Variable effects of the *APOC3* –482C > T variant on insulin, glucose and triglyceride concentrations in different ethnic groups

D.M. Waterworth¹, P.J. Talmud¹, S.E. Humphries¹, P.D. Wicks², G.A. Sagnella³, P. Strazzullo⁵, K.G.M.M. Alberti⁶, D.G. Cook², F.P. Cappuccio⁴

¹ Centre for Cardiovascular Genetics, Department of Medicine, The Rayne Institute, University College London, UK

² Department of Public Health Sciences, St. George's Hospital Medical School, London, UK

³ Department of Medicine, St. George's Hospital Medical School, London, UK

⁴ Department of General Practice and Primary Care, St George's Hospital Medical School, London, UK

⁵ Department of Clinical and Experimental Medicine, Federico II University Medical School, Naples, Italy

⁶ Department of Medicine, University of Newcastle-upon-Tyne, UK

Abstract

Aims/hypothesis. The *apolipoprotein C3*–482C > T variant modulates insulin and glucose concentrations after an oral glucose tolerance test (OGTT) in young healthy white men. We evaluated the effect of this variant in different ethnic groups with different rates of Type II (non-insulin-dependent) diabetes mellitus and coronary heart disease.

Methods. We investigated the –482C > T in a population-based cross-sectional study of white subjects ($n = 462$), South Asians ($n = 442$) and subjects of West African and Afro-Caribbean origin ($n = 462$), whose OGTT and fasting plasma triglyceride concentrations had been measured.

Results. The –482T allele frequency differed between the three groups: 0.25 (95% CI 0.22–0.28) in white subjects, 0.44 (0.41–0.47) in South Asians and 0.71 (0.68–0.74) in black subjects ($p < 0.0001$). A positive association was found between body mass index and genotype in black women ($p = 0.009$) and in black

men ($p = 0.056$) but not in white subjects or South Asians. Associations between –482C > T and fasting insulin were found in white subjects, where –482T allele carriers had higher concentrations (adjusted for age and sex, $p = 0.007$, also including smoking and body mass index, $p = 0.038$). Higher triglyceride concentrations ($p = 0.004$ and $p = 0.007$ in the two models) but not glucose concentrations were also associated with –482C > T. In black subjects, decreased fasting insulin ($p = 0.04$) and fasting glucose ($p = 0.004$) were associated with –482C > T. No relation was observed between genotype and any post-load measures.

Conclusions/interpretation. Allele frequencies of the –482C > T and associations with insulin, glucose and triglyceride concentrations vary considerably among ethnic groups. Although the results are consistent among white subjects across different studies, the associations among black subjects and South Asians differ. [Diabetologia (2001) 44: 245–248]

Keywords *APOC3*, insulin, glucose, triglyceride.

The frequencies of Type II (non-insulin-dependent) diabetes mellitus, atherosclerosis and components of -syndrome X, such as insulin resistance and dyslipidaemia, vary considerably among ethnic groups. South Asians and black subjects have a higher prevalence of Type II diabetes and hypertension than white

subjects. Black subjects, however, have a favourable lipid profile and thus a lower risk of coronary heart disease (CHD) than South Asians, who have a high-risk lipid profile and consequently a high prevalence of CHD [1–3]. Risk factors such as obesity and smoking also vary widely between ethnic groups: black women have a high prevalence of obesity while white subjects have a high prevalence of smoking [1]. Some of these differences in disease susceptibility might depend on cultural inheritance or on the environment, but genetic variation could also play a part.

We have previously shown that a polymorphism in the insulin response element (IRE) of the apolipoprotein CIII (*ApoCIII*) gene promoter (–482C > T)

Received: 29 March 2000 and in revised form: 30 August 2000

Corresponding author: Prof. Francesco P. Cappuccio, MD, Wandsworth Heart & Stroke Study, Department of General Practice & Primary Care, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK

Abbreviations: ApoCIII, apolipoprotein CIII.

is associated with higher insulin and glucose concentrations after an OGTT in young, healthy white European men participating in the second European Atherosclerosis Research Study [4]. The presence of the $-482T$ allele has been shown in vitro to abolish the normal down-regulation of ApoCIII by insulin [5]. We have also found that this variant is associated with increased triglyceride concentrations in smokers in a large group ($n = 2700$) of middle-aged white men [6].

Given the importance of insulin resistance and hypertriglyceridaemia in the metabolic syndrome and its consequences of Type II diabetes and CHD, we have studied the *ApoC3* $-482C > T$ variant in a population-based multi-ethnic cohort to establish if this variant could be, at least in part, responsible for some of these observed differences in disease risk.

Methods

The sampling from south London general practitioners' registers of age and sex has been described previously [1, 7]. Briefly, 1577 men and women aged 40 to 59 years were screened, from the three main ethnic groups living in the area. These were white subjects ($n = 462$) people of South Asian origin ($n = 442$) and people of West African and Afro-Caribbean origin ($n = 462$). The South Asians and the subjects of African origin were all first generation immigrants. Both parents of subjects of black-African origin were born in the country of origin. The ethnic group was recorded at the time of the interview. The study protocol was approved by the local ethics committee and informed consent was obtained from all participants. Procedures were followed as described previously [1, 7]. Briefly, participants not known to be diabetic underwent an OGTT and venous samples were taken 2 h later (± 10 min). Information on medical history and drug treatment was obtained and recorded in a questionnaire. Hypertension was defined as a blood pressure of 160 or more or of 95 mmHg or more, or being on drug therapy for hypertension. Impaired glucose metabolism was defined as the combination of diabetes and IGT by the World Health Organization (WHO) criteria [1, 9]. Fasting insulin, glucose and triglyceride measurements were obtained in all subjects and 2-h insulin and glucose in 78 % of the white subjects, 71 % of South Asians and 71 % of black subjects. Genomic DNA was isolated from whole blood [8] and assay conditions were as described previously [4]. Data were tested for deviation from Hardy-Weinberg equilibrium using a chi-squared test (on 1 degree of freedom). Allele frequencies and 95 % confidence intervals (CI) were calculated using the Z-transformation. Triglycerides, insulin and glucose were not normally distributed and were $\log_e$ -transformed for the analysis. Linear regression models with a $\log_e$ -transformed outcome were fitted for insulin, glucose and triglyceride concentrations for each ethnic group separately. The genotype was entered into the model as a categorical variable and the p value for linear trend was calculated. Both models were also tested for interaction between ethnicity and genotype. Adjustment was made for possible confounding factors such as age and sex (Model 1) and with the addition of smoking and BMI (Model 2). Regression coefficients (β) were exponentiated and are presented as a percentage increase in the measured variable above the baseline genotype. A p value of less than 0.05 was considered statistically significant.

Results

Black subjects had higher BMI than South Asians and white subjects. White subjects had the highest proportion of smokers for men and women. Subjects of African descent had the highest prevalence of hypertension and the proportion of hypertensives did not differ with sex in each ethnic group. South Asians had the highest prevalence of Type II diabetes and IGT [1, 7]. For all ethnic groups the frequency of hypertension, diabetes and IGT was similar among genotypes (data not shown). There was a statistically significant relation, however, between BMI and $-482C > T$ in the group of black women (CC: 26.9, CT: 29.7, TT: 29.6 kg/m², $p = 0.009$) and in black men (CC: 25.8, CT: 27.4, TT: 26.2 kg/m², $p = 0.056$), but not in white subjects or South Asians (interaction: women, $p = 0.05$; men, $p = 0.28$).

There was a statistically significant difference in $-482T$ allele frequency among the three groups, being lowest in the white subjects [0.25 (95 % CI 0.22–0.28)], highest in the black subjects [0.71 (0.68–0.74)] and intermediate in the South Asians [0.44 (0.41–0.47)] ($p = 0.0001$). In white subjects and South Asians, the genotype distributions were in Hardy-Weinberg equilibrium, but in the black subjects it was not ($p = 0.01$). When tested separately, the lack of Hardy-Weinberg equilibrium was confined to the Afro-Caribbean group ($p = 0.015$) rather than the West African group ($p = 0.14$). The genotype frequencies, however, were not statistically significantly different between the two groups ($p = 0.4$).

Linear regression models were fitted in each ethnic group separately to evaluate the relation between genotype and insulin, glucose and triglycerides. Hypertension treatment was not considered to be a confounder as there was no association with genotype. As BMI was, however, associated with genotype in blacks and thus was considered a possible confounder, separate models were fitted that either excluded (Model 1) or included BMI (Model 2). In addition, model 2 also included smoking as a confounder. The regression models (Table 1) show the percentage difference relative to the baseline CC genotype for both heterozygotes and TT homozygotes by ethnic group. The models confirmed the associations between $-482C > T$ and fasting insulin in white subjects, with statistically significant evidence of a linear trend in both model 1 (CT 14.2 % higher and TT 21.9 % higher than CC, $p = 0.007$) and model 2 (CT 10.9 % higher, TT 12.8 % higher; $p = 0.038$). In blacks only model 2, however, showed a significant relation with genotype (CT 2.7 % higher, TT 10.3 % lower; $p = 0.04$) which was the reverse to the association in whites. No relation was observed between genotype and insulin in South Asians. These differences between ethnic groups in insulin were confirmed by a statistically significant test for interaction between

Table 1. Percentage mean (95 % CI) difference in fasting insulin, glucose and triglycerides within each ethnic group according to the *ApoC3* -482C > T genotype.

Genotype	White subjects (<i>n</i> = 462)		Black subjects (<i>n</i> = 456)		South Asians (<i>n</i> = 430)	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Fasting insulin ¹						
CC	0	0	0	0	0	0
CT	14.2 (2.1, 26.2)	10.9 (0.3, 21.5)	18.9 (-0.5, 38.3)	2.7 (-14.4, 19.8)	-11.1 (-24.5, 2.2)	-8.7 (-20.5, 3.1)
TT	21.9 (-0.5, 44.3)	12.8 (-6.9, 32.5)	3.4 (-15.3, 22.2)	-10.3 (-26.8, 6.2)	1.7 (19.1, 0)	5.2 (-10.1, 20.6)
* <i>p</i> value	0.007	0.038	0.37	0.04	0.87	0.78
Fasting glucose ²						
CC	0	0	0	0	0	0
CT	3.1 (-1.1, 7.4)	0.3 (-1.2, 7.2)	-4.3 (-11.2, 2.5)	-6.4 (-13.2, 0.4)	6.1 (1.4, 10.8)	6.3 (1.6, 10.9)
TT	1.9 (-0.6, 9.8)	0.6 (-7.2, 8.4)	-8.0 (-14.6, -1.3)	-9.6 (-16.2, -3.0)	3.4 (-2.8, 9.4)	3.7 (-2.3, 9.7)
* <i>p</i> value	0.23	0.33	0.01	0.004	0.14	0.10
Fasting triglycerides ³						
CC	0	0	0	0	0	0
CT	5.4 (-3.5, 14.4)	5.5 (-0.3, 14.1)	7.5 (-6.9, 21.9)	1.2 (-12.7, 15.0)	7.2 (-2.6, 17.2)	7.4 (-0.2, 16.9)
TT	26.8 (10.1, 43.4)	23.2 (7.2, 39.1)	4.9 (-8.9, 18.9)	0.6 (-12.8, 13.9)	11.4 (-1.4, 24.2)	12.9 (0.7, 25.2)
* <i>p</i> value	0.004	0.007	0.79	0.99	0.065	0.032

Model 1 is adjusted for age and sex. Model 2 is adjusted for age, sex, smoking and body mass index. **p* values for linear trend

Both models were also tested for interaction between ethnicity and *ApoC3* genotype: ¹ Model 1, *p* = 0.025; Model 2, *p* = 0.013; ² Model 1, *p* = 0.006, Model 2, *p* = 0.003; ³ Model 1, *p* = 0.15; Model 2, *p* = 0.11

ethnicity and genotype (model 1, *p* = 0.025; model 2, *p* = 0.013). For glucose, a statistically significant linear relation was observed in the black subjects only, for both model 1 (CT 4.3 % lower and TT 8 % lower than CC, *p* = 0.01) and model 2 (CT 6.4 % lower, TT 9.6 % lower; *p* = 0.004) (Table 1). Both models indicated a strong interaction between ethnicity and genotype with glucose (model 1, *p* = 0.006; model 2, *p* = 0.003). No statistically significant relations were observed with genotype and the available post-load glucose concentrations (data not shown). Similar relations between genotype and triglycerides were observed in white subjects for model 1 (CT 5.4 % higher and TT 26.8 % higher than CC, *p* = 0.004) and model 2 (CT 5.5 % higher, TT 23.2 % higher; *p* = 0.007) but there was no relation between genotype and triglycerides in black subjects (Table 1). South Asians showed a statistically significant linear relation between genotype and triglycerides in model 2 (CT 7.4 % higher, TT 12.9 % higher; *p* = 0.032) but not in model 1 (CT 7.2 % higher, TT 11.4 % higher; *p* = 0.065), though a similar trend was evident (Table 1). The test for interaction between ethnicity and genotype for triglycerides was not statistically significant for either model (model 1, *p* = 0.15; model 2, *p* = 0.11).

Discussion

In white subjects and South Asians, genotype distributions were in Hardy-Weinberg proportions but in the black group it was not. This is probably due to the heterogeneity of this group, which is comprised

of 38 % of people from West Africa who had Hardy-Weinberg proportions and the remainder (62 %) from the Caribbean who did not. This could reflect a higher degree of admixture in this latter group or could be merely a chance finding. The genotype frequencies, however, were not significantly different between the two groups and thus were treated in further analyses as one group. The marked frequency differences between ethnic groups are possibly the result of population bottlenecks and later expansions, which is consistent with the 'out of Africa' hypothesis [9]. It is also possible that selection pressure helped to create the allele frequency differences observed.

These results confirm our previous findings of an association with the *T-482* allele and hyperinsulinaemia in white subjects, though in the original study the association was with insulin after an OGTT [4] and not with fasting concentrations as in this study. Although the previous study was larger (*n* = 800), it was carried out in healthy young men as opposed to the middle-aged men and women (*n* = 462) who participated in this study. Thus the effects on fasting concentrations in the young men might not reflect the association and only when stressed after the OGTT was the effect observed.

There was an association between genotype and insulin concentrations in black subjects only when BMI and smoking were included in the model. This can be explained by the significant association between BMI and genotype which was observed in black subjects only, indicating a complex interaction between BMI and genotype with insulin concentrations. The -482T allele, which is the most common allele in black subjects, is associated with decreased in-

sulin concentrations than the baseline genotype. This is in contrast to white subjects where the *-482T* allele is associated with an increased concentration of insulin.

In contrast to the previous study [4], no association between genotype and glucose in white subjects was observed. It is not clear why the previous association was not replicated as it has been for insulin. The black subjects showed, however, a strong association between genotype and glucose, supporting the findings of an association with insulin. As with insulin, the *-482T* allele was associated with a decreased concentration of glucose in black subjects.

In white subjects, a strong raising effect of the *-482T* allele on fasting triglyceride concentrations was observed, of a similar magnitude to results obtained in a sample of 2700 white middle-aged men [6]. There was a similar trend for increased triglycerides in South Asians, which was statistically significant only after BMI and smoking were included in the model, though the magnitude of the effect was similar in both models. No effect of genotype was observed in black subjects, which might depend on the overall low mean concentrations of triglycerides seen in this group, which would consequently lead to a diminution of any effect of genotype.

The *-482T* allele in the *ApoC3* gene promoter is unresponsive to insulin, as compared with the *C-482* allele which responds to insulin by down-regulating *ApoC3* expression by 40 to 50% [5], suggesting that inappropriately increased concentrations of ApoCIII are responsible for the in vivo effects. The mechanism by which ApoCIII affects insulin and glucose is, however, as yet undetermined, but the synergistic relation between carbohydrate and lipid metabolism is probably crucial.

Why these relations with insulin and glucose should differ between black subjects and white subjects, and also why an association with BMI is evident only in the black subjects is not clear but it is possible that there are polygenic determinants of these traits with many other genetic variants similar to the *ApoC3 -482C > T*, which have differing frequencies between the two populations, such as in the case of the *LPL* promoter variant (*-93T/G*) [10–11]. It is possible that the *-482C > T* variant is only rate-limiting in certain ethnic and gender-specific groups because of lifestyle and hormonal differences.

Further characterisation of gene-gene and gene-environment interactions in different ethnic groups in larger studies with adequate statistical power to examine such interactions, might enable us to understand the different ethnic susceptibilities to Type II diabetes, dyslipidaemia, CHD and hypertension, common diseases which have a major effect on public health.

Acknowledgements. D.M. Waterworth, P.J. Talmud and S.E. Humphries are funded by the British Heart Foundation (RG/95007, PG/96184). The Wandsworth Heart & Stroke study received support from the Wandsworth Health Authority, the South West Thames Regional Health Authority, the NHS Research and Development Directorate, the British Heart Foundation, the British Diabetic Association and the Stroke Association. We thank the participating general practitioners for their co-operation (list in [7]), L. Ashworth for carrying out insulin measurements and R.W. Atkinson, C. Chazot, S. Choudary, S. Cooke, J. Cox, E.J. Folkard, R. Iacone, M.J. Rothwell and the staff in the Blood Pressure Unit for their help. G.A. Sagnella, D.G. Cook and F.P. Cappuccio are members of the St. George's Hospital Medical School Cardiovascular Research Group.

References

1. Cappuccio FP, Cook DG, Atkinson RW, Strazzullo P (1997) Prevalence, detection and management of cardiovascular risk factors in different ethnic groups in South London. *Heart* 78: 555–563
2. Whitty CJ, Brunner EJ, Shipley MJ, Hemingway H, Marmot MG (1999) Differences in biological risk factors for cardiovascular disease between three ethnic groups in the Whitehall II study. *Arteriosclerosis* 142: 279–286
3. Cappuccio FP (1997) Ethnicity and cardiovascular risk: variations in people of African ancestry and South Asian origin. *J Hum Hypertens* 11: 571–576
4. Waterworth DM, Ribalta J, Nicaud MA, Dallongeville J, Humphries SE, Talmud PJ (1999) ApoCIII gene variants modulate postprandial response to both glucose and fat tolerance tests. *Circulation* 99: 1872–1877
5. Li WW, Dammerman MM, Smith JD, Metzger S, Breslow JL, Leff T (1995) Common genetic variation in the promoter of the human apoCIII gene abolishes regulation by insulin and may contribute to hypertriglyceridemia. *J Clin Invest* 96: 2601–2605
6. Waterworth DM, Talmud PJ, Bujac SR, Fisher RM, Miller GJ, Humphries SE (2001) The contribution of apoCIII gene variants to the determination of triglyceride levels and interaction with smoking in middle-aged men. *Arterioscler Thromb Vasc Biol* (in press)
7. Cappuccio FP, Cook DG, Atkinson RW, Wicks PW (1998) The Wandsworth Heart and Stroke Study – A population-based survey of cardiovascular risk in different ethnic groups. Methods and baseline findings. *Nutr Metab Cardiovasc Dis* 8: 371–385
8. Sagnella GA, Rothwell MJ, Opininla AK, Wicks PD, Cook DG, Cappuccio FP (1999) A population study of ethnic variations in the angiotensin-converting enzyme I/D polymorphism: relation with gender, hypertension and impaired glucose metabolism. *J Hypertens* 17: 657–664
9. Cavalli-Sforza LL, Menozzi P, Piazza A (1994) The history and geography of human genes. Princeton University Press, Princeton
10. Talmud PJ, Hall S, Holleran S, Ramakrishnan R, Ginsberg HN, Humphries SE (1999) LPL promoter *-93T/G* transition influences fasting and postprandial plasma triglycerides response in African-Americans and Hispanics. *J Lipid Res* 39: 1189–1196
11. Hall S, Talmud PJ, Cook DG et al. (2000) Frequency and allelic association of common variants in the lipoprotein lipase gene in different ethnic groups. *Genet Epidemiol* 18: 203–216