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Ethnic differences in the association between lipid metabolism genes and lipid levels in black and white South African women

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ABSTRACT

Objective: Dyslipidaemia can lead to the development of atherosclerosis and cardiovascular disease (CVD), however its prevalence has been shown to differ between ethnic groups in South Africa (SA). Therefore the aim of this study was to investigate ethnic differences in the association between serum lipid levels and polymorphisms within genes involved in lipid metabolism in black and white SA women.
Methods: In a convenient sample of 234 white and 209 black SA women of Xhosa ancestry, body composition (DXA) and fasting serum lipids were measured. Participants were genotyped for the cholesteryl ester transfer protein (*CETP*, rs708272, B1/B2), lipoprotein lipase (*LPL*, rs328, S/X), hepatic lipase (*LIPC*, rs1800588, C/T) and proprotein convertase subtilisin/kexin type 9 (*PCSK9*, rs28362286, C/X) polymorphisms.
Results: Compared to white women, black women had lower concentrations of serum total cholesterol (TC, $P < 0.001$), low density lipoprotein cholesterol (LDL-C, $P < 0.001$), high density lipoprotein cholesterol (HDL-C, $P < 0.001$) and triglycerides (TG, $P < 0.001$). There were significant differences in the genotype and allele frequency distributions between black and white women for the *LPL* S/X ($P < 0.001$), *PCSK9* C679X ($P = 0.002$) and *LIPC* 514C/T ($P < 0.001$) polymorphisms. In black women only, there were genotype effects on serum lipid levels. Specifically, women with the *LPL* SX genotype had lower TC and LDL-C and higher HDL-C concentrations than those with the SS genotype and women with the *CETP* B2 allele had lower LDL-C concentrations than those with the B1B1 genotype.
Conclusion: Polymorphisms within the *LPL* and *CETP* genes were associated with a more protective lipid profile in black, but not white SA women. This supports the hypothesis that the more favorable lipid profile of black compared to white SA women is associated with polymorphisms in lipid metabolism genes, specifically the *LPL* and *CETP* genes.

1. Introduction

Obesity has been associated with dyslipidaemia, a condition which is characterized by increased total cholesterol (TC), LDL-cholesterol (LDL-C) and triglyceride (TG) concentrations and decreased HDL-cholesterol (HDL-C) concentrations [1]. Dyslipidaemia is influenced by visceral adipose tissue (VAT) accumulation [2] and insulin resistance [1], amongst other factors, and can lead to the development of atherosclerosis and cardiovascular disease (CVD) [2].

Studies in both the United States of America (USA) and South Africa (SA) have shown that black women exhibit lower TG, TC and LDL-C cholesterol levels than their white counterparts [3–5]. However in the USA, it has been reported that HDL-C levels are higher in black compared to white women [4,5], whereas in SA, HDL-C levels are similar or lower in black compared to white women [3,6,7]. These observations could explain why black SA women have a lower cholesterol-attributed mortality rate (47 in 100 000) compared to white women (152 in 100 000) [8]. This more favorable lipid profile may also be associated with the lower VAT observed in black compared to white women in SA and the USA [4,6]. Despite having lower VAT, black SA women are more insulin resistant than white women. Furthermore, insulin resistance does not correlate with serum lipids in black SA women, unlike white

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women [5,6]. Therefore genetic polymorphisms together with other lifestyle factors may play a role in the more favorable lipid profiles in the black population.

Single nucleotide polymorphisms (SNPs) within the cholesteryl ester transfer protein (*CETP)*, lipoprotein lipase (*LPL)*, hepatic lipase (*LIPC)* and proprotein convertase subtilisin/kexin type 9 (*PCSK9)* genes have been associated with more favorable lipid levels in predominantly American populations [9–13] and a few African populations [14,15]. Specifically, the X allele of the functional *LPL* S/X polymorphism (rs328) is associated with increased lipolytic activity, lower TG and higher HDL-C levels [11,16–19]. The B2 allele of the functional *CETP* Taq 1B (B1/B2) polymorphism (rs708272) is associated with an increase in HDL-C levels [11,20,21]. The X allele of the *PCSK9* C679X polymorphism (rs28362286) is a loss of function mutation associated with reductions in LDL-C levels [13,14,22]. The T allele of the *LIPC* 514C/T polymorphism (rs1800588) has been shown to be correlated with reduced LIPC activity and increased HDL-C concentrations [11,23,24]. A few of these studies have shown ethnic differences in the associations between these gene polymorphisms and lipid levels [9,11–14,25]. However, there are limited studies in Southern African populations, and few that have examined ethnic differences in genotype frequencies of these genes, as well as the ethnic-specific relationships between these polymorphisms and the serum lipid profile.

We therefore hypothesize that ethnic differences in the frequency distribution of polymorphisms within genes involved in lipid metabolism are associated with the differences in serum lipid profiles observed in black and white SA women. Therefore the aim of this study is to investigate the ethnic-specific associations between serum lipid levels (TC, TG, HDL-C, LDL-C) and the *LPL* (S/X), *CETP* (B1/B2), *PCSK9* (C/X) and *LIPC* (C/T) polymorphisms in black and white SA women.

2. Methods

2.1. Subjects

The study participants represent a sub-sample of women reported in a previous study [26], and comprised of a convenience sample of 234 self-reported white SA women and 209 self-reported black SA women of Xhosa ancestry. The inclusion criteria for the study were: 1) 18–45 years of age; 2) not taking chronic medication for any metabolic disorder or infectious diseases; 3) premenopausal (self-reported); 4) not pregnant or lactating; and 4) either of white SA ancestry (both parents born in SA), or black of Xhosa ancestry (both parents being of Xhosa ancestry and born in SA). The study was approved by the Health Sciences Human Research Ethics Committee of the University of Cape Town and written consent was obtained from all subjects prior to initiation of the study. Only women were included because of the high prevalence of overweight and obesity seen in both black and white SA women [27] and because of the differences in serum lipids between black and white women, which have previously been described [6].

2.2. Testing procedures

2.2.1. Body composition assessment

Laboratory testing was conducted following an overnight fast (10–12 h). Basic anthropometric measurements, which included height, weight, waist circumference (at the levels of the umbilicus), and hip circumference (at the largest gluteal area) were taken. Body composition, including fat mass was measured using dual-energy X-ray absorptiometry (Hologic QDR 4500 Discovery-W dual-energy X-ray absorptiometer, Discovery®, software version 12.7.3.7 Hologic Bedford, MD). Abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) areas were measured using computed tomography (Xpress helical scanner; Toshiba, Tokyo, Japan) at the level of L4–L5 lumbar vertebrae.

2.2.2. Biochemical analysis

Fasting venous blood samples were taken from the antecubital vein for the determination of glucose, insulin, TC, HDL-C, LDL-C, and TG levels, as well as DNA extraction. Plasma glucose concentrations were measured via the glucose oxidase method (YSI 2300 STAT Plus; YSI, YellowSprings, OH) and serum insulin via immunochemiluminometric assays using the ADVIA centaur (Bayer Diagnostics, Tarrytown, NY, USA). Serum lipid concentrations were measured via enzymatic colorimetric assays using the Roche Modular autoanalyzer (Roche diagnostics GmbH, Sandhofler Strasse 116, D-68305, Mannheim, Germany). The LDL cholesterol concentrations were determined using the Friedewald formula [28]. The use of the Friedewald calculation was valid since none of the women in this study had a TG concentration above 400mg/dl.

2.3. DNA extraction and genotyping analysis

DNA was extracted from approximately 5 ml of blood using the method described by Lahiri and Nurnberger [29]. *LPL* (S447X) was successfully genotyped in 214 (100%) white and 159 (100%) black women, *CETP Taq IB B1/B2* was genotyped in 199 (93.4%) white and 143 (85.1%) black women, and *LIPC (−514C > T)* was genotyped in 188(97.4%) white and 157 (91.3%) black women using the restriction fragment length polymorphism (RFLP) assay. The DNA samples were amplified using specific primers and conditions for *LPL*, *CETP* and *LIPC* polymorphisms on a PCR thermocycler (Hybaid; PCR Express, Middlesex, UK). After digestion with the appropriate restriction enzyme (supp. Table 1), the RFLP products were visualized after separation by electrophoresis on a 2% agarose gel using *SYBRE Gold* nucleic acid stain (*Invitrogen Molecular Probes*^{TM}, *Oregon*, *USA)* together with a 100 bp DNA molecular weight ladder (Promega Corporation, Madison, Wisconsin, USA). *PCSK9 (C679X)* was successfully genotyped in 181 (99.4%) white and 140 (100%) black women using a custom SNP genotyping assay (Applied Biosystems, Foster City, California, USA) as previously described by Cohen et al., 2006 and performed on a StepOnePlus (Applied Biosystems, Foster City, California, USA) machine.

Several measures were taken as part of the quality control in our genotyping procedure. Several DNA free samples were included in each PCR experiment. Samples which failed to amplify during PCR after two attempts were considered unsuccessfully genotyped and not included in the subsequent analysis.

2.4. Statistical analysis

Data are presented as median and inter-quartile ranges. Non-normally distributed data were normalized by log transformation prior to analysis. A Pearson Chi-square test was used to examine differences in genotype frequencies between black and white women. Ethnic differences in body composition and serum lipid concentrations were determined using a one-way analysis of covariance (ANCOVA), adjusting for age, and age, fat mass and VAT, respectively. Differences in serum lipid concentrations between genotypes were determined using an ANOVA for black and white women separately. In order to determine whether the differences in serum lipids by genotype differed between black and white women, a two way ANOVA including the interaction between genotype and ethnicity, adjusting for age, fat mass and VAT, was performed, using a Fisher LSD post-hoc test. Models were adjusted for age, fat mass and VAT as these differed by ethnicity and were associated with serum lipid levels in both groups. Data was