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The −1131 T > C and S19W APOA5 gene polymorphisms are associated with high levels of triglycerides and apolipoprotein C-III, but not with coronary artery disease: an angiographic study

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Abstract

High plasma concentrations of triglycerides (TG) and apolipoprotein C-III (ApoC-III) are well-known risk factors for cardiovascular disease. Two variants of the recently discovered APOA5, 1131 C > T and S19W, have been associated with hypertriglyceridemia, whereas their relation with coronary artery disease (CAD) remains controversial. Nine hundred and thirteen angiografically defined patients (669 CAD and 244 CAD-free) were genotyped for APOA5 −1131 C > T and S19W polymorphisms.

Carriership of the APOA5 −1131 C allele was identified, by multiple linear regression models, as a significant independent predictor for both TG (standardized β-coefficient = 0.112; $p = 0.010$) and ApoC-III variability (standardized β-coefficient = 0.113; $p = 0.013$). Similarly, APOA5 19W allele carriership was a significant independent predictor for both TG (standardized β-coefficient = 0.113; $p = 0.007$) and ApoC-III variability (standardized β-coefficient = 0.088; $p = 0.045$). Despite the association with at-risk lipid profile, no significant difference was detected in the distribution of both APOA5 gene polymorphisms between subjects with or without CAD. Moreover, homozygous carriers of the APOC3 −455 C, another TG- and ApoC-III raising variant, showed a significant increased risk for CAD (OR 1.90 with 95% CI 1.002–3.62; $p = 0.049$; by multiple logistic regression).

Different genotypes, i.e., APOA5 and APOC3 variants, may lead to similar biochemical phenotypes, namely hypertriglyceridemia, but to contrasting clinical phenotypes such as the presence of angiographically proven CAD.

Keywords: APOA5 polymorphisms; Triglycerides; Apolipoprotein C-III; Coronary artery disease

1. Introduction

High plasma triglyceride (TG) levels are a well-recognized risk factor for cardiovascular disease [1]. TG levels are strongly influenced by genetic factors, although heritability has been observed to vary widely (20–80%) in different studies [2]. Apolipoprotein gene cluster APOA1/C3/A4/A5 on chromosome 11q23 plays a pivotal role in TG metabolism [3] and the recently discovered APOA5 gene has gained attention as a key regulator of TG levels [4]. This gene is exclusively expressed in liver and its product, ApoA-V, is secreted in plasma, where it is associated with high-density lipoproteins (HDL), very low density lipoproteins (VLDL), chylomicrons, but not with low density lipoproteins (LDL) [5,6]. ApoA-V apolipoprotein is not abundant in plasma since its concentra-

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tion ranges from about 20 to 400 μg/l (0.02–0.4% of ApoA-I) [6]. Transgenic mice overexpressing APOA5 gene show decreased TG concentrations and – conversely – APOA5 knockout mice have higher TG levels than control animals. Of note, differences in cholesterol levels were not found in transgenic or in APOA5 knockout mice models [4]. In humans, APOA5 mutations, which generate a truncated protein and ApoA-V deficiency, have been associated with severe hypertriglyceridemia [7] and with hyperchylomicronemia [8]. The function of ApoA-V is not well known, thus far. However recent observations support the view that such protein may function as an activator of intravascular triglycerides hydrolysis process through lipoprotein lipase [8,9]. Furthermore, recent studies reported that APOA5 is a highly responsive peroxisome proliferator-activated receptor alpha (PPARα) target gene and fibrates can increase APOA5 expression in hepatocytes via a PPARα pathway [10].

At present, some common and potentially functional variants of APOA5 gene have been identified: a −1131 T>C substitution, located upstream the proximal promoter, and a 56 C>G mutation, causing a serine to tryptophan change at protein codon 19 (S19W). Both of these polymorphisms have been associated with hypertriglyceridaemia in several studies [11–21]. The −1131 C allele has been also related to decreased HDL levels [13,14]. Both of these variants are relatively frequent since approximately 25% of Caucasians, 35% of African-Americans and 50% of Hispanics are carriers of at least one of the mutant alleles [12].

Considering the association between high TG levels and atherosclerosis and the high prevalence of APOA5 variants in different populations, it is biologically plausible to hypothesize a link between APOA5 gene and atherosclerotic cardiovascular risk. However, whereas the association between APOA5 gene polymorphisms and TG levels has been confirmed in several studies, the relation with coronary artery disease (CAD) is at present still controversial, as shown by the different findings according to diverse gender or ethnic background [14–21].

In the present study, we investigated the distribution of −1131 T>C and S19W APOA5 polymorphisms in a sample of subjects with coronary angiography documentation, most of them with severe CAD. Concomitantly, we evaluated the impact of APOA5 gene variants on lipid profile and, in particular, on ApoC-III apolipoprotein concentrations.

ApoC-III is an essential constituent of circulating particles rich in TG (i.e., chylomicrons and VLDLs) and inhibits the hydrolysis of TG-rich particles by the lipoprotein lipase and their hepatic uptake mediated by ApoE. Thus, high levels of ApoC-III can cause hypertriglyceridemia [22]. However, the potential relationship between ApoC-III plasma levels and APOA5 gene variants has never been investigated, so far.

Two SNPs in APOC3 gene, −455 T>C and −482 C>T promoter variants, which are in strong linkage disequilibrium, have been shown to relate to a reduced affinity for the nuclear transcription factors mediating the down-regulating response to insulin, the so called “insulin resistance” at gene level [23]. Both variants have been associated with high levels of ApoC-III and hypertriglyceridemia. In previous studies, we demonstrated that APOC3 −455 C polymorphism is associated with high levels of TG and ApoC-III, and confers an increased risk for CAD, especially in subjects affected by metabolic syndrome [24,25]. Noteworthy, both APOA5 and APOC3 are sited rather closely in the APOA1/C3/A4/A5 gene cluster, and a linkage disequilibrium between APOA5 −1131 T>C and APOC3 −482 C>T variant has been also described [11]. Because a linkage between APOA5 and APOC3 gene variants could act as a confounding factor on lipid profile and CAD risk, the effects of APOC3 −455 T>C polymorphism were also considered.

2. Materials and methods

2.1. Study population

The Verona heart project is an ongoing study aimed to identify new risk factors for CAD and myocardial infarction (MI) in a population of subjects with angiographic documentation of their coronary vessels. Details about the enrolment criteria have been described elsewhere [24]. In the present study we present data on a total of 913 subjects, for whom APOA5 gene polymorphisms (−1131 T>C; S19W) were analysed. Among these 913 subjects, 669 subjects had angiographically documented severe coronary atherosclerosis (CAD group), i.e., at least one major epicardial coronary artery with >50% lumen stenosis and the majority of them being candidates to coronary artery bypass grafting.

Two hundred and forty-four remaining subjects had completely normal coronary arteries (CAD-free), and were examined for reasons other than CAD, mainly valvular heart disease. Controls were also required to have neither history nor clinical or instrumental evidence of atherosclerosis in vascular districts beyond the coronary bed. Given that the primary aim of our selection was to provide an objective and clear-cut definition of the atherosclerotic phenotype, subjects with non-significant coronary stenosis (<50%) were not included in the study. The angiograms were assessed by two cardiologists unaware that the patients were to be included in the study.

All participants came from the same geographical area (Northern Italy), with a similar socio-economic background. At the time of blood sampling, a complete clinical history was collected, including the assessment of cardiovascular risk factors such as obesity, smoking, hypertension and diabetes.

The study was approved by our local Ethical Committee. Informed consent was obtained from all the patients after a full explanation of the study.

2.2. Biochemical analysis

Samples of venous blood were drawn from each subject after an overnight fast. Serum lipids and the other rou-