Frequencies of the T-455C and C-482T apoCIII gene polymorphisms in different South African population groups and their relationship to fasting serum triglyceride levels

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ABSTRACT

Studies have demonstrated that serum triglyceride levels are higher in Indian and White than Black South African subjects. Polymorphisms in the apoCIII gene have been associated with raised triglyceride levels. This study investigated the prevalence of apoCIII polymorphisms and their effect on triglyceride levels in three South African population groups and in subjects with fasting hypertriglyceridaemia (HT). Two apoCIII polymorphic sites (T-455C and C-482T) were studied in 78 European, 24 Indian and 25 African subjects. Each ethnic group included HT and non-HT (control) patients. Although triglyceride levels were much higher in the HT subjects, no significant differences were noted between the groups for allele or genotype frequencies at either apoCIII locus. Furthermore, at neither locus was there an association between the genotype and serum triglyceride levels. The HT and control subjects were therefore combined and ethnic differences in allele frequencies were investigated. The African subjects had a higher frequency (0.79) of the unfavourable C allele at position 455 than both Indian (0.56; $p<0.05$) and European (0.41; $p<0.0005$) subjects. Furthermore, African subjects had a higher frequency (0.77) of the unfavourable T allele at locus 482 than Indian (0.44, $p<0.005$) and European (0.36, $p<0.0005$) subjects despite triglyceride levels being lower in the African (0.60 [1.60] mmol/l) than European (0.90 [0.40] mmol/l; $p<0.05$) control subjects. These results suggest that the apoCIII polymorphisms studied do not contribute to the raised triglyceride levels in HT subjects and do not explain the ethnic differences observed in fasting serum triglyceride levels. SAHeart 2009; 6:162-167

INTRODUCTION

Apolipoprotein CIII (apoCIII), is an essential constituent of very low density lipoprotein (VLDL), high density lipoprotein (HDL) and chylomicrons and has been shown to be an important regulator of intravascular triglyceride metabolism through the inhibition of lipoprotein lipase and interference with apoE mediated triglyceride–rich lipoprotein uptake by hepatic receptors.[1] In-vitro and transgenic animal studies have demonstrated that overexpression of apoCIII causes delayed clearance of triglyceride rich lipoproteins from plasma resulting in overt hypertriglyceridaemia.[2] Furthermore, the results from a large clinical study have indicated that apoCIII levels were better predictors of risk for the development and progression of CAD than traditionally measured serum triglyceride levels.[3] Recent interest has focused on the possible involvement of genetic variations in genes regulating lipid metabolism in causing hyperlipidaemia[4-6] and thus predisposing to CAD. The gene encoding apoCIII is therefore an obvious candidate gene for dyslipidaemia.

The gene encoding apoCIII lies on the long arm of chromosome 11 (11q23-qter) within a cluster of related genes (apoAI, apoCIII, apoAIV and apoAV) that function in the control of lipid metabolism.[7] Fibrates have been shown to reduce serum triglyceride levels by inhibiting apoCIII gene transcription. This effect occurs through activation of the peroxisome proliferator-activated receptor (PPAR)-α which in turn leads to transcriptional repression of hepatocyte nuclear factor (HNF)-4α, 8 a transcription factor that is necessary for apoCIII gene transcription.

Various studies have shown that hyperlipidaemia and particularly hypertriglyceridaemia may have a genetic predisposition, but the genes responsible have not yet been fully elucidated.[9-11] Olivieri and co-workers,[5] identified apoCIII polymorphisms that are believed to play an important role in the metabolism of circulating triglyceride rich lipoproteins. Several variant alleles of the apoCIII gene have been investigated as possible genetic markers of hypertriglyceridaemia.[12] Two polymorphic nucleotides located at positions -455 (T to C) and -482 (C to T) in the 5' apoCIII promoter region have been associated with elevated plasma triglyceride levels.[5]

In South Africa, dyslipidaemia and CAD are far more common in the Indian and European than African populations.[13-15] Furthermore, Indians have been shown to exhibit a higher prevalence of the metabolic syndrome and CAD when compared to other ethnic groups, worldwide.[16] Therefore, the aims of the current study were to determine the frequencies of the apoCIII T-455C and C-482T polymorphisms and observe their effects on serum triglyceride concentrations in African, Indian and European South African subjects with and without raised fasting triglyceride levels.

MATERIALS AND METHODS

Subjects

A total of 78 (47 males) European, 24 (6 males) Indian and 25 (6 males) African subjects were included in the study. This total cohort consisted of those with a fasting triglyceride level > 3mmol/l and a group of control subjects. Newly diagnosed hypertriglyceridaemia (HT) patients who had not previously received any lipid lowering agents were recruited from the lipid clinic of the Johannesburg Hospital with a fasting serum triglyceride cut-off value of > 3mmol/l used as a selection criteria. This group comprised of 57 European, 10 Indian and 4 African individuals. Control, healthy subjects with no history of diabetes, cardiovascular disease or dyslipidaemia was recruited to the study via advertisement and included 21 European, 14 Indian and 21 African subjects. The study was explained to the volunteers and written informed consent obtained. The investigation was approved by the University of Witwatersrand Ethics committee.

Biochemical measurements

All blood samples were taken from subjects after an overnight fast of not less than 10 hours. Total serum cholesterol concentrations were determined by an enzymatic method (CHOD-PAP, Roche Diagnostics, Mannheim, Germany), as were triglyceride concentrations (GOD-PAP, Roche Diagnostics, Mannheim, Germany). High density lipoprotein-cholesterol (HDL-C) was determined using the HDL-C Plus Third Generation Assay (Roche Diagnostics, Mannheim, Germany). This is a homogeneous assay in which cholesterol esterase and cholesterol oxidase enzymes are conjugated to polyethyleneglycol to enhance the specificity of the assay to HDL-C thus reducing cross reaction with cholesterol in the LDL particles. Fasting blood glucose levels were determined by a glucose oxidase method (glucose GOD-PAP, Boehringer Mannheim) using a Hitachi 717 auto-analyser. Diabetes was defined as a fasting glucose >7mmol/l in subjects who were diagnosed with the disease during the course of the study whilst pre-diagnosed diabetics were defined as such according to the use of oral anti-diabetic agents or insulin injections.

Genotyping

Genomic DNA was extracted using a salting-out method.[17] The apoCIII -455 (T to C) and -482 (C to T) polymorphisms were detected using restriction fragment length polymorphism based polymerase chain reaction.[5] Each PCR reaction contained 100ng genomic DNA with PCR buffer containing 1.5mM $MgCl_2$, 3.2 mM of dATP, dCTP, dGTP, dTTP, the two primers (10pmol) and 1 unit of Taq polymerase in a 50µl reaction. The PCR reaction was initiated at 94°C for 5min, thereafter 35 temperature cycles of 94°C for 60sec, 60°C for 60sec and 72°C for 60 sec, was carried out. A final elongation step at 72°C for 10 min was performed to ensure maximum product yield. The primers used have been described previously:[11] forward primer, 5'-ACAGGTTAATATAGTGAAAAG-3' and reverse primer 5'-TACCCTGAGTTCAGTTCCGTC-3'. The PCR products (10µl) were digested in two separate reaction tubes using the endonuclease restriction enzymes *Fok I* for T-455C and MSP I for C-482T at 37°C for 2 hours. The DNA fragments were separated on a 2% agarose gel and the bands visualised under 300nm ultraviolet trans-illumination after staining with ethidium bromide. The restriction enzyme *Fok I* cleaves the -455 wild type T allele and appears as two bands on the gel electrophoresis (133bp and 129bp), and the variant C allele appears as a single 196bp fragment. The -482 wild type C allele is cleaved by *Msp I* and appears as a 143bp band and the variant as a 159bp band.

Statistical analysis

Data that was not normally distributed was transformed by taking log or reciprocal values for use in parametric statistical tests. The differences in lipid levels between patients and control subjects were analysed using analysis of co-variance (ANCOVA) with adjustment for age, gender and ethnicity. Lipid levels were compared across ethnic groups using ANCOVA adjusted for age, gender and diabetes whilst lipid levels were compared across genotypes using ANCOVA adjusted for age, gender, ethnicity and diabetes. A χ^2 test was used to analyse the frequency of the different polymorphisms. Data in the text and tables is expressed as median [interquartile range] or mean ± SD.

RESULTS

Lipid levels in study groups

The hypertriglyceridaemic (HT) patients were older (48.7 ± 11.1 vs. 36.5 ± 9.6 years; p<0.0005) and had higher fasting serum triglyceride (10.8 [11.5] vs. 0.86 [0.44] mmol/l; p<0.0005) and total cholesterol levels (8.10 [3.50] vs. 4.55 [0.99] mmol/l; p<0.0005) but lower fasting serum HDL levels (0.90 [0.40] vs. 1.70 [0.80] mmol/l; p<0.0005) compared with the control group.

The control group included no diabetic subjects whilst the HT group contained 20 type 2 diabetic subjects and one subject with impaired fasting glucose (IFG). The diabetic and IFG subjects were combined (dysglycaemic group) and lipid levels compared with the non-diabetic, HT subjects. The dysglycaemic group had higher fasting triglyceride (15.1 [26.8] vs. 8.55 [10.8] mmol/l; p<0.005) and total cholesterol (8.50 [5.11] vs. 7.70 [3.44] mmol/l; p=0.058) levels compared to the non-diabetic subjects, whilst HDL levels (0.90 [0.20] vs. 0.90 [0.60] mmol/l; p=0.72) did not significantly differ.

Table 1 shows data for lipid levels in control and HT subjects within each ethnic group. In all 3 groups' triglyceride and cholesterol levels were significantly higher whilst HDL levels were lower in HT than control subjects. Triglyceride levels were significantly higher (p<0.05) in the European than the African control group whilst cholesterol levels were significantly higher in Indian (p<0.05) and European (p<0.005) than African control subjects.

Genotype and allele frequencies

No difference was found for genotypic or allelic frequencies of the C-482T polymorphism between HT and control subjects within each ethnic group or when all ethnic groups were combined. Thus, the frequency of the T allele was 0.49 in control subjects and 0.44 in HT patients (p=0.44) when the ethnic groups were combined. Similar results were found for the T-455C polymorphism with the frequency of the C allele being 0.58 in control subjects and 0.48 in HT subjects (p=0.13). Therefore the control and HT groups were combined when comparing genotypic and allelic frequencies across the 3 ethnic groups.

The frequencies of the apoCIII C-482T genotypes for the different ethnic groups are shown in Figure 1. The CC genotype was significantly rarer in the African population compared to both the European (p<0.0005) and the Indian (p<0.05) subject groups whilst the TT genotype was more common in African than Indian (p<0.005) and European (p<0.005) subjects. The CT genotype was more common in Indian than European subjects (p<0.05). The T allele frequency was 0.77 in Africans, 0.44 in Indians (p<0.005 vs. Africans) and 0.36 in Europeans (p<0.0005 vs. Africans).

The frequencies of the apoCIII T-455C genotypes for the 3 ethnic groups are shown in Figure 2. The TT genotype was significantly more frequent in European than both African (p<0.005) and

TABLE 1: Serum lipid levels in control and hypertriglyceridaemic (HT) subjects within each ethnic group

Variables	Indian		European		African	
	Controls	HT	Controls	HT	Controls	HT
N number	14	10	21	57	21	4
Triglyceride	0.95 [1.19]	6.45 [11.0]*	0.90 [0.40]†	10.8 [11.3]***	0.60 [1.60]	18.8 [18.7]***
Cholesterol	4.56 [0.60]†	7.89 [3.20]*	4.90 [1.10]††	8.20 [3.50]***	4.30 [1.40]	10.3 [9.40]*
HDL	1.60 [0.64]	0.91 [0.40]	1.50 [0.40]	0.90 [0.41]**	1.40 [0.60]	0.55 [0.72]*

*Data expressed as median [interquartile range]. *p<0.05, **p<0.005, ***p<0.0005 vs. control of same ethnic group; †p<0.05, ††p<0.005 vs. African controls.*

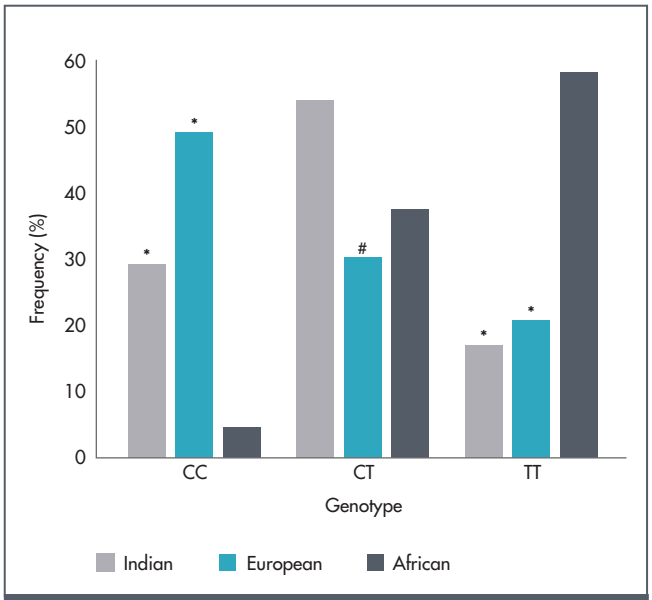

FIGURE 1: The percentage prevalence of the apoCIII C-482T genotypes in the Indian, European and African subjects.

*#p<0.05 vs. Indian subjects; *p<0.05 vs. African subjects.*

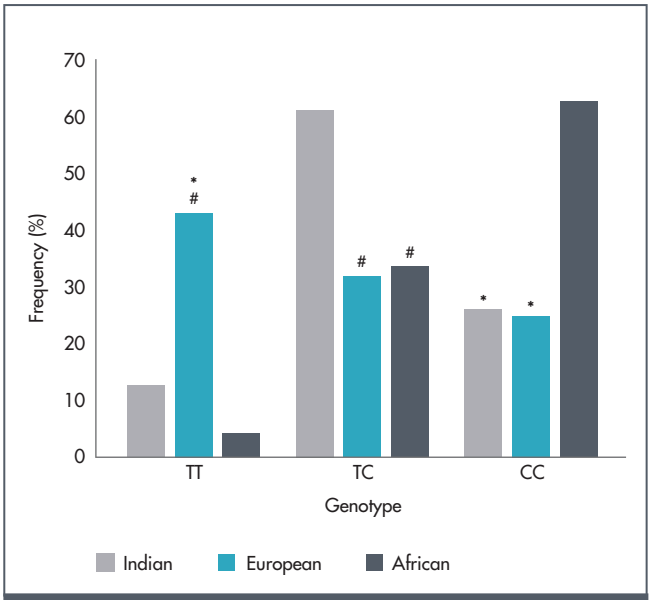

FIGURE 2: The percentage prevalence of the apoCIII T-455C genotypes in the Indian, European and African subjects.

*#p<0.05 vs. Indian subjects; *p<0.05 vs. African subjects.*

Indian (p<0.05) subjects, whilst the CC genotype was more common in African than both Indian (p<0.05) and European (p<0.005) subjects. The TC genotype was more common in Indian than both African (p=0.06) and European (p<0.05) subjects. The C allele frequency was 0.79 in African subjects, 0.56 in Indian subjects (p<0.05 vs. Africans) and 0.41 in European subjects (p<0.0005 vs. Africans).

Triglyceride levels across genotypes

The data in Table 2 shows that at both the -482 and -455 loci of the apoCIII gene there was no significant variation in triglyceride levels with genotype. Thus, for the C-482T polymorphism ANCOVA gave a p-value of 0.10 in the control group and p=0.51 in the HT subjects and with the T-455C polymorphism, p=0.15 in the control group and p=0.81 in the HT group. Furthermore, neither total cholesterol nor HDL serum levels varied across the genotypes at either locus. If triglyceride levels were compared between the homozygous form of the unfavourable allele at each loci and the other two genotypes combined i.e. TT versus CT+CC at -482 and CC versus CT+TT at -455, no significant differences in triglyceride, total cholesterol or HDL levels were observed. The same analysis within each ethnic group gave similar results.

TABLE 2: Triglyceride concentrations across genotypes at the C-482T and T-455C loci of the apoCIII gene in control and hypertriglyceridaemic (HT) subjects.

Genotypes	Control subjects		HT subjects	
	C-482T	T-455C	C-482T	T-455C
CC	1.03 [0.55]	0.80 [0.65]	11.1 [9.85]	10.5 [15.4]
	N=16	N=19	N=28	N=20
CT	0.76 [0.50]	0.75 [0.57]	9.00 [13.0]	6.20 [32.1]
	N=22	N=22	N=22	N=23
TT	0.80 [0.70]	1.00 [0.51]	13.1 [16.3]	11.1 [9.64]
	N=15	N=11	N=18	N=24

Data is expressed as median [interquartile range] and units are mmol/l.

DISCUSSION

The present study is the first to describe and compare the allelic frequencies for the apoCIII T-455C and C-482T polymorphisms in South African subjects of European, Indian and African ancestry. The results of this study clearly show that allelic and genotypic frequencies at the polymorphic -482 and -455 loci of the apoCIII gene differ across ethnic groups. This finding is supported by data from a previous study by Waterworth et al.[18] demonstrating that the allelic frequencies of the C-482T polymorphism differs between European, South-Asian and Afro-Caribbean/West African subjects resident in the UK. That study gave very similar allelic frequencies for the C-482T polymorphism to those described in the current study however, the T-455C polymorphism was not analysed.[18] The allelic frequencies observed for the C-482T and T-455C polymorphisms for the European population in the current paper are also very similar to those reported previously for a Dutch population.[19]

The two apoCIII gene promoter polymorphisms examined in this study lie within an insulin response element (IRE). An in vitro study has shown that the presence of the T allele at locus -482 and the C allele at locus -455 led to attenuation of the ability of insulin to down-regulate apoCIII gene transcription.[20] It has therefore been hypothesised that the polymorphisms in the IRE of the apoCIII gene lead to elevated serum triglyceride levels by reducing the ability of insulin to down regulate apoCIII gene transcription, in vivo. It is also known that transcription of the apoCIII gene can be increased by TGF-β and reduced by TNF-α and IL1-β.21 Furthermore, the intra-cellular kinase, AMP-activated protein kinase (AMPK) can decrease hepatic apoCIII transcription by reducing cellular levels of the transcription factor, HNF-4α.[22] This is intriguing because the anti-diabetic agent metformin is known to activate AMPK in hepatocytes.[23]

No differences were detected in fasting triglyceride, total cholesterol or HDL levels across the genotypes at either apoCIII locus. This is in contrast to other publications where an association has been observed of raised serum triglycerides levels with the -482T and -455C alleles.[5,11,13,18] However, there are studies that failed to show such associations.[24-26] The present study was also unable to find differences in allelic frequencies at either locus between hypertriglyceridaemic and control subjects, again suggesting that neither of these polymorphisms play a prime role in determining fasting triglyceride levels. This is further confirmed by the finding that the unfavourable -482T and -455C alleles were more frequent in African subjects than either of the other two population groups even though fasting triglyceride levels were lower in African compared to the Indian and European subject groups. Previous South African studies have also shown lower fasting triglyceride levels in African compared to Indian or European subjects.[14,15]

The polymorphisms in the apoCIII gene that were investigated in the present study do not cause any change in function of the lipoprotein but rather change its level of expression[20] as shown by the higher levels of serum apoCIII in subjects carrying the T-455C variant.[5] Thus, the phenotype associated with such a polymorphism will not be as marked as that seen when the genetic change causes a complete loss of function of the associated peptide. These small changes in phenotype make the detection of an association between such polymorphisms and the phenotype more difficult to observe and may explain why our study and others have found no link between the apoCIII polymorphisms and fasting serum triglyceride levels.[24-26]

It is also possible that no association was found between serum triglyceride levels and the genotypes at either apoCIII locus because of the low n number of study subjects. Furthermore, other genetic polymorphisms and a variety of environmental, anthropometric, ethnic and demographic factors are known to influence triglyceride metabolism[27] and such factors may 'drown out' the influence of the apoCIII polymorphisms in the present investigation, especially with a small study cohort. Also, the selection criteria for hypertriglyceridaemia used in the current study i.e. > 3mmol/l may have led to the inclusion of subjects with a wide array of different disease aetiologies including simple obesity, type 2 diabetes and other genetic forms of hypertriglyceridaemia. Thus, identification of the specific effect of the apoCIII gene polymorphisms on fasting triglyceride levels within this cohort may have been obscured.

A major drawback of the current investigation is that body mass index (BMI) was not measured in the study participants. BMI is known to be a major determinant of triglyceride levels and there-

fore it is possible that the lack of association between the apoCIII promoter polymorphisms and serum triglyceride levels is due to differences in BMI across the genotypes. However, one study has shown that the apoCIII C-482T polymorphism has no relationship with BMI in European and South Asian subjects but does in a mixed group of Afro-Caribbean/West African subjects where BMI was higher in the presence of the -482T allele.[18] However, adjusting for BMI had minimal effects on the statistical outcomes for the relationship between the C-482T genotype and triglyceride levels, in any of the 3 ethnic groups.[18]

The current study was unable to show associations between either apoCIII polymorphism and lipid levels. This may be a result of the low n number. However, the study was sufficiently powered to demonstrate ethnic differences in allele frequencies at both loci and also to show ethnic differences in fasting serum lipid levels. Thus, it is possible to conclude that neither of the apoCIII polymorphisms can explain ethnic differences in lipid levels but we cannot rule out the possibility that within each ethnic group these polymorphisms may play a role in influencing lipid metabolism.

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