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SNPs at the *APOA5* gene account for the strong association with hypertriglyceridaemia at the *APOA5*/*A4*/*C3*/*A1* locus on chromosome 11q23 in the Northern Irish population

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Abstract

Serum triglyceride levels (TG) are important independent risk factors for coronary heart disease. The apolipoproteins C-III (apoCIII) and A-V (apoAV) are central to normal TG metabolism and the complete sequence analysis of these genes was carried out in severe cases (TG > 9 mmol/l) and controls (TG < 2 mmol/l). A total of 53 SNPs were identified in these genes with 17 being novel to this study. Further analysis defined four *APOC3* SNPs and three *APOA5* SNPs showing strong association with TG levels. Analysis of the two major SNPs from *APOA5* [c.56C > G, c.-3A > G] and from *APOC3* [c.102C > T, c.340C > G] using THESIAS has identified two major haplotypes relative to the most common CACC haplotype showing very strong association with hypertriglyceridaemia, CGTG and GATC (odds ratio 7.45 and 5.26). Logistic regression analysis of these four SNPs revealed that, carriage of the *APOA5* c.56 G allele (odd ratios 4.49) and the *APOA5* c.-3 G allele (odds ratio 3.23) were strong independent predictors of hypertriglyceridaemia ($P < 0.001$), whereas in contrast, carriage of the *APOC3* c102 T allele (odds ratio 1.35) and the *APOC3* c.340 G allele (odds ratio 1.37), did not show any significant effects that were independent of *APOA5*.

Keywords: Hypertriglyceridaemia; Apolipoprotein C-III; Apolipoprotein A-V; *APOC3*; *APOA5*

1. Introduction

Coronary artery disease is the single most common cause of death in the Western World. Serum total and LDL cholesterol levels are significantly associated with risk, however, a large body of evidence also points towards triglyceride (TG) levels as being an independent risk factor for its development ([1], reviewed in [2]). Due to the complexity of hypertriglyceridaemia (HTG), there are many possible origins of the disorder, including secondary causes such as alcohol, diabetes mellitus, obesity and prescription drugs. However, it is thought that predisposing genetic factors play an important role in TG metabolism and regulation and thus candidate genes are being investigated.

The main candidate gene for HTG is lipoprotein lipase (LPL), however, extensive studies have failed to provide convincing evidence of common variants that play a major role in HTG (reviewed in [3]). Another candidate is apoC-III (reviewed in [4]). ApoCIII is an inhibitor of LPL as well as being an inhibitor of hepatic remnant uptake. The evidence for apoCIII affecting TG levels has come from biochemical studies and animal model studies. Linkage studies have also shown Familial Combined Hyperlipidaemia to be linked to the chromosomal region of 11q23, which includes the *APOA1*, *APOC3* and *APOA4* genes (Fig. 1). Since 1983 association studies have shown HTG to be associated with *APOC3* [5]. The association studies have mainly focused

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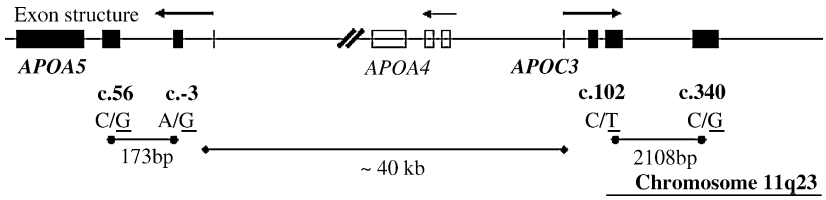

Fig. 1. The structure of the genome region encoding the *APOC3* and *APOA5* genes.

on the single-nucleotide polymorphisms (SNPs) that occur within the exons and promoter region of *APOC3* (reviewed in [6]).

Recently, it has been discovered by comparative sequencing that a new apolipoprotein gene, *APOA5*, is located 40 kb downstream of the *APOC3* gene (Fig. 1), with SNPs across the locus found to be significantly associated with plasma TG levels [7,8]. This variation in *APOA5* has been suggested to be independent of those reported for *APOC3* [7,9]. Transgenic mice studies showed that over expression of *APOA5* decreased TG levels, while mice lacking *APOA5* had a 400% increase in plasma TGs [7]. ApoAV has been suggested to function intracellularly to modulate hepatic VLDL synthesis and/or secretion.

In order to try to fully understand the role of the *APOC3* and *APOA5* gene regions, an in-depth sequence analysis of both genes was carried out in 24 patients with severe HTG and 24 controls, in line with recent suggestions [10–12]. These regions spanned 6 and 5.6 kb, respectively, including exons, introns and over 1 kb on either side.

2. Materials and methods

2.1. Subjects

The patients and controls were Caucasians living in Northern Ireland. Patients free from secondary causes of HTG were recruited from the Lipid Clinic of the Royal Victoria hospital (Belfast, Northern Ireland), after obtaining informed consent and ethical approval.

Genotyping was performed using an initial patient group and follow-up studies used an extended patient group. The patients for the initial genotyping met the following criteria. Cases ($n = 84$): age 54 ± 11 years; male/female ratio 0.62/0.38; TG > 5 mmol/l, average = 15.8 ± 12.8 mmol/l. Controls ($n = 106$): age 39 ± 12 years; male/female ratio 0.57/0.43; TG < 2 mmol/l, average = 1.1 ± 0.35 mmol/l.

The patients for the extended genotyping included the initial patient group and met the following criteria. Cases ($n = 138$): age 51 ± 11 years; male/female ratio 0.7/0.3; TG > 5 mmol/l, average = 14.1 ± 14.6 mmol/l. Controls ($n = 521$): age 26 ± 18 years; male/female ratio 0.53/0.47; TG < 2 mmol/l, average = 0.99 ± 0.43 mmol/l.

Biochemical analysis—fasting triglycerides were measured using standard enzymatic techniques on a Johnson and Johnson Vitros analyser in the Biochemistry Laboratory, Royal Victoria Hospital, Belfast.

DNA was extracted from venous blood, using either the standard Guanadine HCl method [13] or the Whatman Biosciences Ltd., SNAPS system for DNA extraction from whole blood (according to the manufacturer's instruction).

2.2. Sequencing of the APOC3 and APOA5 genes

The genes were screened for SNPs by direct fluorescent sequencing in both directions in 24 unrelated HTG subjects (TG > 9 mmol/l) and 24 controls (TG < 2 mmol/l). The protocol was carried out using a 3100 DNA Genetic Analyser (Applied Biosystems), as described for the version 2 Big Dye Terminator cycle sequencing protocol. The Sequencher software (Gene Codes Corporation) was used to align the sequences to facilitate the detection of the polymorphisms. Nucleotides were numbered as suggested by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/) [14], with nucleotide number 1 being the A of the ATG methionine initiator codon.

2.3. Genotyping SNPs in the APOC3 and APOA5 genes

A number of the SNPs identified in the sequenced populations were then genotyped in larger case and control populations. These SNPs were chosen using a number of criteria. Firstly, SNPs that showed a major frequency difference between the extreme cases and controls were selected (Tables 1 and 2), along with SNPs that covered the entire gene region, taking into consideration the linkage disequilibrium evident in the raw sequencing data and the haplotype analysis (data not shown). Some SNPs were also selected for comparison with previous studies.

The *APOC3* −1236G > A, c.371T > G (previously known as 3206) and 3′ + 1313G > T variations represent detectable changes in *Sty*I, *Bbv*I and *Rsa*I restriction sites, with the minor alleles producing an extra restriction site. Sequences of the primers used for PCR direct sequencing and for initial genotyping are available in the supplementary material.

The *APOC3* −482C > T, c.34–164G > C, c.102C > T (previously known as 1100) and c.340C > G (previously known as 3175, or *Sst*I) variations along with the *APOA5* −600T > C (previously known as −1131 or SNP3), c.-3A > G and c.56C > G variations were determined using the Taqman technology on the 7000 Sequence Detection System (Applied Biosystems), as described in their protocol. Sequences of the primers and probes for Taqman genotyping are available in the supplementary material.