA) TAQ polymerase in a 50 µL solution. **PCR conditions:** 95°C for 5 min, 95°C for 30 sec, annealing temperature specified for each marker in °C for 15 sec, 72°C for 30 sec, then steps 2 through 4 repeated 34 times, 72°C for 5 min and 10°C “forever”.

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**3. ETHNIC DIFFERENCES IN ASSOCIATION OF HEPATIC LIPASE GENE –
514C>T POLYMORPHISM AND NMR LIPOPROTEIN SUBCLASSES IN OLDER
CAUCASIAN, AFRICAN-AMERICAN AND AFRICAN-CARIBBEAN MEN**

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3.1. ABSTRACT

Despite higher rates of risk factors for coronary heart disease (CHD), including hypertension and diabetes, men of African origin have less coronary atherosclerosis, as measured by coronary calcification, than Caucasians. In part, this is thought to be due to the less atherogenic lipoprotein profile observed in men of African origin, characterized by lower triglycerides (TG) and higher high density lipoprotein cholesterol (HDL-C). The -514C>T polymorphism in the hepatic lipase gene (LIPC), has been found to be associated with HDL-C and LDL-C, and in some cases with their subclasses and sizes. However, these effects vary among different populations. We hypothesized that the frequency of the putative protective LIPC -514T allele is more common in men of African origin than in Caucasian men, and that -514C>T polymorphism plays a significant role in determining a less atherogenic lipid profile. We studied the -514C>T polymorphism in 532 Caucasian, 97 African-American and 205 Tobago African-Caribbean men, older than 65 years, from two population-based studies. Lipoproteins and lipoprotein subclasses were determined by nuclear magnetic resonance (NMR) spectroscopy. Men of African origin had a more favorable lipoprotein profile than Caucasians. Additionally, TG levels and VLDL subclasses and sizes were remarkably lower in African-Caribbean men than in either African-Americans or Caucasians. The frequency of the -514T allele was very high in Tobago men (57.3%), almost three times higher than in Caucasians (19.8%, $p<0.0001$), and slightly higher than in African-Americans (49.0%, $p=NS$). The -514C>T interacted with ethnicity to affect the levels of HDL-C ($p=0.013$), large HDL ($p=0.012$) and diameters of HDL ($p=0.043$) and LDL particles ($p=0.044$). After adjusting for age, waist circumference and smoking, the -514T allele

was associated with elevated HDL-C in African-Americans ($p=0.014$). Further, the T allele was associated with elevated large HDL in African-Americans ($p=0.0027$) and to some extent in African-Caribbeans ($p=0.056$), and with a greater diameter of HDL particles ($p=0.024$ and 0.012 respectively). Slightly greater diameter of LDL particles was observed in African-American and African-Caribbean men, however, these differences only approached statistical significance. In contrast, among Caucasians, no significant associations were found, except a slightly lower small HDL in carriers of the T allele, but this difference was not significant. Our findings suggest that ethnicity is a strong moderator of LIPC effects on lipoprotein levels, and that the higher frequency of the protective LIPC -514T allele in men of African origin contributes to the more favorable distribution of the HDL subclasses compared with Caucasians.

3.2. INTRODUCTION

Differences in coronary heart disease (CHD) incidence and mortality rates have been observed between different ethnic groups (Vermaak et al. 1991; Zoratti 1998). Some studies suggest that CHD incidence rates are lower in African-American than in Caucasian men (Keil et al. 1995; Gillum et al. 1997), while other studies report similar CHD incidence in men of both ethnicities (Jones et al. 2002). Overall mortality rates from CHD in African-American men have increased over time, and in 2002 were higher for African-Americans than in Caucasians of all ages (National Heart 2002). However, compared with Caucasians, African-American men have higher rates of death from CHD at younger ages and lower rates at older age (Gillum et al. 1997; Corti et al. 1999; National Heart 2002). These observations are consistent with post-mortem studies that found more evidence of coronary atherosclerosis (more fatty streaks) in young African-

American men, but less evidence of coronary atherosclerosis (less fibrous plaques) in middle aged African-American men, than in Caucasian men (Strong et al. 1984; Freedman et al. 1988). Electron-beam computed tomography studies have shown that African-American men have less coronary atherosclerosis, measured by coronary artery calcium (CAC) (Newman et al. 2002; Lee et al. 2003). Furthermore, men of African origin have consistently been found to have lower percentage of central obesity for the same degree of body mass index (BMI), and most importantly to have a more favorable (less atherogenic) lipoprotein profile, characterized by lower levels of triglycerides (TG) and higher levels of high density lipoprotein cholesterol (HDL-C) (Donahue et al. 1989; Webber et al. 1991; Brown et al. 1993; Zoratti 1998). The underlying reasons for these differences in populations of African descent remain unclear. Several investigators have reported that polymorphisms in candidate genes, involved in lipid metabolism, affect lipoprotein levels. Still, the degree of the impact of this favorable lipoprotein profile on less severe coronary atherosclerosis, and the extent to which lipoprotein levels are affected by genetic and environmental factors, remains controversial.

It is possible that higher HDL-C is due to, at least in part, lower hepatic lipase (HL) activity that has been observed in African-American men (Vega et al. 1998). HL is a lipolytic glycoprotein that has a central role in lipid metabolism. This enzyme hydrolyzes phospholipids and TG carried in large HDL, intermediate density lipoprotein (IDL) and large low density lipoprotein (LDL) particles, resulting in smaller HDL, and small, dense, more atherogenic LDL lipoproteins (Bensadoun and Berryman 1996; Perret et al. 2002). HL also assists the uptake of lipoproteins (HDL particles, TG-rich lipoprotein remnants and LDL) by specific receptors on the hepatocytes (Bensadoun and Berryman 1996; Perret et al. 2002).

The human hepatic lipase gene, LIPC, is located on chromosome 15 (15q21.3) and it comprises 9 exons and 8 introns (Ameis et al. 1990). It has been estimated that the LIPC gene accounts for 25% of variability in HDL-C levels (Cohen et al. 1994) and this fact led to the hypothesis that variants in the LIPC gene play an important role in the metabolism of HDL and observed differences in plasma HDL-C concentrations. One particular variation upstream of the promoter of the LIPC gene has been studied extensively. This functional, non-coding, -514 C>T single nucleotide polymorphism (SNP) has been associated with lower post-heparin HL activity (Vega et al. 1998; Zambon et al. 1998), increased levels of HDL-C (Guerra et al. 1997; Murtomaki et al. 1997; Inazu et al. 2001), increased levels of only large HDL (not necessarily increased HDL-C) (Zambon et al. 1998; Couture et al. 2000; Juo et al. 2001), and with higher levels of large, more buoyant LDL particles (Zambon et al. 1998). Elevated levels of small LDL and small HDL lipoprotein subclasses, and/or reduced levels of large HDL subclasses, are thought to be strongly associated with the presence of CHD, and have been widely accepted as better predictors of atherosclerosis and CHD risk than lipids obtained by the standard tests (total cholesterol, LDL-C and HDL-C) (Freedman et al. 1998; Lamarche et al. 2001; Rosenson et al. 2002).

The frequency of the LIPC T allele at -514 position varies across ethnic groups, ranging 17-27% in Caucasians (Jansen et al. 1997; Zambon et al. 1998; Hokanson et al. 2003; Carr et al. 2004), 44-54% in African-Americans (Vega et al. 1998; Juo et al. 2001; Chen et al. 2003; Carr et al. 2004), 47 % in US Hispanics (Hokanson et al. 2003), 35-44% in Koreans (Hong et al. 2000; Park et al. 2003) and 50% in Japanese (Inazu et al. 2001; Carr et al. 2004).

Although, several studies suggested that the -514 C>T polymorphism in the LIPC promoter has an influence on HDL phenotypes, its role in lipoprotein metabolism and

atherosclerosis is still ambiguous. Despite the fact that many studies have shown that the -514 C>T polymorphism is associated with a more protective lipoprotein profile (Isaacs et al. 2004), some studies reported no association between this LIPC marker and HDL-C (Isaacs et al. 2004), whereas others reported the association to be much stronger in women (Couture et al. 2000), or limited to women (Park et al. 2003).

Additionally, studies of the effect of the LIPC -514C>T on clinical phenotypes are inconclusive. Some studies reported that the presence of the -514T allele may indicate an increased risk of CHD even in cases where the T allele was associated with high HDL-C and low HL activity (Dugi et al. 2001; Andersen et al. 2003; Hokanson et al. 2003). Others found no association between the T allele and CHD risk (Shohet et al. 1999; Couture et al. 2000), or an association of the -514C allele with increased carotid intima-media thickness (marker of early atherosclerosis) (Rundek et al. 2002), and with unstable carotid plaque (risk factor for ischemic stroke) (Faggin et al. 2002), suggesting that this polymorphism could have an effect on a risk of cerebrovascular events. The effect of the LIPC -514 C>T has been found to be modified by various environmental factors such as diet (Ordovas et al. 2002; Tai et al. 2003) and physical activity (Hokanson et al. 2003) and these interactions could partially explain the controversial observation by some investigators on the increased CHD risk in -514 T allele carriers.

The objectives of the present study were to determine ethnic differences in the frequencies of the LIPC -514 C>T promoter polymorphism and to determine and describe an association of the LIPC -514C>T with nuclear magnetic resonance (NMR) lipoprotein subclasses in older Caucasian, African-American and African-Caribbean men. We hypothesized that the frequency of the putative protective hepatic lipase T allele at position -514 of the LIPC gene, is higher in men of African origin than in Caucasian men, and that the -514 T allele is associated

with a more favorable lipoprotein profile characterized by higher concentrations of large HDL and large LDL.

An increased understanding of the association between LIPC polymorphisms and NMR lipoprotein concentration and particle sizes will contribute to our knowledge of the etiology of the CHD, and improve our understanding of the differences in CHD risk patterns observed between African-Americans and Caucasians.

3.3. METHODS

3.3.1. Study populations

The Tobago Health Study was derived from the Tobago Prostate Survey, a population-based prostate cancer screening survey of African-Caribbean men on the Caribbean island of Tobago with population of 46,435, according to the 1990 census data (Tobago 1993). The target recruitment population for this study was all males aged 40 to 79 years (5121 men), of whom 3375 participated in the survey. In 1990, the population of Tobago was 92% African descent, 4.5% mixed, 2% Asian Indian, 0.4% white and 1% other (Tobago 1993). Men learned of the Tobago Prostate Survey by word of mouth, posters, flyers, public health announcements, and from health care workers. After obtaining written informed consent from each participant, a questionnaire was administered by trained interviewers. The questionnaire was designed to gather demographic and anthropometric data, and to assess history of smoking and alcohol consumption, medical history, personal and family cancer history, and occupational history. The data forms were based on the questionnaires used in the Prostate, Lung, Cervix and Ovary Screening Study (PLCO) (Prorok et al. 2000) and were adapted for the needs of this study.

Aliquots of frozen serum, drawn from fasting subjects, were shipped on ice packs or dry ice by express courier and stored at the University of Pittsburgh -70°C freezers for later measurements of serum prostate specific antigen (PSA), sex steroid hormones and lipoproteins. The present study population includes a random sample of 205 Tobago men over the age of 65 (mean age 73.1 years), not diagnosed with prostate cancer, who reported three or more grandparents of African descent.

The Cardiovascular Health Study (CHS) is a population-based, longitudinal study of 5888 people aged ≥ 65 year recruited from a stratified random sample of Medicare recipients from four US communities. 5201 CHS participants underwent baseline assessments of cardiovascular risk factors in 1989-1990 (Fried et al. 1991). An additional 687 African-Americans were recruited to CHS in 1992-1993 (Kuller et al. 2002). Originally, CHS was designed to evaluate risk factors and noninvasive measures, and to describe and predict atherosclerotic events in older adults (Fried et al. 1991). Participants have had extensive baseline and follow-up evaluations. At the baseline examination, anthropometric measurements, medical and lifestyle histories, blood collection, echocardiography, resting 12-lead ECG, carotid ultrasonography and ankle-brachial index were obtained. Prevalence and extent of CVD were assessed (Fried et al. 1991). The present study population consists of a sample of 532 Caucasian men (mean age 73.1 years), and 97 African-American men (mean age 72.9 years) from a large sample of the original cohort members who were previously selected for a case-cohort evaluation of an association of NMR lipoproteins and risk of CHD.

3.3.2. Laboratory methods/NMR Spectroscopy

Lipoprotein concentrations and subclass distributions were determined from frozen serum specimens, by nuclear magnetic resonance (NMR) spectroscopy at LipoScience, Inc (Raleigh, NC). This method has been described in details in reports by Otvos et al (Otvos et al. 1991; Otvos et al. 1992; Otvos et al. 2002). The NMR method uses the distinguishing signals broadcast by lipoprotein subclasses of different sizes as the base of their quantification. These signals originate from the combined number of terminal methyl groups on the lipids. The intensities of signals are proportional to the lipid mass of the particles. These intensities are converted into more familiar concentration units (mg/dl). NMR spectroscopy measures 11 subclasses of VLDL, LDL and HDL. Additionally, each profile provides calculated estimates of the concentrations of total cholesterol, total triglyceride, LDL cholesterol, HDL cholesterol, LDL particle numbers, and average VLDL, LDL, and HDL particle size. Reported LDL particle concentration is the sum of the concentrations of the LDL subclasses including IDL. Total particle concentrations (in units of nmol/l) are the sums of the particle concentrations of each subclass.

3.3.3. Genetic analysis

DNA isolation from Tobago participants' blood clots from a 15 cc coagulation tube was accomplished by mechanical disruption of the clot, protease K digestion and isolation on a Qiagen column (Qiagen, Inc., Santa Clara, CA). The genotype at position -514 of LIPC promoter was determined by polymerase chain reaction (PCR)-RFLP based approach first described by Guerra et al (Guerra et al. 1997). The following primers were used: forward, 5'-TACTTTTCAGTCCTCTACACAGC-3'; reverse, 5'-GTCAGGCTCTTACCTGGTTTCA-3'. PCR reaction mixture included Invitrogen (Carlsbad, CA) 1x PCR reaction buffer, 1.5 mM of

magnesium chloride, 200 μ M of dNTP (PGC Scientific, Frederick, MD), 0.3 μ M of forward primer, 0.3 μ M of reverse primer (University of Pittsburgh Sequencing Facility, Pittsburgh, PA), 1 units of Invitrogen Life Tech (Carlsbad, CA) TAQ polymerase in a 50 μ L solution. The PCR steps were as following: 95°C for 5 min, 95°C for 30 sec, annealing temperature 51°C for 15 sec, 72°C for 30 sec, then steps 2 through 4 repeated 34 times, 72°C for 5 min and 10°C “hold”. After digestion with Nla III restriction endonuclease at 37°C overnight, electrophoresis was performed on 2% agarose gel stained with florescent dye ethidium bromide, using tris-borate (TBE) buffer. Photographs of ethidium bromide stained gels were made using UV illumination.

3.3.4. Statistical analysis

Allele frequencies were estimated by direct gene counting. The observed genotype frequencies were compared with those expected under Hardy-Weinberg equilibrium by the goodness-of-fit chi-square test. Comparison in allele frequencies between the three ethnic groups was evaluated using a chi-square test. Initial descriptive data analysis showed that most of the NMR lipoprotein subclasses and sizes (except small HDL) exhibited markedly skewed distributions. Prior to statistical analysis, we transformed the lipoprotein subclasses and sizes by square root, inverse, or natural logarithms in order to reduce the non-normality of the distributions of lipoproteins. Analysis of variance (ANOVA) was performed to test any significant difference of baseline demographic (continuous) characteristics among three ethnic groups as well as among three genotypes within ethnic groups. Categorical variables were compared using chi-square testing. Analysis of covariance (ANCOVA), which adjusted for age, waist circumference and smoking, was conducted using the general linear model procedure (GLM) to test for possible differences among ethnic groups in mean levels of NMR lipoproteins. We further used a two-way ANOVA

to test for a possible interaction between the LIPC -514 C>T polymorphism and ethnicity on NMR lipoproteins subclasses or sizes. ANCOVA was also performed to test for mean differences in NMR lipoprotein levels among different LIPC genotypes within the three ethnic groups. To evaluate the statistical significance with regard to multiple testing and phenotypes that are correlated, we adjusted the p-value to reflect the empirical data. The p-value <0.015 was considered to be truly significant. Additionally, in case of significant differences, group means were compared by Fisher's Least Significant Difference (LSD) test for multiple pairwise comparisons. We also used a trend analysis to test for linear trends across the genotypic means within each ethnic group. Because the population distributions of small and medium LDL and large VLDL were highly skewed, we used the Kruskal-Wallis median test to test for possible differential effects among genotypes and ethnic groups. The Statistical Analysis System (SAS, version 8.2; SAS Institute, Cary, NC) and the Statistical Package for the Social Sciences (SPSS, version 12.0.1; Chicago, IL) were used for statistical analysis.

3.4. RESULTS

3.4.1. Participants' characteristics and comparison of NMR lipoprotein levels across ethnic groups

The three populations were similar in age and BMI (table 3-1). Waist circumference was significantly lower in Tobago men than either US population, while smoking was much more frequent in African-American men (table 3-1).

Significant differences across the three ethnic groups were found for all NMR lipoproteins, subclasses and sizes, except for small HDL (table 3-2). African-Caribbean men had a more favorable lipoprotein profile than African-Americans or Caucasians, characterized by lower TG,

lower total cholesterol, lower LDL-C, as well as fewer LDL particles, and lower small VLDL. Both populations of African men had similar levels of HDL-C, large HDL, and medium VLDL compared to each other, but when compared with Caucasian men, HDL-C and large HDL were higher, and medium VLDL was lower in men of African origin. Large LDL was considerably higher in African-American men, even when compared to Tobago men. The lipoprotein particle sizes are also shown in table 2. The average size of VLDL particles was smaller in African-Caribbeans when compared to Caucasians and African-Americans. Average LDL and HDL size were significantly greater in both groups of African men when compared to Caucasian participants. These lipoprotein patterns, observed in African men, outline a more favorable lipoprotein profile than what we observed in Caucasians.

3.4.2. LIPC -514 T allele frequency

None of the three ethnic groups showed significant deviation from HWE in relation to LIPC -514 C>T genotypes. The LIPC -514 T allele frequency was almost three times higher in Tobago men (57.3%) than in Caucasians (19.8%, $p<0.0001$), and although not statistically significant, somewhat higher than in African-Americans (49.0%, $p=0.21$).

3.4.3. Ethnic group by LIPC genotype group interaction effects on NMR lipoproteins

We further tested the interaction between the LIPC -514 C>T polymorphism and ethnicity in determining lipoprotein levels and sizes. This interaction (gene-ethnicity) significantly affected HDL-C ($p=0.013$, figure 3-1), large HDL ($p=0.012$, figure 3-2), HDL size ($p=0.043$, figure 3-3) and LDL size ($p=0.044$, figure 3-4) in the whole population. Because significant heterogeneity of

the polymorphism effect was observed among the ethnic groups, especially in distributions of HDL-C and subclasses and size, we carried further analyses separately for each ethnic group.

3.4.4. Differences in NMR lipoprotein levels between LIPC genotypes within the different ethnic groups

Within each ethnic group, three LIPC -514 C>T genotypes, were similar with respect to mean age, BMI, and waist circumference, and the presence of smoking, with exception of the mean age among Tobago men, where slight differences were observed (TT, 74.1 years; CT, 72.4 years; CC, 73.75 years; $p=0.06$). First, we tested differences in levels of the standard NMR lipoprotein measurements (TC, TG, LDL-C and HDL-C), between the LIPC genotypes within each ethnic group. No significant associations were found, except a protective effect of the LIPC -514 C>T on HDL-C in African-Americans ($p=0.014$), with a gene dose trend (p for trend= 0.04), but this was not observed in Caucasians or African-Caribbeans (table 3-3). Further we tested VLDL, LDL and HDL subclasses. There was no difference in VLDL subclasses between the LIPC genotypes in any of the ethnic groups. In African-American men, compared with non-carriers, both homozygous and heterozygous carriers of the T allele (TT and CT) had higher large HDL ($p=0.0027$), with a significant gene dose effect (p for trend= 0.0006) (table 3-3). In Tobago men, large HDL subclass distribution was observed in the same pattern, but the difference between genotype groups only approached statistical significant ($p=0.056$, p for trend= 0.024) (table 3-3). In Caucasians, in contrast to men of African origin, no association was found between the LIPC polymorphism and HDL and LDL lipoprotein phenotypes (table 3-3). Caucasian carriers of the CT genotype had slightly lower small HDL than those with the CC genotype (LSD pairwise p -value= 0.027), even though the overall difference between LIPC genotypes did not reach statistical significance ($p=0.076$) (table 3-3).

3.4.5. Differences in NMR lipoprotein particle sizes between LIPC genotypes within the different ethnic groups

In Caucasians, no significant associations were observed between the LIPC polymorphism and VLDL, HDL or LDL particle diameter size. Observed patterns in groups of African origin were very similar to each other and differed from Caucasians. African-American carriers of the T allele had slightly greater diameter of HDL particles ($p=0.024$, only approached significant level), and HDL size was evidently greater in African-Caribbean carriers of the T allele ($p=0.012$) (table 3-3). In both ethnicities, these differences were associated with the number of T alleles (p for trend 0.007 in African-Americans and 0.005 in African-Caribbean respectively). LDL size tended to be greater in carriers of the T allele in both men of African origin, with a significant gene dose effect, though these differences did not reach statistical significance (in African-Americans $p=0.08$, p for trend 0.033; in African-Caribbeans $p=0.055$, p for trend 0.022) (table 3-3).

3.4.6. Interaction between BMI and LIPC 514 C>T genotypes in relation to HDL subclasses

Because the BMI and waist circumference were highly correlated, we have not included BMI as a covariate in the ANCOVA model. However, BMI might confound associations of the LIPC and lipoproteins. When considering an interaction of BMI with the LIPC 514 C>T polymorphism, BMI was analyzed as a categorical variable, grouped into three levels (<24.4, 24.4-26.9, >27.9). The interaction between the LIPC -514 C>T polymorphism and BMI (according to tertiles of BMI) in determining level of large HDL and HDL size was tested by two-way Anova. No significant interaction between the LIPC genotypes and BMI was found.

3.5. DISCUSSION

The present study has shown that older men of African origin, from both US and Caribbean environments have a more favorable distribution of the NMR lipoprotein particle numbers and sizes than Caucasian men. In addition, levels of TG, and large and small VLDL, as well as VLDL particle size, were remarkably lower in Tobago African-Caribbean men than in either CHS-African-American, or CHS-Caucasian men. The patterns of lipoprotein subclasses observed in men of African origin from the Cardiovascular Health Study and the Tobago Health Study are believed to be protective against atherosclerosis. Our findings are consistent with results from several previous studies which reported that people of African origin across all geographic regions have lower TG levels and higher HDL-C than Caucasians. A number of well designed epidemiological studies reported an increased level of TG as an independent CHD risk factor (Malloy and Kane 2001). The inverse association between HDL-C and the development of atherosclerosis and CHS is also well established (Barter and Rye 1996; Gotto 2001). However, VLDL-C, LDL-C and HDL-C have different metabolic and vascular effects, and beneficial or harmful effect on the process of atherosclerosis may depend on particle size. It has been shown that the presence of CHD was more strongly associated with HDL particle size distribution than with the low HDL-C level (Cheung et al. 1991; Drexel et al. 1992) and that small HDL had a positive association (Cheung et al. 1991), and large HDL a negative association (Ballantyne et al. 1982) with the development of CHD. Additionally, the growing body of evidence suggests that large VLDL is another predictor of the risk of atherosclerosis, independently of TG levels or other VLDL subclasses (Freedman et al. 1998). Therefore, the NMR analysis of lipoprotein subclasses and sizes can provide additional, valuable information on the relation of lipoproteins to atherosclerosis, and help in earlier identification of high-risk individuals. To our knowledge,

this the first study that provides information on NMR lipoprotein subclasses and sizes in men of African origin living in the Caribbean.

We have shown that older men from Tobago had a lower degree of central obesity and were less likely to smoke compared with CHS men (table 3-1). One hypothesis is that the lower degree of abdominal adiposity observed in men of African origin is associated with an increased anti-lipolytic effect of insulin, which could account for low triglyceride levels and high HDL-C (Zoratti 1998). However, even after taking into consideration some of the risk factors such as anthropometric traits or lifestyle risk factors, differences in lipoproteins subclasses among the three ethnic groups were still present in our study. We speculate that African-Caribbeans live in a different environment than African-Americans, which could potentially explain observed ethnic differences. However, lipoprotein profile was also favorable in African-Americans from our study, when compared to Caucasians, and similar to the one observed in Tobago men, suggesting that genetic factors may play an important role in determining a more favorable lipoprotein profile in African men from our study.

In the present study, we observed significant variations in the distribution of hepatic lipase -514 C>T allele frequencies between three populations. In African-Caribbeans, the frequency of the T allele was almost three times higher than in Caucasians, and somewhat higher than in African-Americans. The observed frequencies in CHS-African-American and CHS-Caucasian men are consistent with the findings from previous studies (Jansen et al. 1997; Vega et al. 1998; Zambon et al. 1998; Hong et al. 2000; Inazu et al. 2001; Chen et al. 2003; Hokanson et al. 2003; Park et al. 2003; Carr et al. 2004). Very high frequency observed in Tobago men is, however, the highest reported so far.

In men of African descent from our study, independent of geographic origin, the LIPC -514 C>T polymorphism was associated with a more cardio-protective lipoprotein profile, characterized by higher levels of HDL-C, higher large HDL, and greater diameter of HDL and LDL particles. Contrary to these observations, the -514 T allele in CHS Caucasian men had little impact on NMR lipoprotein distributions. Only a slight lowering effect of the T allele on small HDL was observed in Caucasians, an effect that was not seen in African men. However, this finding suggests that it is possible that we would find a significant protective affect of the T allele on small HDL subclass, in a larger sample of Caucasians. On the other hand, the inspection of the other lipoprotein subclasses, suggests that Caucasian TT genotype carriers seem to have a less favorable lipoprotein profile (lower levels of HDL-C, large HDL and large LDL), but no significant association with the LIPC polymorphism was found. Given the small sample size of Caucasians with the TT genotype (N=28), the effects in this ethnic group must be interpreted with caution.

Our analysis of the data suggested that genetic variation at the LIPC gene interacts with ethnicity to affect the levels of HDL-C, and its subclasses and size, and possibly LDL size. There are number of possible reasons for the strong ethnicity-gene interaction and significant associations that were found only in men of African origin. One possibility is that the LIPC-514 C>T polymorphism may not be the causal modulator of the observed differences in lipoprotein phenotypes and that some other causal polymorphism, or polymorphisms which are actually reducing the LIPC expression, are in strong linkage disequilibrium with the -514 C>T variant in men of African origin, but not in Caucasian men. Sequencing of the whole LIPC gene and flanking regions in our populations, could potentially clarify our findings. Second, our discrepancy might be due to other genes related to lipoprotein metabolism. Third, the association

of the LIPC polymorphism in African men from our study was found after controlling for some factors known to be associated with lipoprotein variations. Some other factors known to influence HDL-C, such as alcohol consumption, diet or physical activity, were not assessed in our study. It is possible that they would modify the genetic effect through interaction. Still, we believe that it is more likely that African-Americans share similar environment with the US Caucasians, than with the Tobago African-Caribbeans. Another possibility for explaining the inconsistent observations of significant associations among different populations, proposed by Juo et al., is the fact that the LIPC promoter variants influence HDL particle size only indirectly, through HL activity (Juo et al. 2001). The simplest explanation would be that the lack of positive association between the T allele and HDL subclasses and lipoprotein diameters in Caucasians is related to relatively small number of the T allele homozygotes. Finally, the participants in our study were older than 65 years. It is possible that the LIPC -514 T allele influences survival. However, the LIPC genotypes were in Hardy-Weinberg equilibrium in all populations, and age did not differ significantly by genotype.

It is widely accepted that individual responses to different drugs vary within a population and differences in drug response have been observed between ethnic populations as well (Burroughs et al. 2002). Genetic variation in drug metabolizing enzymes, such as hepatic lipase, could be a possible modulators (Burroughs et al. 2002). An earlier study reported that middle-aged Caucasian men with dyslipidemia and established CHD responded differently to lipid-lowering therapy, depending on the LIPC -514 C>T polymorphism (Zambon et al. 2001). Homozygous -514 C allele patients experienced a greater increase in LDL buoyancy with lipid-lowering therapy than both homozygous and heterozygous carriers of the -514T allele. Screening for this variant in the HL gene could clarify possible lower responsiveness to the lipid-lowering

therapy in African-Americans, and identify CHD patients who will benefit the most from the lipid-lowering strategies (Zambon et al. 2001).

Conflicting results have been reported regarding the increased or decreased CHD risk in carriers of the -514 T allele. The majority of the participants in studies of CHD risk and the -514 C>T polymorphism were Caucasians. In order to explain the contradictory association between high HDL-C and increased CHD risk, Andersen et al. proposed that it is possible that higher HDL-C found in carriers of the -514 T allele is caused by reduced HL activity, and that this reduced activity would decrease catabolism of large HDL particles (Andersen et al. 2003). High accumulation of large HDL could potentially reduce the capacity of the reverse cholesterol transport system and therefore, cause an insufficient removal of cholesterol from the arterial intima. In our study the -514T allele was associated with a more cardio-protective lipoprotein profile in men of African origin, but not in Caucasians. Further large studies with non-Caucasian men, are needed to clarify whether these protective effects of LIPC polymorphism on lipoprotein subclasses translate to reduced CHD risk in men of African origin.

Additionally, future studies should not only include larger samples of African populations, and other candidate genes involved in lipoprotein metabolisms, but also the haplotype profiles in the LIPC promoter in different ethnic groups. Populations of African descent have smaller haplotype blocks, a greater diversity of common haplotype, fewer sites being in linkage disequilibrium and greater recombination than non-African ethnicities (Tishkoff and Williams 2002). In Caucasians, the -514 T allele is 2-3 fold less common than in Africans or Asians. It is possible that due to the high frequency of the T allele in African populations, this polymorphism, together with other polymorphisms, could be variously combined in haplotypes,

and these haplotypes could therefore, be differently distributed in populations of Caucasian and African descent.

In conclusion, the present study demonstrates significant ethnic differences in NMR lipoprotein subclasses distributions, with similar patterns of more favorable, less atherogenic lipoprofile in older African-American and Tobago African-Caribbean men, compared with older Caucasian men. Additionally, the present investigation shows a significant effect of the LIPC - 514C>T polymorphism on HDL subclasses and sizes, and possibly LDL size, in older men of African origin, living in different environments, but not in older Caucasian men. The current findings suggest that further evaluation of the effects of LIPC gene polymorphisms on lipoprotein levels should consider ethnicity as a potential moderator of those effects. Our findings have important implications in understanding of the observed Black-White differences in lipoprotein distributions, and are likely to increase our understanding of the mechanisms behind the ethnic differences in susceptibility to atherosclerosis.

Table 3-1 Participants' characteristics according to the ethnicity

	CHS-Caucasians (N=528)	CHS-African- Americans (N=97)	Tobago-African- Caribbeans (N=202)	P-value
Age (years)	73.1±0.24	72.9±0.57	73.1±0.34	0.95
BMI (kg/m ²)	26.4±0.16	27.1±0.4	26.8±0.32	0.17
Waist (cm)	98.0±0.42	98.1±1.14	93.9±0.9	<0.0001
Current smokers (%)	9.7	21.7	5.9	0.0001

Values are means ± SEM; P-value obtained in the comparison among ethnic groups (ANOVA test for means and chi-square test for percentages)

Table 3-2 Comparison of NMR Lipoprotein Distributions of Tobago and CHS Men

(Mg/dl)	CHS Caucasians (N=528)	CHS African-Americans (N=97)	Tobago African- Caribbean (N=202)
CHOL ^{A,2,3}	225.6±1.9	227.1±3.5	179.2±2.6
TG ^{C,1,2,3}	155.5±3.3	129.2±6.2	78.7±2.1
LDL-C ^{A,2,3}	144.9±1.7	139.9±1.4	113.7±2.2
HDL-C ^{A,1,2,3}	45.8±0.7	54.0±2.2	49.9±0.96
Large HDL ^{A,1,2}	27.9±0.8	36.2±2.4	32.2±0.98
Small HDL	17.96±0.26	17.8±0.7	17.7±0.3
Small VLDL ^{A,2,3}	33.3±0.7	32.0±1.5	15.0±0.7
Medium ^{A,1,2} VLDL	50.3±1.6	31.6±3.0	26.7±1.2
Large VLDL*	33.2±2.2	26.5±3.8	3.4±0.6
Small LDL*	38.7±2.0	27.7±4.2	14.7±1.6
Medium LDL*	41.1±1.7	30.5±3.8	28.8±1.4
Large LDL ^{A,1}	64.0±2.0	81.0±4.7	68.3±2.6
LDL Particles ^{A,2,3} (nmol/L)	1659.5±22.0	1557±42.7	1183±26.5
HDL Size ^{B,1,2} (nm)	8.95±0.03	9.25±0.07	9.4±0.04
LDL Size ^{B1,2} (nm)	20.8±0.03	21.1±0.08	21.2±0.04
VLDL Size ^{B,1,2,3} (nm)	47.9±0.4	45.3±0.9	39.7±0.3

Values are unadjusted means ± SEM; P-value obtained by ANCOVA after adjustment for age, waist and current smoking status showed significant differences across three ethnic groups for all NMR lipoproteins (P<0.0001, large LDL p=0.002) except for small HDL; *significant non-parametric Kruskal-Wallis' p-value; ^Asquare root transformed; ^Breciprocal transformed; ^Clog transformed; ¹Caucasians different than AA, ²Caucasians different than Tobago, ³AA different than Tobago

Table 3-3 Associations between LIPC -514 C>T polymorphism and NMR lipoproteins within ethnic groups

Mg/dl	Caucasians N=528				African-Americans N=97				Tobago Afro-Caribbeans N=202			
	TT N=28	CT N=152	CC N=348	p-value	TT N=24	CT N=47	CC N=26	p-value	TT N=62	CT N=108	CC N=32	p-value
HDL-C ^A	44.1 ±2.6	46.9 ±1.5	45.5 ±0.8	0.55	64.2 ± 5.4	53.5 ±2.7	45.5 ±3.5	0.014 ^{T,1,3}	51.6 ±1.8	50.1 ±1.3	45.9 ±2.1	0.13 ^T
LDL-C ^A	141.5 ±8.0	145.5 ±3.06	145.0 ±2.02	0.79	139.1 ± 9.3	137.6 ±4.4	144.6 ±5.1	0.68	116.7 ±4.4	110.7 2.7	118.2 ±6.2	0.25
Small HDL	17.5 ±1.05	17.1 ±0.5	18.4 ±0.3	0.076 ²	16.2 ±1.3	17.6 ±1.1	19.6 ±1.3	0.25	17.1 ±0.5	17.8 ±0.5	18.5 ±0.9	0.45
Large HDL ^A	26.6 ±2.9	29.8 ±1.7	27.1 ±0.9	0.26	48.0 ±5.6	35.9 ±2.8	25.9 ±4.1	0.0027 ^{T,1,2,3}	34.4 ±1.8	32.4 ±1.4	27.4 ±2.0	0.056 ^{T,1,2}
Small LDL*	44.5 ±9.9	38.5 ±3.7	38.4 ±2.4	0.72	11.6 ±5.1	27.1 ±6.3	43.7 ±9.2	0.03	12.1 ±2.8	13.9 ±1.9	22.3 ±6.0	0.075
Large LDL ^A	57.04 ±8.6	63.7 ±3.6	64.7 ±2.5	0.71	94.7 ± 9.9	79.0 ±7.0	71.9 ± 7.9	0.41	77.2 ±5.1	64.6 ±3.3	63.3 ± 6.3	0.16
HDL Size ^B (nm)	8.94 ±0.11	9.01 ±0.05	8.93 ±0.03	0.26	9.5 ±0.13	9.3 ±0.1	8.9 ±0.11	0.024 ^{T,1,2}	9.51 ±0.07	9.42 ±0.05	9.23 ±0.08	0.0125 ^{T,1,2}
LDL Size ^B (nm)	20.63 ±0.16	20.8 ±0.07	20.8 ±0.04	0.63	21.45 ±0.11	21.1 ±0.13	20.85 ±0.16	0.08 ^{T,1}	21.4 ±0.08	21.15 ±0.06	21.06 ±0.11	0.055 ^{T,1}

Values are unadjusted means ± SEM; p-value obtained by ANCOVA after adjustment for age, waist circumference and current smoking status (a p-value <0.015 was considered to be statistically significant); *p-value obtained by non-parametric Kruskal-Wallis test; ^T Test for linear trend across genotypes was significant; ^A square root transformed; ^B reciprocal transformed; ^C log transformed ¹ TT different than CC; ² CT different than CC; ³ TT different than CT

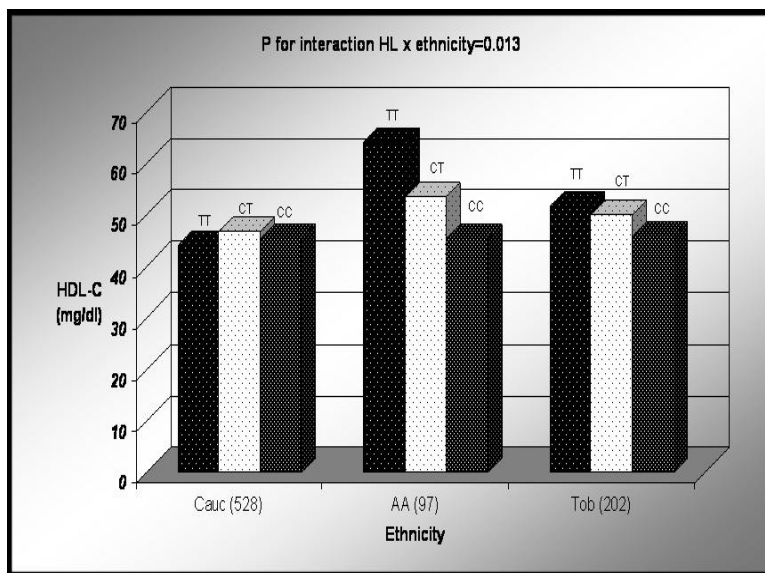


Figure 3-1 HDL-C according to the hepatic lipase genotypes in CHS and Tobago men

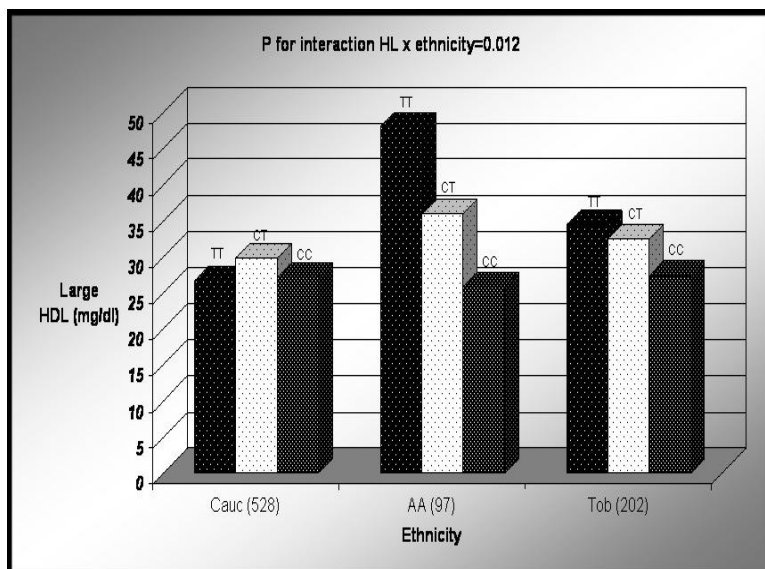


Figure 3-2 Large HDL according to the hepatic lipase genotypes in CHS and Tobago men

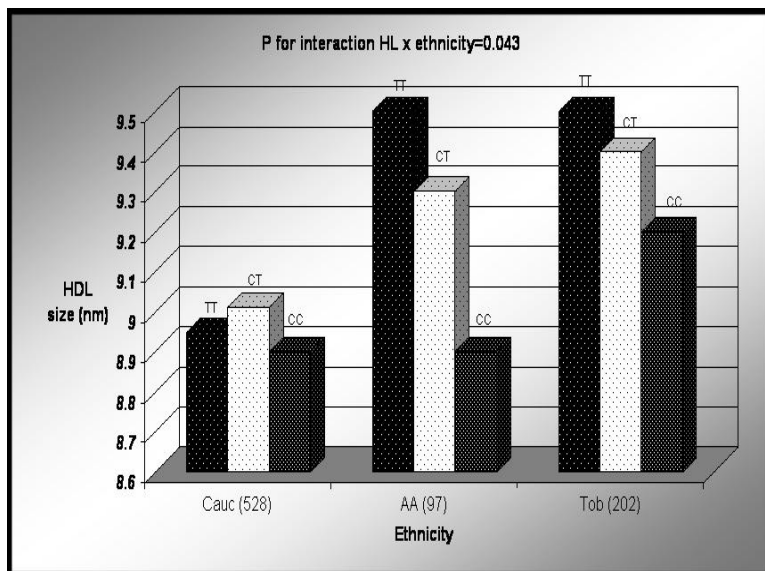


Figure 3-3 HDL size according to the hepatic lipase genotypes in CHS and Tobago men

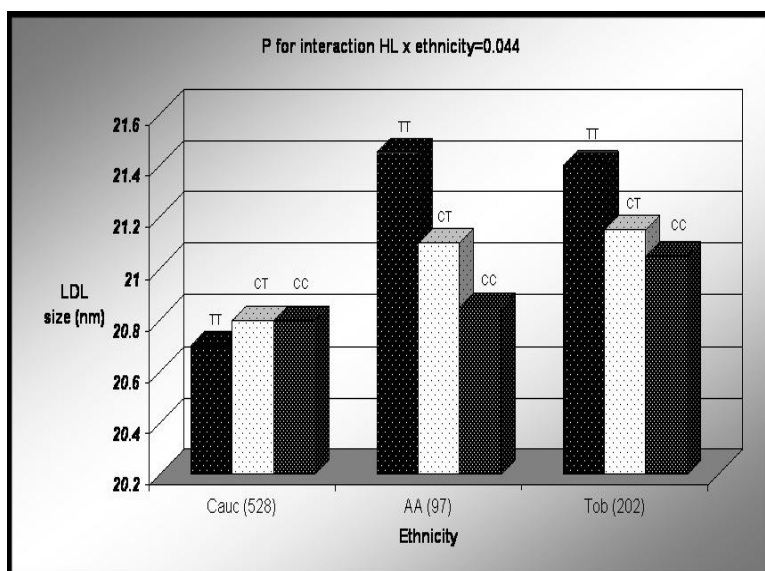


Figure 3-4 LDL size according to the hepatic lipase genotypes in CHS and Tobago men

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4. ENDOTHELIAL LIPASE 584 C>T POLYMORPHISM IN OLDER CAUCASIAN, AFRICAN-AMERICAN AND TOBAGO AFRICAN-CARIBBEAN MEN: FREQUENCIES AND ASSOCIATION WITH THE NMR LIPOPROTEIN SUBCLASSES

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4.1. ABSTRACT

Genetic factors may play an important role in determining high density lipoprotein cholesterol (HDL-C) levels and HDL subclasses. Recent research has focused on lipoprotein subclasses, and their link to coronary heart disease (CHD). It has been shown that the presence of atherosclerosis and CHD was more strongly associated with HDL particle size distribution than with the HDL-C level. Men of African origin have consistently been shown to have higher levels of HDL-C and lower levels of triglycerides (TG). Recent discovery and characterization of endothelial lipase (LIPG) gene suggested that the LIPG 584C>T (also known as Thr111Ile) was more common among individuals with elevated HDL-C levels. However, the reports on the association between this polymorphism and HDL-C or HDL subclasses have been inconsistent. The objectives of the present study were to assess the LIPG 584T allele frequency in 585 Caucasian, 98 African-American and 200 Tobago African-Caribbean older men, and to determine an association of LIPG 584 C>T polymorphism with nuclear magnetic resonance (NMR) measured lipoprotein concentrations, subclasses and sizes in these populations. The T allele frequency in Caucasians (29.0%) was two times more common ($p=0.003$) than in African-American men (13.8%), and five times ($P<0.0001$) more common than in Tobago men (5.75%). There was no statistically significant association of the LIPG 584 C>T polymorphism with any of the NMR lipoproteins in Caucasian or African-American men. Despite the low frequency in Tobago men, this allele was associated with significantly decreased small HDL (Ancova $p=0.015$), greater diameter of HDL particles (Ancova $p=0.02$) and lower large VLDL and medium LDL (Kruskal-Wallis $p=0.007$ and $p=0.02$ respectively). In conclusion, our results suggest that the LIPG 584C>T

polymorphism has significant protective effect on small HDL and HDL size in Tobago African-Caribbean, but not in Caucasian or African-American men. However, the frequency of the protective 584 T allele in African-Caribbean population is too low to explain the more favorable lipoprotein profile observed in this population.

4.2. INTRODUCTION

Coronary heart disease (CHD) is the leading cause of morbidity and mortality and one of the most important causes of physical disability in older individuals. Plasma lipoprotein abnormalities are central to the development of atherosclerotic lesions in CHD (Daugherty and Schonfeld 1985). A large number of epidemiological studies have consistently shown that men of African origin have a more favorable, less atherogenic lipoprotein profile than Caucasian men, characterized by lower levels of triglycerides (TG) and higher levels high density lipoprotein cholesterol (HDL-C). (Tyroler et al. 1980; Watkins et al. 1986; Donahue et al. 1989; Webber et al. 1991; Brown et al. 1993; Zoratti 1998). The inverse association between HDL-C and the development of atherosclerosis and CHD is well established (Gordon and Rifkind 1989; Badimon et al. 1992; Barter and Rye 1996; Gotto 2001; Assmann and Nofer 2003). An earlier study reported that in elderly people, HDL-C, but not total cholesterol (TC) or low density lipoprotein cholesterol (LDL-C), was associated with mortality from CHD (Corti et al. 1995). Furthermore, it has been shown that the presence of CHD was more strongly associated with HDL particle size distribution than with the low HDL-C level (Cheung et al. 1991; Drexel et al. 1992). Indeed, lipoprotein characteristics that contribute to the development of atherosclerosis include both particle number and size (Freedman et al. 1998). Elevated levels of small, dense

LDL and small HDL lipoprotein subclasses, or reduced levels of large HDL subclasses, have been widely accepted as better predictors of CHD risk than lipid concentrations obtained by the standard assays (total cholesterol (TC), LDL-C and HDL-C) (Freedman et al. 1998; Lamarche et al. 2001; Rosenson et al. 2002). In addition, some studies reported that large HDL has an inverse and stronger association with atherosclerosis and CHD than small HDL (Miller et al. 1981; Ballantyne et al. 1982). Thus, the measurement of lipoprotein subclasses can significantly improve the assessment of CHD risk.

Both genetic and environmental factors play an important role in the etiology of CHD. Recently the search for the genetic markers which influence lipoprotein levels has become the primary focus of the CHD prevention studies. Numerous studies so far suggested that genetic factors may be very important in determining HDL-C, LDL-C and TG levels, and consequently the CHD risk.

The most recently discovered member of the triglyceride lipase family is endothelial lipase (EL). This enzyme is synthesized in endothelial cells, and is 45% homologous to lipoprotein lipase (LPL), 40% to hepatic lipase (HL), and 27% to pancreatic lipase (PL) (Jaye et al. 1999). EL has a low triglyceride lipase activity, but a significant phospholipase A1 activity (Rader and Jaye 2000). In a mouse model, over-expression of EL significantly reduced levels of plasma HDL-C and apolipoprotein A-I (ApoAI) (Jaye et al. 1999); conversely, inhibition of EL increased plasma HDL-C levels (Jin et al. 2003). Treatment of cultured human endothelial cells with inflammatory cytokines, like tumor necrosis factor- α (TNF- α) or interleukin-1 β (IL-1 β), as well as physical forces, increased the expression of EL. These findings could potentially explain the reduction of HDL-C levels in infection and suggest that endothelial lipase could be involved in the process of atherosclerosis. It has also been shown that in the presence of heparin sulfate

proteoglycans, and with the regulation of ligand clustering, EL directly mediates binding and uptake of plasma lipoproteins, independently of its enzymatic activity (Fuki et al. 2003). Finally, in human macrophages, with oxidized LDL, EL was found to be over-expressed (Rader and Jaye 2000).

Endothelial lipase gene (LIPG) is located on chromosome 18 (18q21.1). DeLemos et al. were among the first to hypothesize that functional polymorphisms and mutations in the LIPG might contribute to the elevated levels of HDL-C (deLemos et al. 2002). The investigators identified 17 LIPG polymorphisms (deLemos et al. 2002). In the present study we have focused on the functional and the most common among these, 584C>T (also known as Thr111Ile) missense polymorphism in exon 3 of the LIPG, responsible for a threonine to isoleucine amino acid change at codon 111 of the LIPG protein. It has been shown that the allele frequency of the LIPG 584 T allele is differently distributed in African-Americans, in Japanese, in Caucasians or in Caucasian individuals with high levels of HDL-C (10.3% in African-Americans (deLemos et al. 2002), 24% in Japanese (Yamakawa-Kobayashi et al. 2003), 31.2% in Caucasian controls (deLemos et al. 2002), and 32.6% in Caucasians with high HDL-C (deLemos et al. 2002)). The reports on the association between this polymorphism and HDL-C or HDL subclasses have been inconclusive. DeLemos and colleagues have found no association between the LIPG 584 C>T and HDL-C (deLemos et al. 2002). Another study reported no association between LIPG 584 C>T and HDL-C, total cholesterol, TG or LDL-C among 340 Japanese participants (Yamakawa-Kobayashi et al. 2003). On the other hand, the Lipoprotein and Coronary Atherosclerosis Study (LCAS) of 372 predominantly Caucasian participants, aged 35-75 years (only 7% African-Americans, N=27), reported a significant positive association between the 584 T allele and HDL-C, but no association of this allele with atherosclerosis after 2 and half years of follow-up (Ma et

al. 2003). The Quebec Family Study examined the association between the LIPG 584 C>T polymorphism and HDL subclasses, in addition to HDL-C, in 281 Caucasian women and 216 Caucasian men. The investigators reported no association between HDL-C and 584 T allele (Paradis et al. 2003). However, homozygous TT women had significantly increased large HDL subclasses, a result that was not observed among men (Paradis et al. 2003). Finally, a study by Halverstadt et al. has found that the carriers of the 584 T allele (TT/CT) may have a more protective lipoprotein profile than the homozygous CC individuals, characterized by decreased small HDL subclasses and slightly increased large HDL subclasses (Halverstadt et al. 2003).

The objectives of the present study were to examine the frequencies of the LIPG 584 C>T (Thr111Ile) polymorphism in older Caucasian, African-American and African-Caribbean men, to determine an association of LIPG 584 C>T polymorphism with nuclear magnetic resonance (NMR) measured lipoprotein subclasses in these populations, and finally, to describe potential ethnic differences in these associations. We hypothesized that the LIPG 584C>T is associated with HDL-C and HDL subclasses

4.3. METHODS

4.3.1. Study populations

The Tobago Health Study was derived from the Tobago Prostate Study, designed for prostate cancer survey of the male population of Tobago, aged 40-79. In 1990, the population of Tobago was 92% African descent, 4.5% mixed, 2% Asian Indian, 0.4% white and 1% other (Tobago 1993). All protocols were reviewed and approved by the Institutional Review Board of the University of Pittsburgh and the Tobago Ministry of Health. Written informed consent was

obtained from each participant. The questionnaire was designed to gather demographic and anthropometric data, and to assess history of smoking, medical history, personal and family cancer history, and occupational history. Peripheral blood was drawn from fasting subjects. Aliquots of frozen serum were shipped to the University of Pittsburgh, and stored in -70°C freezers for later measurements. The present study population includes a random sample of 200 Tobago men over the age of 65 (mean age 73.2 years), not diagnosed with prostate cancer, who reported three or more grandparents of African descent.

The Cardiovascular Health Study (CHS) is a population-based, longitudinal study of 5888 people aged ≥ 65 year randomly recruited from Medicare eligibility lists from four US communities. Potential participants were excluded if they were institutionalized or were severely ill. 5201 CHS participants underwent baseline assessments of cardiovascular risk factors in 1989-1990 (Fried et al. 1991). An additional 687 similarly aged African-Americans participants were recruited to CHS in 1992-1993 (Kuller et al. 2002). All participants signed consent forms upon entry into the study. Originally, CHS was designed to evaluate risk factors and noninvasive measures, and to describe and predict atherosclerotic events in older adults (Fried et al. 1991). Participants have had extensive baseline and follow-up evaluations. At the baseline examination, anthropometric measurements, medical and lifestyle histories, blood collection, echocardiography, resting 12-lead ECG, carotid ultrasonography and ankle-brachial index were obtained. Prevalence and extent of CVD were assessed (Fried et al. 1991). The present study population consists of a sample of 585 Caucasian men (mean age 73.1 years), and 98 African-American men (mean age 72.8 years) from a large sample of the original cohort members who were previously selected for a case-cohort evaluation of an association of NMR lipoproteins and risk of CHD.

4.3.2. Laboratory methods/NMR Spectroscopy

Plasma lipoprotein concentrations and subclass distributions were determined by nuclear magnetic spectroscopy (NMR) at LipoScience, Inc (Raleigh, NC). This method has been described in detail by Otvos et al (Otvos et al. 1991; Otvos et al. 1992; Otvos et al. 2002). Each lipoprotein subclass is quantified using the distinguishing NMR signals broadcast by lipoprotein subclasses, which differ in frequency and shape, depending on the diameter of the lipoprotein particles. The intensities of signals are proportional to the quantity of particles and are further multiplied by a standard lipid amount to provide the results in milligrams per deciliter (mg/dl) of cholesterol. The NMR spectroscopy measures 6 subclasses of VLDL, 4 subclasses of LDL and 5 subclasses of HDL in fresh or frozen plasma or serum specimens. Additionally, each profile provided calculated estimates of the concentrations of total cholesterol, total triglyceride, LDL-C, HDL-C, LDL particle number, and average VLDL, LDL, and HDL particle size. Reported LDL particle concentration is the sums of the concentrations of the LDL subclasses including IDL.

4.3.3. Genetic Analysis

DNA isolation from Tobago participants' blood clots from a 15 cc coagulation tube was accomplished by mechanical disruption of the clot, protease K digestion and isolation on a Qiagen column (Qiagen, Inc., Santa Clara, CA).

The genotype at position 584 of the LIPG exon 3 was determined by the fluorescence polarization (FP) (Chen et al. 1999). The following unique sequence flanking primers were used for amplification: F: 5'-GGGAAGAGGGTCATATAAG-3', and R: 5'-CATGACCTGCATTTCTTA-3'. Polymerase chain reaction (PCR) was conducted with 25 mM

of magnesium chloride, 1.25 mM of dNTP, 2.5 uM of forward primer, 2.5 uM of reverse primer (University of Pittsburgh Sequencing Facility, Pittsburgh, PA), 1 unit of Invitrogen Life Tech (Carlsbad, CA) Taq polymerase. The PCR steps were as following: 94°C for 10 min, 94°C for 30 sec, annealing temperature 51.8°C for 30 sec, 72°C for 1 min, then steps 2 through 4 repeated 34 cycles. The excess primers and dNTP were removed by addition of 10 µL mix containing 0.1 U of exonuclease (USB, Inc., Cleveland, OH) and 1 U of shrimp alkaline phosphatase (Roche, Nutley, NJ) and incubation at 37°C for 1.5hr and inactivation of enzymes at 95°C for 15 min. The primer detection reaction was performed with 10 uM of detection (internal) primer 5'-ACTCGTGTCAGCCCTGCACA-3', 25 uM of appropriate dye dNTP combination (r110 U/tamra C) (Perkin Elmer-Life and Analytical Sciences, Boston, MA) and 0.2 U of thermosequense (USB, Inc., Cleveland, Ohio), followed by another round of 30 cycles of 94°C for 1 min, 94°C for 15 sec, 50°C for 30 sec and 10°C “hold”. Fluorescence was measured using the Analyst HT Assay Detection System (Molecular Devices, Sunnyvale, CA) and FP genotypes were assigned using the Allele Caller software.

4.3.4. Statistical analysis

Allele frequencies were estimated by direct gene counting. The observed genotype frequencies of the 584 T and 584 C alleles were compared with those expected under Hardy-Weinberg equilibrium (HWE) by the goodness-of-fit chi-square test. Ethnicity allele frequency differences were analyzed by chi-square test. Initial descriptive data analysis showed that most of the NMR lipoprotein subclasses and sizes (except small HDL) exhibited markedly skewed distributions. Prior to statistical analysis, we transformed the lipoprotein subclass and sizes by square root, inverse, or natural logarithms in order to reduce the non-normality of the distributions of

lipoproteins. Analysis of variance (ANOVA) was performed to test any significant difference of baseline demographic (continuous) characteristics among ethnic groups. Categorical variables were compared using chi-square testing. Analysis of covariance (ANCOVA), which adjusted for appropriate covariates, was conducted using the general linear model procedure (GLM) to test for possible differences among LIPG genotypes on mean levels of NMR lipoproteins. To evaluate the statistical significance with regard to multiple testing and phenotypes that are correlated, we adjusted the p-value to reflect the empirical data. A p-value <0.015 were considered to be truly significant. Because the population distributions of small and medium LDL and large VLDL levels were highly skewed, we used non-parametric Kruskal-Wallis test to analyze the possible differential effects among genotypes and ethnic groups. Finally, we used a two-way ANOVA to test for a possible interaction between the LIPG 584C>T polymorphism and ethnicity on NMR lipoproteins subclasses or sizes. The Statistical Analysis System (SAS, version 8.2; SAS Institute, Cary, NC) and the Statistical Package for the Social Sciences (SPSS, version 12.0.1; Chicago, IL) were used for statistical analysis.

4.4. RESULTS

The distribution of LIPG 584 C>T genotypes is shown in table 4-1. The observed genotype distributions were in Hardy-Weinberg equilibrium in all three populations. The LIPG 584 T allele frequency differed significantly across all three ethnic groups. In Caucasians T allele (29%) was twice as common ($p=0.003$) than in African-American men (13.8%), and five times ($P<0.0001$) more common than in Tobago men (5.75%).

Three ethnic populations were similar in age and BMI. Waist circumference was significantly lower in Tobago men than either US population (ANOVA $p < 0.0001$), while smoking was more frequent in African-American men (overall chi square $p = 0.0003$).

Table 4-2 shows the NMR lipoprotein subclasses and sizes, and their association with the LIPG 584 C>T genotype within each ethnic group. No significant associations were found between the LIPG 584 C>T genotypes and levels of standard lipoprotein measurements (TC, TG, LDL-C and HDL-C).

In Caucasians, we found no significant associations between the LIPG 584 C>T genotype and the NMR lipoprotein subclasses.

Due to the small number of the T allele homozygotes in African-Americans (TT=4), the T allele carriers, TT and CT genotype group were combined for statistical analysis. No significant associations were found. The number of LDL particles in the TT/CT group was slightly lower than in the CC group, but this difference did not reach statistical significance ($p = 0.065$).

There were no T allele homozygotes among Tobago men. Carriers of the CT genotype were associated with a significantly lower small HDL subclass ($p = 0.015$). Additionally, HDL diameter was slightly greater in carriers of the T allele than in non-carriers ($p = 0.02$). Large VLDL subclass and medium LDL subclass were lower in the CT genotype group than in the CC genotype group, (Kruskal-Wallis $p = 0.007$ and $p = 0.02$ respectively).

No statistically significant interaction was observed between the LIPG 584 C>T polymorphism and ethnicity in determining NMR lipoprotein levels and sizes.

4.5. DISCUSSION

To our knowledge, this report is unique in that we evaluated the prevalence of the LIPG 584 T allele in the largest sample size of Caucasian men to date, and also, we characterized two population samples of men of African origin, living in the differing environments. In addition to the reported frequencies of the LIPG 584 T allele, we also described the association between NMR lipoprotein subclasses and the LIPG 584 C>T polymorphism in each of these populations.

This study shows that the frequency of the LIPG 584 T allele differed significantly among ethnic groups. The frequencies that we observed in Caucasian men were similar to those reported previously (deLemos et al. 2002; Halverstadt et al. 2003), and the frequencies that we observed in African-Americans were somewhat higher than those reported previously (deLemos et al. 2002). This was the first study to report the frequency of this allele in African population living outside of the US. In Tobago men, the frequency of the 584 T allele is lower than in any population so far.

In the present study on large population samples, the LIPG 584 C>T genotype displayed very little association with the NMR lipoproteins, subclasses and sizes in Caucasians and African-Americans. On the other hand, we found lower small HDL subclass, and increased size of HDL particles in African-Caribbean T allele carriers. Small HDL is an atherogenic HDL-C subclass that shows a positive association with CHD. In contrast, larger diameter HDL particles are thought to be less atherogenic and possibly cardio-protective. Therefore, we speculate that the LIPG 584 C>T could contribute to a more cardio-protective lipoprotein profile, with respect to HDL subclasses, in African-Caribbean men, but not in the US Caucasian and African-American men from our study. The findings in Tobago men are in agreement with the results by Halverstadt et al. who found this allele to be associated with lower levels of small HDL, although

majority of the 83 participants in their study were Caucasians (Halverstadt et al. 2003). However, in the Quebec Family Study only women homozygous for the T allele, and not men, had significantly increased large HDL subclasses, suggesting that the association of this polymorphism with HDL subclasses could be gender related, at least in Caucasians (Paradis et al. 2003).

It is not clear if small or large VLDL subclasses are atherogenic, and if their role in atherosclerosis is direct. Some investigators suggested that large VLDL is positively associated with CHD and coronary artery lesion progression (Freedman et al. 1998). However, others suggested that small VLDL, rich in cholesterol ester, unlike large VLDL which is rich in triglycerides, has a strong atherogenic potential (Mack et al. 1996; Hodis 1999). The indirect role of large VLDL in atherosclerosis is established. Increased levels of large VLDL can promote the formation of small, dense LDL (Packard 2003). LDL particles derived from large VLDL have been shown to have a prolonged plasma life compared with LDL particles derived from smaller VLDL subclasses (Packard 2003). In African-Caribbeans, the large VLDL subclass was lower in the T allele carriers. Further studies may be necessary to clarify whether these effects of LIPG polymorphism on large VLDL subclass, without an effect on TG, occurred by a chance, and whether this outcome, if real, would further translate into reduced CHD risk.

Although, EL has been found to be a key enzyme in HDL metabolism, some of the very recent studies focused on the EL role in catabolism of ApoB-containing lipoproteins. So far only a small number of studies using a mouse model reported a significant effect of EL on LDL-C (Ishida et al. 2003; Broedl et al. 2004), and one of these studies reported this effect only in males (Ishida et al. 2003). This effect has not yet been studied or confirmed in humans. Our results indicate that in older men of Caucasian and African origin, the LIPG 584 C>T polymorphism is

not associated with LDL-C, LDL subclasses and LDL particle size. The possible effect of this polymorphism on medium LDL subclass in Tobago men, without an association with LDL-C or any other LDL subclasses, and the possible role of medium LDL in the development of the atherosclerosis are not clear and should be further investigated.

We did not observe any significant gene-ethnicity interactions. The small numbers of men of African origin, and the low frequency of the LIPG 584T allele limited our power to detect significant gene-ethnicity interactions. Another limitation was the lack of information on some other important factors that can modify lipoproteins and interact with the gene, such as dietary and alcohol intake, and physical activity. Finally, there is a possibility of survivor's bias in the present study because our sample included men older than 65 years. However, the frequencies observed in our Caucasian group are consistent with those previously reported. The 584C>T polymorphism was slightly more common among African-Americans in the present study than in only other study that included African-Americans.

The reasons for the discrepancies between the genetic association studies on the LIPG 584C>T polymorphism are not clear. The protective effect on HDL subclasses, found only in a study population with the lowest frequency of the 584 T allele, suggest that the LIPG 584 C>T variation may not be the causal modulator of the observed lipoprotein phenotypes, and that some other polymorphism is in strong linkage disequilibrium with the 584 T allele in some populations, but not in others, independent of ethnicity. Another possibility is that some environmental factors, that we did not account for, could vary among the study samples and may play an important role in affecting the association of this LIPG variant with lipoproteins.

In conclusion, in the present study we have found no evidence which would suggest that the LIPG 584C>T polymorphism significantly affects HDL-C or LDL-C lipoproteins and their

subclasses in older Caucasian and African-American men. On the contrary, a significant protective association was found between the 584T allele and small HDL, HDL size and large VLDL in African-Caribbean men. However, even though the LIPG 584T allele is protective in African-Caribbean men, its rarity suggests that it is unlikely that this allele accounts for a more favorable lipoproteins observed in these men compared to Caucasians. Further studies are needed to confirm potential protective effect of this polymorphism on HDL, LDL or VLDL lipoproteins and their subclasses. These studies should include men and women over a broader age range, and larger samples of minority populations.

Table 4-1 Genotype distribution of LIPG 584 C>T and frequency of the T allele by ethnic group

LIPG 584 C>T Genotypes	CHS-Caucasians N=585	CHS-African-Americans N=98	Tobago African- Caribbean N=200
TT	47	4	0
CT	243	19	23
CC	295	75	177
T allele frequency ^{1,2,3}	0.29	0.14	0.058
P value for the test of HWE	0.76	0.07	0.39

¹ Caucasians different from African-Americans (P<0.003)

² Caucasians different from Tobago African-Caribbeans (p<0.0001)

³ African-Americans different from Tobago African-Caribbeans (p=0.035)

Table 4-2 Associations between LIPG 584 C>T polymorphism and NMR lipoproteins within ethnic groups

Mg/dl	Caucasians N=576				African-Americans N=98			Tobago African-Caribbean N=197		
	TT N=44	CT N=241	CC N=291	p-value	TT/CT N=23	CC N=75	p-value	CT N=23	CC N=174	p-value
CHOL ^A	227.5 ±6.8	220.7 ±2.7	229.2 ±2.6	0.077	223.9 ±7.5	228.8 ±4.2	0.57	180.2 ±8.2	179.05 ±2.7	0.86
TG ^C	145.0 ±8.6	156.9 ±5.6	157.5 ±4.1	0.73	123.8 ±11.9	131.1 ±7.2	0.56	70.8 ±3.8	79.9 ±2.3	0.28
LDL-C ^A	146.1 ±6.0	141.5 ±2.3	147.5 ±2.3	0.21	132.4 ±8.0	144.3 ±3.6	0.13	114.2 ±7.6	113.6 ±2.3	0.93
HDL-C ^A	48.4 ±2.1	45.11 ±1.0	45.7 ±0.9	0.62	58.2 ±4.2	51.3 ±2.1	0.13	52.4 ±3.3	49.6 ±1.0	0.47
Small HDL	18.9 ±0.9	18.0 ±0.4	17.9 ±0.3	0.56	18.6 ±1.8	17.7 ±0.8	0.67	15.5 ±0.98	18.0 ±0.35	0.015
Large HDL ^A	29.6 ±2.4	27.1 ±1.1	27.8 ±1.01	0.83	39.6 ±4.5	33.7 ±2.3	0.21	37.0 ±3.5	31.6 ±1.1	0.14
Small VLDL ^A	32.8 ±2.1	31.3 ±1.01	35.1 ±0.99	0.04	33.3 ±3.1	31.6 ±1.67	0.66	14.6 ±2.1	14.9 ±0.8	0.84
Medium ^A VLDL	51.6 ±4.8	50.3 ±2.5	50.1 ±2.2	0.68	26.3 ±4.9	34.5 ±3.6	0.22	21.3 ±2.2	27.7 ±1.4	0.16
Large VLDL*	21.4 ±4.2	36.9 ±3.9	33.3 ±2.5	0.33	25.9 ±7.9	26.5 ±4.3	0.83	0.91 ±0.22	3.7 ±0.7	0.007
Small LDL*	30.8 ±6.9	39.2 ±2.8	41.7 ±2.9	0.25	16.5 ±6.4	33.8 ±5.5	0.1	12.8 ±5.2	15.4 ±1.8	0.1
Medium LDL*	37.5 ±5.3	38.3 ±2.3	42.8 ±2.3	0.19	34.8 ±7.8	28.0 ±4.2	0.4	19.4 ±3.5	29.7 ±1.6	0.02
Large LDL ^A	76.5 ±7.5	62.8± 3.0	62.2 ±2.6	0.34	81.1 ±8.3	81.6 ±5.5	0.80	80.7 ±8.0	66.4 ±2.8	0.088
LDL Particles ^A (nmol/l)	1628 ±81.3	1626 ±30.5	1701 ±31.4	0.28	1419 ±87.5	1620 ±52.5	0.065	1149 ±94.8	1189 ±27.8	0.61
HDL Size ^B (nm)	9.1 ±0.9	8.9 ±0.04	8.9 ±0.03	0.81	9.3 ±0.14	9.2 ±0.08	0.87	9.65 ±0.12	9.4 ±0.04	0.02
LDL Size ^B (nm)	21.05 ±0.11	20.8 ±0.05	20.77 ±0.05	0.18	21.25 ±0.16	21.05 ±0.1	0.32	21.4 ±0.11	21.2 ±0.05	0.085
VLDL Size ^B (nm)	46.05 ±0.97	48.3 ±0.5	48.1 ±0.56	0.68	45.5 ±2.3	45.4 ±0.96	0.84	38.3 ±0.8	39.9 ±0.35	0.11

Values are unadjusted means ± SEM; p-value obtained by ANCOVA after adjustment for age, waist circumference and current smoking status (a p-value<0.015 was considered statistically significant); *p-value obtained by non-parametric Kruskal-Wallis test; ^a square root transformed; ^b reciprocal (inverse) transformed; ^c log transformed

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5. GENERAL DISCUSSION/FUTURE RESEARCH

In 2000, the Healthy People 2010 program was established for building strategies for elimination of health disparities. Since then, the identification of genetic contributions to health disparities has become a very important part of this initiative. A report from the US National Human Genome Research Institute emphasizes that allele frequencies for some disease-associated polymorphisms, differ considerably across populations and could significantly contribute to some existing health disparities (Collins et al. 2003). The authors anticipated that extensive research in genetics and genetic epidemiology would become an important part of public health strategies in order for health disparities to be eliminated, although they still acknowledged that social, environmental and behavioral factors contribute significantly to disparities in health status. The present project was designed in an effort to unravel the genetic contributions to differences in lipoprotein phenotypes observed between men of African origin and Caucasian men, and as a result, to improve our knowledge of the etiology of the coronary heart disease.

A very high African genetic contribution (~94%), and low European contribution (~6%), found in the first phase of this project, suggest that the Tobago population derives mostly from the African parental population, and that European parental population contributes very little to this population's gene pool. The Tobago population, therefore, may carry a higher burden of high-risk alleles of African origin for certain diseases, than the more admixed African-American population. Conversely, the Tobago population may benefit from a higher prevalence of protective alleles of African origin. Therefore, this African-Caribbean population is a valuable

resource for studying the genetics of syndromes or diseases, that show considerable Black-White differences in prevalence, such as, dyslipidemia, atherosclerosis, hypertension, diabetes, prostate cancer, or osteoporosis.

The second part of the present study has shown that older men of African origin, from both US and Caribbean environments, have a more favorable, less atherogenic distribution of the NMR lipoproteins and their subclasses and sizes than the US Caucasian men. In addition, TG levels and VLDL subclasses were found to be remarkably lower and VLDL particle size smaller, in Tobago African-Caribbean men, than in either CHS men, African-Americans or Caucasians. We have shown that older men from Tobago had a lower degree of central obesity and were less likely to smoke compared with CHS men. In general, it is possible that the lower central obesity, observed in men of African origin is associated with an increased anti-lipolytic effect of insulin, which could account for low triglyceride levels and high HDL-C (Zoratti 1998). However, the significant differences between ethnicities were found after adjusting for age, waist circumference and smoking. Other factors that can influence TG levels, such as alcohol, diet and physical activity, were not available in this study. We can speculate that African-Caribbeans live in a different environment than African-Americans. However, the lipoprotein profile was also found to be favorable in African-Americans from our study, when compared to Caucasians, and distribution of LDL and HDL subclasses was very similar to the one observed in Tobago African-Caribbean men. In addition, the average waist circumference in African American men was not significantly different from that observed in Caucasian men. These findings suggest that genetic factors may play an important role in determining a more favorable lipoprotein profile in African men from our study.

We further investigated polymorphisms in two candidate genes, hepatic lipase (LIPC) and endothelial lipase (LIPG), both associated primarily with variations in HDL-C and its subclasses and sizes, in order to test if these variants could in part explain variations in lipoproteins observed, both within and between ethnic groups, in our study.

The frequency of the LIPC -514 T allele in African-Caribbeans was almost three times higher than in Caucasians, and is the highest reported so far (57.3%). Furthermore, in both groups of African descent from our study, the LIPC -514 C>T polymorphism was associated with a more cardio-protective lipoprotein profile, characterized by higher large HDL subclass and greater diameter of HDL particles and to some extent LDL particles. Additionally, in African-Americans the -514 T allele was also associated with elevated level of HDL-C. Contrary to these observations, the -514 T allele in CHS Caucasian men had little impact on NMR lipoprotein distributions. Thus, our findings suggest that ethnicity is a potential moderator of the effect of the LIPC gene polymorphisms on lipoprotein levels. Moreover, this hepatic lipase polymorphism could potentially explain higher levels of cardio-protective large HDL subclass and greater HDL size in men of African origin from our study.

This significant interaction between genetic variation at the LIPC gene with ethnicity affected the levels of HDL-C, its subclasses and size, and LDL size. There are number of possible reasons for the strong gene-ethnicity interaction, and for significant associations that were found only in men of African origin. One possibility is that the LIPC-514 C>T polymorphism may not be the causal modulator of the observed differences in lipoprotein phenotypes and that some other causal polymorphism, or polymorphisms which are actually reducing the LIPC expression, are in strong linkage disequilibrium with the -514 C>T variant in men of African origin, but not in Caucasian men. Sequencing of the whole LIPC gene and

flanking regions or direct measuring of hepatic lipase activity in our populations, could potentially clarify our findings. Second, our discrepancy might be due to other genes related to lipoprotein metabolism (i.e. gene-gene interactions). It is possible that polymorphisms not associated individually with lipoprotein phenotypes, might be associated with them only if they are in combination with some polymorphisms in other genes involved in lipoprotein pathways (Anderson and Carlquist 2003). Third, due to the high frequency of the T allele in African populations, this polymorphism, together with other polymorphisms, could be variously combined in haplotypes, and these haplotypes could therefore, be differently distributed in populations of Caucasian and African descent. Further, the association of the LIPC polymorphism in African men from our study was found after controlling for some factors known to be associated with lipoprotein variations. Some other factors known to influence HDL-C, such as alcohol consumption, diet or physical activity, were not included in our study. It is possible that they would modify the genetic effect through interaction. Still, we observed that, despite probable differences in environment and lifestyle, African Americans were more similar to Afro-Caribbeans than to Caucasian Americans. Another possibility for explaining the inconsistent observations of significant associations among different populations, proposed by Juo et al., is the fact that the LIPC promoter variants influence HDL particle size only indirectly, through HL activity (Juo et al. 2001). Further, the discrepancy reported by the present study, as well as some other studies, may as well be due to the selection bias resulting from different schemes of sampling strategies. Finally, the simplest explanation might be that the lack of positive association between the T allele and HDL subclasses and lipoprotein particle diameters in Caucasians is related to the relatively small number of the T allele homozygotes. Our results differ from the results of the Framingham Study, where majority of participants were

Caucasians. The investigators reported that the T allele was significantly associated with large HDL and HDL size, although this association was much stronger in women (N=1353, TT=56), than in men (N=1314, TT=49).

Another inconclusive aspect is related to the question of functionality of LIPC 514 C>T. When different associations between genotype and phenotype are observed, and the polymorphism is non-functional, it is likely that these associations are actually due to another polymorphism which is in linkage disequilibrium with this non-functional one. However, Botma et al. reported that -514 C>T is functional, and that reduced HL activity found in carriers of the T allele, is due to a reduced transcription, but that this effect is relatively small, and may become apparent only in combination with some other polymorphisms in the LIPC promoter (Botma et al. 2001). Measuring transcription in men with and without the T allele from our study might provide additional valuable information.

Some studies reported unexpected, and contradictory, increased CHD risk in carriers of the LIPC -514 T allele. Some of these studies were well designed, large cohort studies, but the participants were mainly Caucasians. In order to explain this paradoxical association between high HDL-C and increased CHD risk, Andersen et al. proposed that it is possible that higher HDL-C found in carriers of the -514 T allele is caused by reduced HL activity, and that this reduced activity decreases catabolism of large HDL particles (Andersen et al. 2003). High accumulation of large HDL could potentially reduce the capacity of the reverse cholesterol transport system and therefore, cause an insufficient removal of cholesterol from the arterial intima. In our study the -514 T allele was associated with a more cardio-protective lipoprotein profile in men of African origin, but not in Caucasian men. Further large cohort studies in non-

Caucasian men are needed to clarify whether these protective effects of LIPC polymorphism on lipoprotein subclasses translate to reduced CHD risk in men of African origin.

We further evaluated the frequency of the 584 C>T polymorphism, also known as Thr111Ile, in exon 3 of the endothelial lipase gene (LIPG). The present study was the first study to report the frequency of the LIPG 584 T allele in African population living outside of the US. Additionally, there is only one other study that has investigated this polymorphism in African-Americans (deLemos et al. 2002). In Tobago African-Caribbean men, contrary to the results related to the hepatic lipase polymorphism, the frequency of the LIPG 584 T allele is lower than in any populations studied so far (5.75%).

As was the case with the hepatic lipase variant, we found very little impact of the LIPG 584 C>T on the NMR lipoproteins, subclasses and sizes in Caucasians. However, we also found no significant association between this allele and lipoproteins in African-Americans. The T allele African-Caribbean carriers had lower small HDL, and greater size of HDL particles, the findings that are in agreement with the results from a recent study in Caucasians (Halverstadt et al. 2003), and that actually characterize a more cardio-protective lipoprotein profile.

Furthermore, in African-Caribbeans, large VLDL was lower in the T allele carriers. Though we have to take into consideration that this result, due to the highly skewed distribution of large VLDL, was obtained by a less powerful, Kruskal-Wallis's non-parametric test, our inspection of the CT and CC genotype groups, did not show any differences in the degree of BMI, waist circumference or smoking. This finding, related to large VLDL, reported for the first time, could be significant, due to the fact that large VLDL may be positively associated with CHD and coronary artery lesion progression (Freedman et al. 1998). Increased levels of large VLDL can promote the formation of small, dense LDL (Packard 2003). LDL particles derived

from large VLDL have been shown to have a prolonged plasma life compared with LDL particles derived from smaller VLDL subclasses (Packard 2003). Further studies may be necessary to clarify whether these effects of LIPG polymorphism on large VLDL subclass, without an effect on TG, occurred by a chance, and whether this outcome, if real, would further translate into reduced CHD risk.

Both LIPC and LIPG polymorphisms had very little effect on routinely measured lipoproteins (total cholesterol, TG, LDL-C and HDL-C). Significant associations that we found between the LIPC and LIPG polymorphism and lipoprotein subclasses and sizes would not be apparent if only standard lipoproteins were measured.

We also need to address some limitations of this study. Studies of older individuals are, by definition, studies of survivors. However, the frequencies observed in Caucasians and African-Americans from this study are consistent with those previously reported. Additionally, the LIPC and LIPG genotypes were in Hardy-Weinberg equilibrium in all populations, and age did not differ significantly by genotype. It is possible that associations between the LIPC and LIPG polymorphisms and lipoprotein levels in both groups of African origin, or lack of associations found in Caucasians, were related to sample size. However, in men of African origin, positive findings were not only significant in both groups, but also showed the same tendency between genotypes and lipoprotein phenotypes. On the other hand, the small number of the LIPC -514 T allele carriers, in Caucasians, could lower our power to detect significant results obtained in previous larger studies in Caucasian men that looked at this LIPC polymorphism and lipoproteins, such as the Copenhagen City Heart Study (N=4023, CT=1335, TT=194) (Andersen et al. 2003) and the Framingham Offspring Study (N=1314, CT=435, TT=49) (Couture et al. 2000). The effect of the LIPC -514 C>T has been found to be modified by various environmental

factors such as diet (Ordovas et al. 2002; Tai et al. 2003) and physical activity (Hokanson et al. 2003). The lack of information on some other important factors that can modify lipoproteins and interact with the gene, such as dietary and alcohol intake, and physical activity evaluation, especially in case of the LIPC variant, was another limitation of our study.

Finally, despite many advantages of the NMR spectroscopy, there are also some limitations associated with this methodology. This method provides lipoprotein values that are derived from the direct measurement of the size of the lipoprotein particles carrying the lipids, and not their chemical compositions (Otvos et al. 2002). The assumption is that an individual has lipoprotein particles of normal compositions (Otvos et al. 2002). However, chemical composition may vary among different individuals, especially in some pathological syndromes, such as diabetes. Therefore, if a person has particles depleted of cholesterol and enriched in TG, the NMR-derived value will be higher than the one obtained by the chemical measurement (Otvos et al. 2002). Despite these differences, several studies have shown close correlation between the LDL, HDL and VLDL subclasses measured by the NMR spectroscopy and the standard chemical method (Grundy et al. 1999; Blake et al. 2002; Tsai et al. 2004).

Further studies are needed to clarify whether the protective effects of the LIPC and the LIPG polymorphisms on lipoprotein subclasses in men of African origin would translate to reduced CHD risk. Additionally, these studies should include larger samples of African populations, both genders, and wide range of age. The future efforts should broaden, currently insufficient, knowledge about the contribution of genetics to diseases in African populations. The search for other candidate genes that cause lipoprotein ethnic variations should be persistent, and combinations of polymorphisms in multiple genes involved in lipoprotein metabolism should be further assessed. Furthermore, single nucleotide polymorphisms do not occur in random

combinations, but in fixed patterns within regions of DNA delimited by areas of reduced recombination. Therefore, many believe that haplotype-based approach to completely scan the relevant gene, and surrounding areas, in a world-wide variety of ethnic groups, would provide a greater power for understanding the role that genetics play in ethnic differences in complex diseases. It is known that populations of African descent have smaller haplotype blocks, a greater diversity of common haplotype, with fewer sites being in linkage disequilibrium and greater recombination than non-African ethnicities (Tishkoff and Williams 2002). In the near future, we can expect that the International Haplotype Map Project (HapMap) could potentially facilitate the ultimate identification of candidate genes (2003). In this project, individuals of all ethnicities are undergoing complete genomic sequencing to identify SNP and large scale genotyping to identify SNP that define the most common haplotypes and the extent of haplotype blocks.

In the present study, we estimated the degree of European and African admixture in Tobago African-Caribbean men, using autosomal markers. However, an earlier study reported that the European genetic contribution from males to African-Americans was considerably higher than the female contribution, supporting the possibility of sex-biased gene flow (Parra et al. 2001). Future studies should include mtDNA haplogroups, which are maternally inherited, and informative Y-chromosome-specific markers transmitted only from father to son. These two methods would allow the evaluation of male and female specific gene flow in this population.

In our study, Tobago African-Caribbeans have been found to have much lower triglyceride levels and VLDL subclasses than either Caucasians or African-Americans. Although, our findings could in part explain favorable distribution of HDL-C and its subclasses, we were not able to explain observations related to the extraordinarily favorable TG and VLDL subclasses distributions. At this point, we can only speculate about several possible rationales for

this finding. Obesity, and in particular, abdominal obesity, contribute to both triglyceride and HDL-C levels. African-Caribbean men were less centrally obese than Caucasians or African-Americans from the present study. However, we observed these differences in TG and VLDL, even after adjusting for waist circumference. Future studies in this population should incorporate a precise quantification for adipose tissue compartments, by using the computed tomography (CT) scanning. It is also possible that common mutations in lipoprotein lipase (X447, 93 T>G), Apo E (E2 and E4 alleles) or some other candidate genes, might have a strong effect on plasma triglyceride levels in Tobago men, especially since it has been shown that the distributions of these polymorphism differ between African-Americans and Caucasians. Screening for these variants in Tobago population could potentially add more information to the reasons for higher TG levels. Finally, environmental factors, such as diet, alcohol consumption and physical activity, could interact with genetic determinants to modulate triglyceride levels. Beside these factors, there are probably many others, still unknown, that are causing these significantly lower TG levels and low VLDL subclasses in this population. The finding of the possible cause, or causes, for these observations could significantly improve the strategies for the prevention of atherosclerosis and CHD.

Additionally, increased TG level, in combination with decreased HDL-C level, are often found to be a major syndrome in insulin resistance. Despite a less atherogenic lipoprotein profile in African-Americans, they are found to be more hyperinsulinemic than Caucasians, and at any given level of insulin sensitivity, African Americans are likely to experience greater insulin action (Haffner et al. 1996). Additionally, emerging evidence suggests that adiponectin plays a protective role against insulin resistance and atherosclerosis by stimulating fatty acids oxidation, decreasing plasma TG, increasing HDL-C, and improving glucose metabolism by increasing

insulin sensitivity (Thamer et al. 2002; Gil-Campos et al. 2004). However, it was also reported that plasma adiponectin levels were reduced in African-American boys than in Caucasian boys, suggesting that low adiponectin may predispose African-American young men to a greater risk of diabetes and CHD. Yet, this was one of the rare studies that looked at Black-White differences in adiponectin levels. Therefore, the future research in the Tobago Health Study should also include assessment of glucose, insulin, and adiponectin levels, in addition to TG levels. We would test whether insulin would be elevated, or adiponectin decreased, as we might expect considering the reports in African-Americans. Much remains to be learned about genetic control of insulin and adiponectin, especially in populations of African descent.

There is also some evidence that abdominal fat cell size, independent of fat mass, visceral abdominal fat, subcutaneous abdominal tissue, and femoral fat area, is a good predictor for plasma triglyceride and LDL Apo B levels in middle-aged men (Imbeault et al. 1999). Additionally, another study found that gluteal fat cell size was independently correlated with insulin, triglyceride and HDL-C levels in African-American women, but not in Caucasian women (Tittelbach et al. 2004). Presuming that the fat cell size could be important factor in determining lipoproteins, their measurement could potentially help explaining the ethnic differences in lipoprotein variations.

Some argue that the higher prevalence of important CHD risk factors in African-Americans may abolish cardio-protective effect of their favorable lipoprotein profile. In that case the definition of what represents an elevated triglyceride level or decreased HDL-C may need to be reevaluated depending on ethnic background, and not only on age or gender.

In order to fully understand the specific genetic susceptibility to disease, dedicated large studies of individuals are critically needed for assessing the severity of atherosclerosis, and to

better quantify the degree of CHD risk associated with HDL-C and its subclasses, particularly in populations of African descent. However, it is possible that the T allele of the 514C>T polymorphism modifies CHD risk even without the important modification of HDL-C levels and HDL subclasses. The availability of the CHD events data in participants from the Cardiovascular Health Study (CHS) will allow further assessment of the CHD presence in carriers and non-carriers of the 514 T allele in CHS men, to test if this polymorphism attributes to increased CHD risk, independently of HDL-C and HDL subclasses.

In the Tobago Health Study atherosclerosis and CHD outcomes were not evaluated. We would propose to evaluate a carotid artery atherosclerosis by carotid artery intima-media thickness (IMT), assessed by high-resolution B-mode ultrasound in 212 African-Caribbean men from the present study. We would further compare IMT cases and controls to test if 514 T allele is a significant predictor of carotid artery intima-media thickness. Furthermore, our findings of less atherogenic lipoprotein profile in Tobago African-Caribbean men suggest that this population could have lower burden for atherosclerosis. Therefore, we would additionally propose the assessment of carotid artery atherosclerotic disease (IMT) and the assessment of coronary artery calcification, in larger sample of Tobago African-Caribbean men. We would test if the observed, more favorable and less atherogenic lipoprotein profile in Tobago men, would transfer into the lower degree of atherosclerosis.

As clinical interest increasingly focuses on the role of HDL-C in the pathogenesis of the CHD, any future research that involves study of the genes potentially associated with lipoproteins in old individuals could be very significant for longevity research. In old people, HDL-C is the most important predictor of CHD mortality. If elderly men in Tobago have higher than average levels of HDL-C, and additionally, their levels of TG are unusually low, future

studies should also investigate the offspring of these men, to see if they are in fact inheriting similar lipoprotein phenotypes, and variants of the LIPC, the LIPG, and other genes thought to be related to lipoprotein variations. This would not only more accurately describe the genetic and environmental contributions to lipoprotein phenotypes, but would improve our knowledge on longevity.

Finally, in the present project we focused on common polymorphisms in candidate genes for dyslipidemia. However, some very recent studies reported that actually rare alleles might significantly contribute to the low HDL-C levels in the general population (Cohen et al. 2004; Pajukanta 2004), and therefore, strongly suggest that these rare mutations should not be a priori excluded from future studies (Pajukanta 2004).

6. PUBLIC HEALTH IMPLICATIONS

At present, elimination of health disparities among ethnic groups is one the most challenging and important public health priorities. Candidate genes for dyslipidemia and lipoprotein variations among ethnicities, once identified, are likely to be useful for improving CHD risk prediction for different ethnic populations. An increased understanding of the association between hepatic lipase and endothelial lipase alleles and lipoprotein concentrations and particle sizes in older Tobago and CHS men will contribute to our knowledge of the etiology of CHD, and improve our understanding of the differences in CHD risk patterns between African-Americans and Caucasians. Screening for the LIPC and the LIPG variants could potentially contribute to a more efficient characterization of individual risk of coronary and cerebrovascular events.

The present study has confirmed presence of a more favorable, less atherogenic lipoprotein profile in older African-Caribbean men, characterized with not only higher level of HDL-C and lower level of TG, but as well as favorable levels of all VLDL, HDL and LDL subclasses. The same was confirmed in older CHS African-Americans, though their TG level was considerably higher than that observed in African-Caribbeans, but at the same time significantly lower than that observed in Caucasians. These findings are very important for CHD prevention strategies. Today, the major part of this strategy is lipid-lowering therapy. It may be that target lipoprotein goals are different for men of African origin and, and that definitions of normal ranges of triglyceride and HDL-C levels may need to be reevaluated among African-American men. Individual responses to different drugs vary within a population and differences

in drug response have been observed between ethnic populations as well. Genetic variation in drug metabolizing enzymes, such as hepatic lipase, could be possible modulators (Burroughs et al. 2002). An earlier study reported that middle-aged Caucasian men with dyslipidemia and established CHD responded differently to lipid-lowering therapy, depending on the LIPC -514 C>T polymorphism (Zambon et al. 2001). Homozygous -514 C allele patients experienced a greater increase in LDL size with lipid-lowering therapy than both homozygous and heterozygous carriers of the -514 T allele. Therefore, screening for this variant in the HL gene could explain possible lower responsiveness to the lipid-lowering therapy in African-Americans, and identify CHD patients who will benefit the most from the lipid-lowering strategies, as well as individuals for whom a more aggressive LDL-lowering therapy is needed or an overall more aggressive risk-reducing approach might be necessary (Zambon et al. 2001).

One fact is certain. It is critical that research on the genetics of lipoproteins and atherosclerosis continues and eventually leads to clinical exploitation.

7. SUMMARY

In summary, we found that European parental population contributes very little to the Tobago population gene pool and that Tobago African-Caribbean men share considerable genetic background with their West African ancestors. Older men of African origin, from both US and Caribbean environments, have a more favorable, less atherogenic distribution of the NMR lipoprotein particle numbers and sizes than Caucasian men. In addition, we found remarkably lower levels of TG, lower large and small VLDL subclasses, and smaller VLDL particle diameter in Tobago African-Caribbean men, than in either CHS-African-American, or CHS-Caucasian men. Frequency of the *endothelial lipase* 584 T allele in Tobago men was the lowest reported thus far. Despite this finding, the LIPG 584 T allele had a protective affect on small HDL subclass, HDL size, and possibly large VLDL subclasses, in Tobago African-Caribbean, but not in Caucasian or African-American men. However, the frequency of the protective LIPG 584 T allele in African-Caribbean population is too low to explain the more favorable lipoprotein profile observed in this population. In contrast, the -514 T allele in the promoter of the *hepatic lipase gene* was very high in Tobago men, almost three times higher than in Caucasians. Additionally, the present investigation found a protective effect of the hepatic lipase -514 C>T polymorphism on HDL subclasses and sizes, and possibly LDL size, in older men of African origin, but not in older Caucasian men. The current findings suggest that further evaluation of the effects of hepatic lipase gene polymorphisms on lipoprotein levels should consider ethnicity as a potential moderator of those effects. We conclude that the higher frequency of the protective

LIPC -514T allele in men of African origin contributes to the more favorable distribution of the HDL subclasses compared with Caucasians. Both the LIPC and the LIPG polymorphisms had very little effect on routinely measured lipoproteins (total cholesterol, TG, LDL-C and HDL-C) and the significant associations would not be apparent if only standard concentrations were examined. Therefore, our results suggest that lipoprotein subclasses provide information on the role of the hepatic lipase and the endothelial lipase genes on variation in lipoproteins between ethnicities, that cannot be obtained with routine measurements of lipoprotein concentrations.

APPENDIX A: LITERATURE REVIEW OF MOLECULAR EPIDEMIOLOGY OF ADVERSE LIPOPROTEIN PROFILE AND ATHEROSCLEROSIS

Several investigators have reported that polymorphisms in candidate genes, involved in lipid metabolism, affect lipoprotein levels. Candidate genes for adverse lipoprotein profile, atherosclerosis, and lipoprotein variations among ethnicities, once identified, are likely to be useful for improving individual and population CHD risk prediction.

CHOLESTEROL ESTER TRANSFER PROTEIN: CETP GENE

CETP plays a key role in the metabolism of HDL as it mediates the exchange of lipids between lipoproteins, resulting in the net transfer of cholesteryl ester from HDL to other lipoproteins in the subsequent uptake of cholesterol by hepatocytes (Kuivenhoven et al. 1998). It facilitates the transfer of esterified cholesterol from HDL to VLDL, and the transfer of triacylglycerol from VLDL particles to HDL (Kuivenhoven, Jukema et al. 1998). Elevated concentrations of CETP have been associated with reduced concentrations of HDL (Kuivenhoven, Jukema et al. 1998). The CETP gene is located on chromosome 16 and consists of 16 exons and 15 introns (Kuivenhoven, Jukema et al. 1998).

The TaqIB polymorphism in the CETP gene, has been associated with elevations in lipid-transfer activity and reductions of HDL concentration (Yamashita et al. 2001).

The association of CETP deficiency, CETP polymorphisms and atherosclerosis had been examined in 3,469 Japanese-American males in the Honolulu Heart Study (Zhong et al. 1996). This was the largest clinical study in which the D442G polymorphism was examined. 170 men

were heterozygous and 6 were homozygous for the exon 15 missense mutation D442G (Asp442-to-Gly) and only 17 men were heterozygous for the intron 14 splicing defect G14A G (+1)-to-A In14. The overall prevalence of CETP gene mutations in this population sample was 5.6%. Mean HDL cholesterol concentrations were significantly higher in men with intron 14 or exon 15 mutations, compared to men with no CETP gene mutation. Triglyceride concentrations were significantly lower in men heterozygous for the exon 15 mutation D442G. The D442 mutation was associated with only about a 10% mean increase in HDL, and the intron 14A change with a larger 32% change. The initial results of this study indicated that the D442G (A+55G/Ex15) and G14A (G+1A/In14) mutations were associated with increased risk for CHD, but this was only true among men with intermediate levels of HDL. The additional results are indicating that D442G may not be a risk factor in this population, particularly among high-HDL individuals (Zhong et al. 1996). There is a possibility that at very high HDL levels, some protective, anti-atherogenic effects of HDL (anti-oxidant effect) exist.

Together, 15 D442G and intron 14 splicing defect account for 10% of variability in HDL levels (Inazu et al. 1994). In a study in Taiwanese Chinese, the results showed that both D442G mutation and TaqIB2 allele were associated with high HDL concentrations (Hsu et al. 2002). However, some factors (gender, TaqIB1 allele, obesity and high triglyceride concentration) diminished this association. On the other hand, among 474 Taiwanese CHD patients and controls, the CHD risk for 442 DG carriers was 1.69-fold higher than DD carriers, with no significant effect on HDL (Wu et al. 2001).

A study of two hundred myocardial infarction patients investigated association between small, dense, LDL and D442G mutation in CETP gene (Wang et al. 2002). It was found that the major peak size of LDL particles in patients with D442G mutation of the CETP gene was

significantly larger than the in those not having this mutation. This suggested that CETP also plays an antiatherogenic role with respect to LDL particle size.

Another very common CETP polymorphism is I405V. The less-common allele is present in all populations examined and generally occurs at a frequency of over 25%. A large meta-analysis of TaqIB and I405V, reported that those with the less-common 405V allele have lower CETP levels and higher HDL levels than in 405II homozygotes (Boekholdt and Thompson 2003). When studies of TaqIB were subjected to a meta-analysis, the B2B2 homozygotes were found to have higher HDL levels than B1B1 homozygotes (Boekholdt and Thompson 2003).

The role of CETP gene is still subject of controversy. Some investigators have found increased CHD in Japanese-American men with this protective mutation (D442G) despite increased HDL level and concluded that these particles may not be cardio-protective, while others suggest that CETP deficiency is associated with a low prevalence of coronary artery disease (Miller et al. 2003). Possibly the most useful genetic tool used thus far is the Taq1B that results in a protein with wild-type sequence but altered levels of expression (Boekholdt and Thompson 2003).

Interactions:

A study of interaction between genetic and environmental factors in the regulation of plasma HDL cholesterol concentration by determining TaqI (-) polymorphism at the CETP gene locus in 93 male alcohol drinkers and 82 control men has shown that the TaqI-B was associated with elevated HDL-C and decreased CETP activity only in nonsmoker/non-heavy drinkers (Hannuksela et al. 1994). The West of Scotland Coronary Prevention Study (WOSCOPS) was designed to investigate gene-smoking and gene-treatment interactions (Freeman et al. 2003). The

same results were reported: the association between CETP TaqIB genotype and cardiovascular risk is primarily in non-smokers.

Association between HDL-C concentration and polymorphisms at the CETP gene locus was studied in a random population-based sample of 526 Caucasian subjects (259 men, and 267 women) in order to report sex difference in the regulation of plasma HDL (Kauma et al. 1996). It was reported that TaqI-B(-) was associated with elevated HDL-C only in women and in nonsmoker men.

LIPOPROTEIN LIPASE: LPL GENE

The lipoprotein lipase (LPL), extracellularly localized, is the only extrahepatic, intravascular enzyme for the hydrolysis of TG into free fatty acids and mono- and diglycerides (Vance 2002). LPL plays a central role in triglyceride metabolism, hydrolysing circulating triglyceride-rich lipoproteins (chylomicrons and VLDL) primarily in adipose tissue and muscle capillaries (Vance 2002). The hydrolysis of TG-rich lipoproteins also releases apolipoproteins and phospholipids that are precursors of HDL (Vance 2002). An efficient LPL is associated with lower TG and LDL, but higher HDL, and is therefore potentially atheroprotective (Vance 2002).

LPL gene is localized on chromosome 8 (8p22) (Gehrisch 1999). Common mutations in the LPL gene leading to deficient LPL activity have been associated with hypertriglyceridemia and low levels of HDL, placing affected individuals at increased risk for premature CHD (Gehrisch 1999).

In the study of the nucleotide exchange -93T>G, the frequency of the -93G carriers was compared in three populations (Caucasians, South Africans, and Chinese) (Ehrenborg et al. 1997). This polymorphism is distributed very differently among various ethnic groups. The

carrier frequency in the Caucasian population was 1.7% (4/232), while the mutation was not identified in the Chinese population. This was in contrast to the South African Black population, which had a frequency for this allele of 76.4% (123/161) (Ehrenborg et al. 1997) and in Afro-Caribbeans (from another study) of 62.6% (57/91)(Hall et al. 1997). Individuals with the -93GG genotype had significantly lower triglyceride levels compared with carriers of the -93TT genotype. No significant differences were found for HDL or LDL cholesterol levels. It was also found that in the Caucasian population, the nucleotide exchange -93T>G and the amino acid exchange causing the Asp9Asn mutation are located on the same allele (-281T>G; 106G>A) and are found to be in almost complete linkage disequilibrium (Ehrenborg et al. 1997). In contrast, investigations in African-Americans discovered that in the LPL gene, -93G is the original nucleotide that it was not in linkage with the nucleotide change responsible for the Asp9Asn missense mutation (Ehrenborg et al. 1997). The majority of investigations report an association of the minor allele Asn9 with a moderately increased TG level (Fisher et al. 1997).

Several studies confirmed an association of the missense mutation Asn291Ser, caused by the nucleotide exchange 953A>G, with increased TG level (Fisher et al. 1997). In almost all case-control studies the frequency of Asn291Ser mutation carriers was higher in patient groups with elevated TGs compared with normolipidemic or healthy controls (Fisher et al. 1997).

A very high linkage disequilibrium exists among three polymorphisms: PvuII (intron 6), HindIII (intron 8), and Ser447X (Peacock et al. 1992). There is a strong association of the X447 allele with beneficially low TG and high HDL cholesterol levels (Fisher et al. 1997).

When compared in diabetic and non-diabetic Hispanics and non-Hispanic Whites from the San Luis Valley in Colorado, HindIII and PvuII polymorphisms were in strong linkage disequilibrium in both groups (Ahn et al. 1993). HindIII H(-/-) genotype was associated with the

lowest TG and the (+/+) genotype with the highest TG levels; these levels were intermediate in the (+/-) genotype. The HindIII (+/+) genotype was associated with lower levels of HDL-cholesterol in normoglycemic men.

In the meta-analysis of the effect of the Asp9Asn, Asn291Ser, and Ser447Ter substitutions in lipoprotein lipase in the heterozygous state on lipid metabolism and risk of CHD, it was found that compared with non-carriers, carriers of the Asn9 (106 G>A), and Ser291 (953 A>G) polymorphisms have an atherogenic lipoprotein profile, whereas carriers of the Stop447 polymorphism have a protective lipoprotein profile (Wittrup et al. 1999). Asp9Asn, and Asn291Ser heterozygous carriers had increased plasma triglycerides and decreased HDL cholesterol, whereas carriers of the Ser447Ter had decreased triglycerides and increased HDL when compared with non-carriers profile (Wittrup et al. 1999). The Ser447Ter substitution is believed to be more important for the observed effects because it truncates 2 amino acids, whereas the Hind III and Pvu II polymorphisms are intron variants (Wittrup et al. 1999).

Interactions:

In the Bogalusa Heart Study, a community-based sample of 902 Caucasians and 389 African-Americans aged 18 to 41 years, the carrier frequency for the LPL X447 allele was significantly higher in African-Americans than Caucasians (16% vs. 11%) (Xin et al. 2003). Interestingly, the triglyceride-lowering effect of the LPL X447 allele was enhanced further in the presence of the LIPC 514 T allele, but only among Caucasians and in the total sample. The sample size of African-Americans was relatively small and may not have adequate power to detect significant interaction effect of LPL S447X and LIP 514C>T polymorphisms on TG in African-Americans.

The effects of polymorphisms HindIII and S447X and in the apolipoprotein (apo) AI-CIII gene cluster (G75A and C1100T) on levels of fasting plasma triglycerides, apoCIII, HDL-C, and apoAI were examined in 315 healthy men and women from Iceland (Peacock et al. 1997). Non-smoking and smoking men and women were examined separately. Polymorphisms HindIII and apoAI apoCIII loci were associated with levels of triglycerides, apoCIII, HDL-C, and apoAI, but these effects are strongly modulated by smoking and were different between men and women. In those carrying the HindIII H⁺ allele, HDL levels were higher than in those carrying the H⁻ allele. Men and women smokers with the LPL H⁺ allele had higher levels of triglycerides than those with one or more H⁻ alleles. This effect was higher and significant only in the smoking women and was almost significant in the smoking men, but effects were smaller and opposite in the nonsmokers.

A study of 520 men from a representative sample used in a population study in Spain reported that there was a statistically significant interaction between LPL HindIII genotype and smoking on lipid concentrations (Senti et al. 2001). It was found that the presence of the H⁺H⁺ genotype has a harmful effect on lipid profile in an adverse environment such as smoking, and that the expenditure of more than 291 kcal/day in physical activity attenuates this effect. (Clee et al. 2001)

APOLIPOPROTEIN A1: APOA1 GENE

As the primary apolipoprotein of HDL, APO A1 serves as a cofactor for cholesterol esterification and is an important component of reverse cholesterol transport. The apo A1 gene is located at chromosome 11q13. A common variant in the APO A1 promoter (75 G>A) has been

shown to be associated with elevated HDL-cholesterol and apo A1 levels in some but not other studies (Miller et al. 2003).

Interactions:

A recent nutritional study has examined whether dietary fat modulates the association between 75 G>A polymorphism and HDL cholesterol concentrations (Ordovas et al. 2002). The investigators reported a significant gene-diet interaction associated with the APOA1 75 G-A polymorphism, but polyunsaturated fatty acids (PUFA) modulated the effects of the APOA1 G-A polymorphism on HDL-cholesterol concentrations in a gender specific manner (in women only) (Ordovas et al. 2002). In women carriers of the A allele, higher PUFA intakes were associated with higher HDL cholesterol concentrations, whereas the opposite effect was observed in G/G women (Ordovas et al. 2002). In another study, it was found that the A allele associated with elevated HDL-C and apoA-I levels in men nonsmokers (Sigurdsson et al. 1992). This effect was abolished in the men who smoked.

APOLIPOPROTEIN B: APOB GENE

APO B is a component of plasma lipoproteins, particularly LDL (Vance 2002). It mediates binding to LDL receptor (Vance 2002). Apo B exists in human plasma as two isoforms, apo B-48 and apo B-100 (Vance 2002). The Apo B-100 is the major physiological ligand for the LDL receptor (Vance 2002). Mutations occurring in the apo B gene can alter blood cholesterol levels (Humphries and Talmud 1995). Most of the mutations lower blood cholesterol levels due to the production of truncated apo B (Humphries and Talmud 1995).

Human apoB gene is located on chromosome 2p; the coding portion of the gene extends over 43 kb and contains 29 exons and 28 introns (Humphries and Talmud 1995). Several

polymorphisms in the Apo B gene have been defined (Humphries and Talmud 1995). A large meta-analysis was performed to describe the associations between the three most frequently investigated polymorphisms (XbaI, signal peptide insertion/deletion, EcoRI) in the APOB gene, lipid parameters, and the risk of CHD (Boekholdt et al. 2003).

The most widely studied of these polymorphisms is the XbaI polymorphism in exon 26, which does not result in an amino acid substitution (Boekholdt et al. 2003). The XbaI X⁺ allele was significantly associated with higher LDL-C and apoB levels (Boekholdt et al. 2003). Peculiarly, the XbaI X⁺ allele was also associated with a significantly decreased risk of CHD (OR=0.80; 95%CI: 0.66–0.96) (Boekholdt et al. 2003). XbaI site does not cause an amino acid substitution and the observed association with CHD could result from co-segregation with one or more functional polymorphisms in the APOB gene or a gene located nearby.

The apo B signal peptide contains a leucine-alanine-leucine insertion/deletion polymorphism affecting the amino acids 14-16 producing signal peptides with 24 or 27 amino acids (Boekholdt et al. 2003). Homozygosity for the signal peptide deletion (D) allele was associated with increased levels of LDL-C and apoB, and with an increased risk of CHD (OR=1.30; 95%CI: 1.08–1.58) (Boekholdt et al. 2003). The EcoRI restriction fragment length polymorphism in exon 29 is associated with an amino acid change Gln Lys 4154 (Boekholdt et al. 2003). Subjects homozygous for the rare EcoRI A allele had significantly decreased levels of total and LDL cholesterol, but unchanged risk of CHD (Boekholdt et al. 2003).

APOLIPOPROTEIN C III: APOC3 GENE

One of the most important and reliable markers of TG rich lipoproteins levels is apolipoprotein C-III (APOC-III) (Boekholdt et al. 2003). APOC-III is a 79-amino-acid protein synthesized by

liver and intestine, which is an essential constituent of circulating particles rich in triacylglycerol (chylomicrons and VLDLs) (Surguchov et al. 1996). APOC-III inhibits the hydrolysis of TG-rich particles by the LPL and their hepatic uptake mediated by APOE (Surguchov et al. 1996). Therefore, the overexpression of the APOC3 gene on chromosome 11q results in hypertriglyceridemia and hypercholesterolemia (Surguchov et al. 1996).

Seven polymorphisms in the APOC3 gene are in strong linkage disequilibrium with one another: T-455C, C-482T, T-625 deletion, G-630A, C-641A, C1100T in exon 3 and C3238G gene variant Sst I (also known as S1/S2) (Olivieri et al. 2002). SstI is one of the most extensively studied, and has consistently been reported to be associated with hypertriglyceridemia (Olivieri et al. 2002). T-455C is in linkage disequilibrium with the SstI polymorphism (Surguchov et al. 1996).

In a recent case-control study of APOC3 gene polymorphisms and risk of CHD in 800 patients (549 of whom had angiographically documented coronary atherosclerosis, and 251 who had normal coronary arteriograms), the investigators assessed three polymorphisms: T-455C in the insulin-responsive element (IRE) promoter region, C1100T in exon 3, and SstI polymorphic site (S1/S2) in the 3' untranslated region (Olivieri et al. 2002). Each variant influenced triglyceride levels, but only the 455C (in CC homozygotes) and S2 alleles influenced APOC-III levels (Olivieri et al. 2002). In CHD patients, 18.6% were homozygous for the -455C variant compared with only 9.2% in CHD-free group ($P < 0.001$). The TG-raising effect associated with C1100T variant, also observed by other investigators, remains to some extent unexplained, because the base change in exon 3 does not code for an amino acid change (Olivieri et al. 2002). Therefore, it was concluded that the polymorphism may be in linkage disequilibrium with another, unrecognized functional variant.

Interactions:

A study of interaction between the APOC3 gene promoter polymorphisms, saturated fat intake and plasma lipoproteins has shown that a polymorphism -455C modulates the association between saturated fat intake and plasma lipoprotein concentrations. Significant gene–diet interactions were found for plasma total and LDL cholesterol (Brown et al. 2003).

A study of 59 volunteers examined the interaction between smoking and the SstI (Perez-Martinez et al. 2001). The smokers carrying the S1S1 genotype were not influenced by any of the diets, but when the carriers of the S2 allele changed their diet from the diet rich in saturated fatty acids to a diet rich in olive oil or carbohydrates, the atherogenic ratio decreased. It was concluded that smoking interacts with the SstI polymorphism and determines the level of lipid response to dietary changes.

In a large cohort of healthy men (n=2745), Second Northwick Park Heart Study (NPHSII), the effect on TG levels of the SstI polymorphic site at 3238, was strongly influenced by smoking (Waterworth et al. 2000).

APOLIPOPROTEIN E: APOE GENE

Apolipoprotein E (APOE) is a protein constituent of chylomicrons, VLDL, HDL and VLDL remnants (Vance 2002). APOE is a constituent of liver-derived VLDL and serves in the transport and redistribution of lipids among various tissues. The apo E gene is located in chromosome 19q (19q13.2) (Mahley and Rall 2000). The structural gene is polymorphic with three common alleles, E2, E3, and E4, producing three isoforms of the protein, E2, E3, and E4, which are associated with variations in the blood lipid concentrations (Mahley and Rall 2000). The three common human isoforms, apoE2, apoE3, and apoE4, differ in amino acid sequence at positions

112 and 158 in the molecule and have very different metabolic properties and effects on disease (Mahley and Rall 2000). ApoE3 (Cys-112, Arg-158) binds normally to LDL receptors and is associated with normal lipid metabolism, whereas apoE2 (Arg158Cys i.e., Cys-112, Cys-158) binds defectively to LDL receptors and, under certain circumstances, is associated with the genetic disorder type III hyperlipoproteinemia (Mahley and Rall 2000). ApoE4 (Cys112Arg i.e., Arg-112, Arg-158) binds normally to LDL receptors but is associated with elevated cholesterol levels and increased risk for cardiovascular disease. APO E polymorphism modifies plasma lipids partly by affecting the efficiency of cholesterol absorption (Mahley and Rall 2000).

It was shown that LDL levels are 10 to 20 mg/dl lower in E3/E2 compared with E3/E3, accounting for approximately 10% of the population variance in LDL-C levels (Breslow 2000).

Epsilon 3 allele is the most common, and almost 60% of North Americans are homozygous for this genetic variant (Wilson et al. 1996).

The Coronary Artery Risk Development in Young Adults (CARDIA) Study studied an association of apolipoprotein E phenotype with plasma lipoproteins in African-American and white young adults (Howard et al. 1998). 3,485 African-American and Caucasian men and women between the ages of 25 and 37 years participated in the study. African-American men and women had significantly higher frequencies of Epsilon 2 and Epsilon 4 alleles. However, APOE phenotype was associated similarly with differences in lipoproteins distributions in both ethnic groups.

In a meta-analysis of 14 published observational studies in comparison with E3, carriers of E4/ E4 allele have been associated with a 40% increased risk of developing CHD when compared to carriers of E2/E3 (Wilson et al. 1996). The investigators concluded that E4 allele is

associated with higher risk for atherosclerotic disease and the results were similar in men and women.

Another meta-analysis of ApoE gene and TG levels in 45 population samples, has reported that carriers of E2 or E4 alleles had significantly higher TG levels (Dallongeville et al. 1992).

Interactions:

Individuals with the APOE4 allele are more responsive to diet than those carrying the APOE3 or APOE2 alleles (Ordovas et al. 1995). It is possible that this effect is more evident when the total amount of dietary fat is changed (Ordovas, Lopez-Miranda et al. 1995). The APOE2 allele could modulate the effect of habitual saturated fat on VLDL cholesterol and HDL cholesterol in a population with an average habitual total fat intake of less than 30% (Ordovas, Lopez-Miranda et al. 1995).

In a cross-sectional study of 1,029 healthy, Spanish people, the investigators examined the associations between the LPL and APOC3 gene loci (HindIII, S447X, and APOC3-SstI) and plasma lipid levels and their interaction with APOE polymorphisms and smoking (Corella et al. 2002). There was a significant interaction between APOE and LPL variants and HDL-C levels in both genders ($P < 0.05$). This effect was modulated by smoking (interaction HindIII-APOE-smoking, $P = 0.019$), indicating that smoking abolishes the increase in HDL-C levels observed in E4/H⁻ subjects.

A cross-sectional study in a Mediterranean Spanish population consisting of 396 men and 513 women aged 18 to 66 years investigated APOE gene--environment interaction effects on plasma lipid concentrations (Dallongeville et al. 1992). A statistically significant interaction effect between the APOE polymorphisms and physical activity in determining HDL cholesterol

concentrations was observed in men. They have found an effect of the E4 allele on TG levels, but the increasing effect of E2 allele was not detected. This conflicts with the previous studies where both E2 and E4 were associated with TG levels and strongly suggests that the link between APOE polymorphism and TG concentrations also involves interactions with environmental factors.

Significant interactions were also found between Apo E alleles and BMI for total and LDL cholesterol, where the increase in total and LDL cholesterol between E2 and E4 allele carriers was stronger among obese than nonobese subjects (Marques-Vidal et al. 2003). The same study reported significant interactions between the E2 allele and alcohol consumption for apo B levels.

LECITHIN-CHOLESTEROL ACYLTRANSFERASE: LCAT GENE

Any free cholesterol picked up can be converted to cholesterol esters by the enzyme lecithin-cholesterol acyltransferase (LCAT), which is associated with HDL and activated by apoA-I (Vance 2002). The human LCAT enzyme is a monomeric glycoprotein that catalyzes the transfer of an acyl group of phosphatidylcholine to esterify free cholesterol (Vance 2002). Esterification occurs preferentially on the surface of HDL with APOA-1 serving as co-factor (Vance 2002). Cholesteryl ester is incorporated in the core of HDL, which is then either directly incorporated by steroidogenic tissues or transferred to LDL for hepatobiliary excretion (Vance 2002).

LCAT deficiency is either associated with reduced esterification in plasma (classic LCAT deficiency) or in HDL (fish eye disease) (Miettinen et al. 1998). Absence of LCAT leads to accumulation of lecithin and free cholesterol as well as production of an abnormal lipoprotein (Miettinen, Gylling et al. 1998). At least 30 different mutations have now been reported

(Kuivenhoven et al. 1997). However, premature CHD is uncommon, even in the presence of cardiovascular risk factors (Winder et al. 1999).

LCAT gene is located in chromosome 16q. A mutation of LCAT gene changing glycine to arginine in the LCAT protein has been found in a Finish proband and has been associated with low plasma HDL levels (Miettinen et al. 1998). The prevalence of the mutation called LCAT_{fin} is 5 % among Finish men with HDL below 0.7 mmol/l (27 mg/dl) (Miettinen et al. 1998).

ATP CASSETTE-BINDING TRANSPORTER: ABCA1 GENE

The adenosine triphosphate (ATP)-binding cassette (ABC) represent the largest family of transmembrane proteins (Vance 2002). These proteins bind ATP and use the energy to drive the transport of various molecules (ions, vitamins, lipids, and proteins) across all cell membranes (Vance 2002). ABC-1 has been shown to be involved in apolipoprotein-mediated lipid efflux from peripheral cells (Vance 2002). ABCA1 gene on the chromosomal location 9q22-q31 has been found to be the locus for the extremely rare Tangier disease characterized by a near absence of plasma HDL-C, increased triglycerides and also, in some families, for familial hypoalphalipoproteinemia (FHA), a disorder characterized by moderately low HDL-cholesterol and premature CHD (Kakko et al. 2003). In recent population study of FHA subjects (n = 515) no significant association between ABCA1 polymorphisms and low HDL-cholesterol was found (Kakko, Kelloniemi et al. 2003). Another study reported that ABCA1 gene contains polymorphisms that were associated with HDL-C levels (Wang et al. 2000). It was reported that I/M823 (nucleotide 2589A/G) homozygotes (M823/M823 homozygotes had a significantly higher plasma HDL cholesterol compared with subjects with the other genotypes (Wang et al. 2000). ABCA1 polymorphism R219K (Arg219Lys), was associated with decreased TG,

increased HDL-C and, most importantly, a decreased progression of atherosclerosis and a reduced risk of coronary events, suggesting that common genetic variants in ABCA1 may influence these clinical outcomes in the general population (Clee et al. 2001).

In the study of frequency and phenotypic effect of R219K variant in a community-based sample of 887 Caucasians and 390 African-American young adults aged 20 to 38 years, the frequency of the variant allele (K219) was higher in African-Americans than in Caucasians, with carriers (KK+RK) representing 83.8% of African-Americans versus 44.2% of Caucasians (Srinivasan et al. 2003). These results indicate that the frequency of K219 allele differs markedly between African-Americans and Caucasians. Levels of HDL cholesterol and triglycerides were not significantly different between carriers and non-carriers in Caucasians or African-Americans, after adjusting for age, BMI, and sex. Additionally, both groups of African-Americans (carriers and non-carriers) had significantly higher HDL cholesterol and lower triglyceride levels than Caucasians, independent of age, sex, and BMI. Significant interaction between K219 and age on HDL cholesterol ($P<.001$) and K219 and BMI on triglycerides ($P=.029$) was found only in Caucasians. This can be in part explained by the fact that African-Americans have significantly higher LPL activity and lower HL activity, key enzymes associated with metabolism and plasma levels of triglyceride-rich lipoproteins and HDL. Therefore, the phenotypic effects of the R219K may not be expressed in this ethnic group.

SCAVENGER RECEPTOR CLASS B TYPE I: SR-BI GENE

The HDL receptor, scavenger receptor class B, type I (SR-BI), a functional receptor, is a member of the scavenger receptor class B family, whose members also include CD36 and LIMPII (Cao et al. 1997). SR-BI plays a part in the selective uptake of cholesterol ester. A number of ligands

bind with high affinity to SR-BI, including LDL, HDL, VLDL, modified LDL, and apolipoproteins (Cao, Garcia et al. 1997).

The SR-BI gene has been localized to chromosome 12 (12q24.2), it spans 75 kilobase pairs, and contains 13 exons (Cao, Garcia et al. 1997). SR-BI is highly expressed in liver and steroidogenic tissue, and has been localized in atherosclerotic plaques (predominantly in macrophages) (Hirano et al. 1999). To determine its role in humans, Acton et al. have characterized the human SR-BI gene and investigated its genetic variation in 489 European Caucasian men and women (Acton et al. 1999). The investigators have found that the exon 1 variant (4G>A) was significantly associated with higher HDL and lower LDL levels in men but not in women. The exon 8 variant (1050C > T) was associated with lower LDL levels in women but not in men, and the intron 5 variant showed an association with body mass index in women. The authors concluded that SR-BI might influence LDL and HDL levels.

Interactions:

A study was carried to determine whether the exon 1 variant (4G>A) at the SRB-I gene is associated with the lipid response to the content and quality of dietary fat in healthy subjects (65 homozygous for allele 1 (1/1) and 32 heterozygous for allele 2 (1/2)), it was found that carriers of the minority allele, 1/2, are more prone to the presence of saturated fatty acids in the diet because of a greater increase in LDL cholesterol (Perez-Martinez et al. 2003).

Among 2463 nondiabetic (49% men) and 187 diabetic (64% men) participants in the Framingham Study, consistent association between the exon 8 polymorphism and HDL-C concentration and particle size was shown (Osgood et al. 2003). However, only between exon 1 genotypes and type 2 diabetes was found a statistically significant interaction, indicating that diabetic subjects with the less common allele (allele A) have lower lipid concentrations.

A recent study of SR-BI Polymorphisms associated with HDL cholesterol levels in three populations (Finland, Sweden and Israel) with type 2 diabetes, four common polymorphisms were examined: an exon 1 missense (EX1), exon 8 silent (EX8), intron 5 (IVS5) and intron 10 (IVS10) variants (McCarthy et al. 2003). One specific pattern of genotypes (IVS5_T and EX8_C alleles), and absence of the IVS10_G allele, was consistently associated with the lowest mean levels of HDL-C in women from all three populations. These same associations were not found in men. It is possible that polymorphic variation of the SR-BI gene may influence HDL-C levels in a gender-specific manner.

ENDOTHELIAL LIPASE: LIPG GENE

Nascent triglyceride-rich lipoproteins secreted from the liver and intestine (chylomicrons and VLDL) are delipidated first by the LPL, which has a tendency for triglycerides, and then by hepatic lipase (HL) which hydrolyzes both triglycerides and phospholipids (Cohen 2003). Individuals lacking LPL accumulate very large, triglyceride-rich lipoproteins, whereas individuals lacking HL accumulate partially-delipidated lipoproteins of intermediate density (Cohen 2003). Recent studies indicate that HDL metabolism may also include both a lipolytic step, in which the main lipid component is hydrolyzed by endothelial lipase, and a receptor-mediated step, in which cholesterol is removed by careful uptake through the SR-BI (Cohen 2003).

Endothelial lipase (EL) is a recently discovered new member of the triglyceride lipase family. A group of researchers cloned endothelial lipase as a transcript that was upregulated in the human macrophage-like cell line THP-1 exposed to oxidized LDL (Jaye et al. 1999). The investigators inhibited endothelial lipase in mice by administering a polyclonal anti-murine

endothelial lipase antibody, an approach that has been used previously to assess the physiological roles of hepatic lipase and lipoprotein lipase. Inhibition of endothelial lipase activity brought out more changes in plasma lipid levels in hepatic lipase-deficient mice. Adenovirus-mediated overexpression of EL in mice has reduced levels of HDL and Apo A-I in plasma. These data were the first to suggest that EL may play a significant role in HDL catabolism. However, another study by McCoy et al. has shown that EL is inactive in vitro in presence of serum (McCoy et al. 2002).

Both LPL and HL have significant triglyceride lipase activity, but they differ in their phospholipase activity, since HL has considerable, and LPL has very low phospholipase activity (Rader and Jaye 2000). On the other hand, EL has low triglyceride lipase activity, but significant phospholipase activity (Rader and Jaye 2000). EL enzyme was found to be 45% molecularly homologous to LPL, 40% to HL, and 27% to pancreatic lipase [PL] (Jaye et al. 1999; Rader and Jaye 2000; McCoy et al. 2002). Since 1999, there have been number of studies showing that the EL enzyme is involved in HDL-C metabolism (Cohen et al. 2003; Jaye et al. 1999; McCoy et al. 2002; Rader et al. 2000; Choi SY et al. 2002; deLemos et al. 2002; Ma et al. 2003; Halverstadt et al. 2003).

LIPG gene is located on chromosome 18 (18q21.1). DeLemos et al. were among the first to hypothesize that functional polymorphisms and mutations in EL (LIPG) gene might contribute to the elevated levels of HDL-C (deLemos et al. 2002). 17 polymorphic sites in LIPG were reported. The frequencies of 6 potentially functional variants, 4 of which cause amino acid substitutions (Gly26Ser, Thr111Ile, Thr298Ser, and Asn396Ser,) and 2 of which occur in the promoter (-303A/C and -410C/G), were analyzed in 176 African-American controls, 165 Caucasian controls, and 123 white participants with HDL-C levels greater than the 90th percentile. The allele frequency of Thr111Ile (584 C>T) polymorphism was the highest of all

variants with an allele frequency of 10.3% in African-Americans, 31.2% in Caucasian controls, and 32.6% in participants with high HDL-C. It was also reported that Gly26Ser, Thr298Ser, and -303A/C were found only in African-American controls and in Caucasians with high HDL-C, but were not present in the control Caucasian group (deLemos et al. 2002). There was no significant difference in allele frequencies of six polymorphisms in the LIPG gene between Caucasians with normal and those with high HDL-C.

In another study by Ma et al. it was found that there is a significant association between a Thr111Ile (584C/T) polymorphism in the LIPG gene and HDL cholesterol in a population of 372 individuals and suggested that EL is a major determinant of HDL concentration (Ma et al. 2003). Patients with the TT allele had a 14% higher mean HDL-c compared with those with the CC allele. The participants, who were from the Lipoprotein and Coronary Atherosclerosis Study (LCAS), were not selected on the basis of HDL-c and they included only 7% African-Americans in contrast to the study of deLemos et al. Furthermore, participants were between 35 and 75 years old who had LDL cholesterol levels of 115-190 mg/dl and ≥ 1 coronary lesion causing 30-75% diameter stenosis.

As we mentioned previously, the study by deLemos has found no significant difference in allele frequencies of polymorphisms in the LIPG gene between Caucasians with normal and those with high HDL-C but the association of polymorphism Thr111Ile (584 C>T) with HDL subfractions or with individuals with HDL levels within the normal range was not assessed (deLemos, Wolfe et al. 2002). For that reason, the study by Halverstadt et al. proposed the hypothesis that the LIPG Thr111Ile gene polymorphism is associated with HDL-C and its subfractions at the baseline and with the response of HDL-C and its subfractions to exercise training (Halverstadt et al. 2003). The investigators have found an association of the LIPG gene

Thr111Ile polymorphism with initial levels of NMR measured small HDL subclasses with the CT/TT group having 30% and 27% lower initial levels of HDL₂ and HDL_{1,2} than the CC group, respectively. With exercise training, HDL-C levels increased twice as much and large HDL₃ levels increased almost 2-fold greater in the carriers of CC allele compared to the carriers of CT/TT allele. This data suggest that the carriers of TT/CT for Thr111Ile polymorphism may have a more protective lipoprotein profile than the carriers of CC allele and that the effect of LIPG on HDL subclass is modified by exercise training. (Halverstadt et al. 2003).

HEPATIC LIPASE: LIPC GENE

The majority of hepatic lipase (HL) is synthesized and secreted by the liver (Bensadoun and Berryman 1996). HL has a dual role in lipid metabolism; it is involved in chylomicron remnant catabolism and also in the metabolism of HDL (Bensadoun and Berryman 1996; Cohen et al. 1999). HL is a key enzyme involved in lipoprotein metabolism. Its catalytic activity contributes to the hydrolysis of triglycerides and phospholipids in LDL and HDL particles (Bensadoun and Berryman 1996). HL participates also as a ligand in promoting the hepatic uptake of lipoproteins, a process that may lead to their increased uptake by hepatocytes (Bensadoun and Berryman 1996; Cohen et al. 1999). It also influences HDL interconversion, a process essential for cholesterol metabolism (Bensadoun and Berryman 1996; Cohen et al. 1999). Both the catalytic and ligand activities of HL play a major role in promoting the scavenger receptor B1-mediated uptake of HDL-cholesteryl ester. Therefore, HL may contribute to the process of reverse cholesterol transport (Jansen et al. 2002).

Studies on HL enzyme activity provided evidence that hepatic lipase plays a role in human lipoprotein metabolism. Post-heparin plasma activity of hepatic lipase has consistently

been found to be inversely related to plasma HDL-cholesterol concentrations, especially to the HDL₂ subfraction (Cohen, Vega et al. 1999). These observations lead to the hypothesis that HL plays an important role in the metabolism of HDL, and that variation in hepatic lipase activity contributes to intra- and inter-individual differences in plasma HDL-C concentrations (Cohen, Vega et al. 1999).

In cross-sectional studies, high HL activity was associated with an increase in small, dense LDL particles and a decrease in HDL₂ (Zambon et al. 1993; Watson et al. 1994). In another study by Zambon et al. it was reported that in men with high triglycerides, CHD and family history of CHD, who participated in the Familial Atherosclerosis Treatment Study (FATS), a decrease in HL activity was associated with large, buoyant LDL particles correlated with a decrease in coronary stenosis (Zambon et al. 1999) (LDL buoyancy was strongly correlated with decreased HL activity).

HL also affects the metabolism of apoB-100 containing lipoproteins and plays an important role in lipoprotein metabolism independent of its enzymic activity (Zambon et al. 2000). Inactive HL protein appears to affect VLDL and IDL particle concentration, while HL enzymic activity seems to influence VLDL, IDL, LDL and HDL triacylglycerol content and physical properties (Zambon et al. 2000). Recently, HL was found to be present and produced in human macrophages, a finding that may help to explain the role of HL in atherogenesis (Gonzalez-Navarro et al. 2002). HL is synthesized by macrophages as well as liver, and macrophage-synthesized HL may also contribute to foam cell formation and promoting atherosclerosis by enhancing monocyte recruitment and preservation in the arterial wall and facilitating the retention of VLDL remnants, IDL and small, dense LDL in the subendothelial space (Gonzalez-Navarro et al. 2002).

By modulating the metabolism of apo B-100-containing lipoproteins, HL may have pro- as well as anti-atherogenic effects (Jansen et al. 2002). In the presence of hypertriglyceridaemia or an increased LDL concentration, the pro-atherogenic effect of high HL activity (the formation of small, dense LDL) may overcome the potential beneficial effects of high HL levels on VLDL remnant and IDL metabolism (Jansen et al. 2002).

The human hepatic lipase gene, LIPC, is located on chromosome 15 (15q21.3). It comprises 9 exons and 8 introns, and spans a length of more than 60 kb. It encodes a protein of 449 amino acids with a signal peptide of 23 amino acids (Vega et al. 1998). Six polymorphisms have been detected in relation to the human hepatic lipase gene in complete linkage disequilibrium (G250A, C514T, T710C, A763G, A1075C) (Guerra et al. 1997; Jansen et al. 1997; Nie et al. 1998). It has been reported that the C>T base pair substitution at the 514 (T allele) is associated with increased plasma HDL-C, large HDL₂ and large buoyant LDL particles (Jansen et al. 1997). The frequency of the T allele at -514 position is very different among ethnic groups, ranging 18-27% in Caucasians (Jansen et al. 1997; Zambon et al. 1998; Hokanson et al. 2003; Carr et al. 2004), 44-52 % in African-Americans (Vega et al. 1998; Juo et al. 2001; Carr et al. 2004), 47 % in US Hispanics (Hokanson et al. 2003), 44% in Koreans (Hong et al. 2000) and 50% in Japanese (Inazu et al. 2001; Carr et al. 2004).

The study of 1314 male and 1353 female Framingham Offspring Study participants reported the association between the LIPC 514 C>T polymorphism and HDL subclasses (Couture et al. 2000). In men and women, carriers of the -514T allele had higher HDL-C and Apo AI concentrations compared with non-carriers. The higher HDL-C levels associated with the -514T allele was due to an increase in the HDL₂ subfraction (large HDL), and this association was stronger in women compared with men. No relationship between the LIPC polymorphism at

position -514 and the LDL particle size distribution was reported after adjustment for familial relationships, age, body mass index, smoking, alcohol intake, use of beta-blockers, apoE genotype, and menopausal status and estrogen therapy in women (Couture et al. 2000). Lipoprotein subclasses were measured by NMR spectroscopy and gradient gel electrophoresis.

Another study has also used two independent analytical methods (NMR spectroscopy and gradient gel electrophoresis) for measuring HDL subclasses (Grundy et al. 1999). HDL subclass distributions were compared in 11 homozygotes for the -514C allele of HL and in 6 homozygotes for the -514T allele. The terms given to the NMR-derived HDL subclasses and their average diameters were H1, H2, H3, H4, and H5. The size ranges of these subclasses matched those measured by gradient gel electrophoresis (HDL_{3c}, HDL_{3b}, HDL_{3a}, HDL_{2a}, and HDL_{2b}). Both methods specified that HDL_{2b} was significantly higher and HDL_{3a} was significantly lower in -514T homozygotes than in -514C homozygotes. No differences were noted in the other HDL fractions. Although the sample size was small, this was the first study to examine HL polymorphisms and NMR-derived HDL subclasses.

In the study of three ethnic groups the less common A allele at position 250 was compared between 68 healthy US-Whites, 60 unrelated dyslipidemic men with elevated apo B levels, 31 healthy Japanese-Americans and 56 African-Americans (Zambon et al. 1998). The less common 250 A allele (frequency was 0.21 and 0.25 in normal and CHD subjects, respectively) was associated with lower HL activity and buoyant LDL particles ($P \leq 0.01$) in both healthy and CHD groups. The A allele was associated with higher HDL₂ in CHD patients. The LIPC A allele was more frequent in Japanese-Americans (0.47) and African-Americans (0.45) than in whites (0.21).

The LIPC genotypes were also determined in two groups of African Americans and Caucasians in NYC (43 African Americans and 45 Caucasians aged 20 to 40 years) and in

Dallas (54 African Americans and 60 Caucasians) (Zambon et al. 1998). In the first group, the frequency of the -514T allele was significantly higher in African Americans (0.52) than in white Americans (0.17) and almost the same results were observed among participants from Dallas (0.53 in African Americans and 0.18 in Caucasians). The LIPC -514 T allele was associated with low hepatic lipase activity in both populations, and was 3-fold more common among African Americans than Caucasians.

137 subjects with CHD and 124 age-matched controls were recruited from Seoul province, Korea and genotyped for LIPC (Hong et al. 2000). The frequency of -250 promoter A allele was 0.63 and allele frequency of the -514 T allele was 0.44.

A study of 578 African American men from the Coronary Artery Risk Development in Young Adults study (CARDIA) reported that the frequency of the 250A allele was 0.52 (Juo et al. 2001). The study confirmed that the genetic effect on HDL-C levels is due to an effect on HDL₂ but not on HDL₃, which has been also suggested by other studies (Zambon et al. 1998; Couture et al. 2000).

In 299 Japanese a HL promoter polymorphism of -514C/T explained a considerable variance of HDL-C (2.9%) (Inazu et al. 2001). Carriers of -514C allele reported to have high HL activity, had significant effects on decreasing HDL-C levels, whereas -514T allele carriers had a weak effect on increasing HDL-C levels. The frequency of the -514T allele was 0.50. It was concluded that the higher frequency of the HL -514T allele, along with CETP gene mutations, could explain about 9% of phenotypic variability of HDL-C.

In some studies -514C>T is described as -480C>T polymorphism. Among 395 Finnish men, 480 T/T homozygotes did not differ in HDL levels when compared with other genotypes (Tahvanainen et al. 1998). The same was concluded in the study in three Canadian populations

(657 Alberta Hutterites, 328 Ontario Oji-Cree and 210 Keewatin Inuit) (Hegele et al. 1999) and in the study of 823 Chinese (Fang and Liu 2002). The lack of association between the T allele and plasma HDL is thought to be due to unspecified environmental or genetic factors (Hegele et al. 1999).

The most recent study by Carr et al. examined the LIPC allele frequencies in Japanese-American (N=84), African-American (N=94) and US Caucasian (N=110) men and women (Carr et al. 2003). Hepatic lipase activity was associated with LIPC polymorphisms in all three groups. While the Caucasian men had lower HDL-C and HDL3 than the Japanese-American and African-American men, there were no differences in HDL2. Among men, -514T allele frequency was 0.24 in Caucasians, 0.51 in Japanese-Americans and 0.51 in African-Americans.

Forty-nine Caucasian middle-aged men with dyslipidemia and established CHD who were undergoing intensive lipid-lowering therapy were studied in order to examine if CHD patients will respond differently to therapeutic intervention, depending on the HL gene promoter polymorphism (Zambon et al. 2001). Homozygous CC patients experienced a greater decrease in HL activity and a greater increase in LDL buoyancy with lipid-lowering therapy than both homozygous and heterozygous carriers of the T allele. It was concluded that the HL gene promoter polymorphism is responsible for a different lipoprotein and angiographic response to lipid-lowering drugs.

Interactions:

The LIPC -514 C>T SNP has been found to be affected by various environmental factors. Described interactions could potentially explain the controversial observation by some investigators on the increased CHD risk in 514 T allele carriers.

As the amount of visceral adiposity increases, HL activity increases until an apparent maximum is reached in both sexes. A study of 57 premenopausal women reported that the relationship between central obesity and HL activity is modulated by the LIPC promoter polymorphism, such that the presence of the 250 A allele seems to attenuate the increase in HL activity with high levels of intra-abdominal fat (Carr et al. 1999). This study indicates a potential genetic susceptibility to increased atherogenic risk factors in the presence of central obesity.

In the Framingham Study, the T allele was correlated with higher HDL cholesterol concentrations only in individuals who usually consume a low-fat diet (Ordovas et al. 2002). In contrast, the TT genotype was associated with lower HDL cholesterol levels in individuals who usually consume a high-fat diet. This gene-diet interaction was observed for saturated and monounsaturated fat, but not for polyunsaturated fat.

The same gene-nutrient interaction was examined in a Singapore National Health Survey of representative sample of 1324 Chinese, 471 Malays and 375 Asian Indians (Tai et al. 2003). The T allele was associated with higher plasma HDL-C and higher TG. A highly significant interaction between polymorphism and fat intake in determining TG concentration and the HDL-C/TG ratio was reported in the overall sample. However, for HDL-C concentration, the gene-diet interaction was significant only in subjects of Indian origin. These results indicate that the association of -514C>T polymorphism with plasma lipids according to dietary intake depends on ethnic background.

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