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Haplotype analyses of the *APOA5* gene in patients with familial combined hyperlipidemia

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Abstract

Background: Familial combined hyperlipidemia (FCH) is the most common genetic lipid disorder with an undefined genetic etiology. Apolipoprotein A5 gene (*APOA5*) variants were previously shown to contribute to FCH. The aim of the present study was to evaluate the association of *APOA5* variants with FCH and its related phenotypes in Dutch FCH patients. Furthermore, the effects of variants in the *APOA5* gene on carotid intima-media thickness (IMT) and cardiovascular disease (CVD) were examined. *Materials and methods:* The study population consisted of 36 Dutch families, including 157 FCH patients. Two polymorphisms in the *APOA5* gene (−1131T>C and S19W) were genotyped. *Results:* Haplotype analysis of *APOA5* showed an association with FCH ($p=0.029$), total cholesterol ($p=0.031$), triglycerides ($p<0.001$), apolipoprotein B ($p=0.011$), HDL-cholesterol ($p=0.013$), small dense LDL ($p=0.010$) and remnant-like particle cholesterol ($p=0.001$). Compared to S19 homozygotes, 19W carriers had an increased risk of FCH (OR = 1.6 [1.0–2.6]; $p=0.026$) and a more atherogenic lipid profile, reflected by higher triglyceride (+22%) and apolipoprotein B levels (+5%), decreased HDL-cholesterol levels (−7%) and an increased prevalence of small dense LDL (16% vs. 26%). In carriers of the −1131C allele, small dense LDL was more prevalent than in −1131T homozygotes (29% vs. 16%). No association of the *APOA5* gene with IMT and CVD was evident. *Conclusion:* In Dutch FCH families, variants in the APOA5 gene are associated with FCH and an atherogenic lipid profile.

Keywords: Familial combined hyperlipidemia; *APOA5* gene; Haplotype; Triglyceride level; Intima-media thickness; Remnant-like particle

1. Introduction

Familial combined hyperlipidemia (FCH) is the most common lipid disorder of unknown genetic etiology, affecting 2–5% of the general population [1,2]. Major characteristics of FCH include elevated plasma levels of apolipoprotein B (apoB), triglycerides (TG) and/or total cholesterol (TC). Other phenotypes include decreased levels of high-density lipoprotein cholesterol (HDLc) and the presence of small, dense low-density lipoproteins (sdLDL). In addition, FCH patients have an increased risk of cardiovascular disease (CVD) and are often obese and insulin resistant [3].

Despite decades of research, the complex genetics of FCH are still not fully understood. Several linkage analyses were performed, leading to the identification of multiple loci for FCH [4–9]. One repeatedly identified locus is located on chromosome 11q, a site involved in modulating the expression of FCH [10–12]. This region includes the apolipoprotein *A1–C3–A4–A5* gene cluster [13]. The apolipoprotein A5 (*APOA5)* gene encodes the apolipoprotein AV protein (APOAV), which

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is exclusively expressed in the liver. APOAV is found on very low-density lipoprotein (VLDL), HDL and chylomicrons and is, compared to other apolipoproteins, present in very low plasma concentrations [14,15]. Variations in the *APOA5* gene are related to TG levels, however, the underlying mechanism is not yet fully understood [16]. One hypothesis, based on mouse studies, suggests that APOAV modulates TG levels by guiding VLDL and chylomicrons to proteoglycan-bound lipoprotein lipase for lipolysis and by increasing VLDL clearance [17,18].

In the human *APOA5* gene, several single-nucleotide polymorphisms (SNPs) have been identified (−1131T>C, −3A>G, S19W (56C>G), IVS3+476G>A and 1259T>C) [19,20]. The three major haplotypes, representing approximately 98% of all haplotypes in these populations, were defined by the −1131T>C and the S19W SNPs [19,20]. In Caucasians, the rare alleles of these two SNPs were associated with elevated plasma levels of TC, TG, remnant-like particle cholesterol (RLPc), LDL cholesterol (LDLc) and apoB, decreased HDLc levels and the presence of sdLDL [13,20,21]. In patients with FCH, *APOA5* polymorphisms were associated with an increased risk of FCH and related lipid phenotypes, including TG, apoB and HDLc levels and LDL particle size [11,22,23]. The relationship between variants in the *APOA5* gene and RLPc levels was not previously studied in FCH patients.

Hypertriglyceridemia is an independent risk factor for the development of CVD [24]. Despite its effect on plasma triglycerides, the role of *APOA5* in the development of CVD remains controversial [20,25–29]. In 372 Finnish men with CVD, who participated in the LOCAT study, it was demonstrated that only the rare allele of the S19W SNP (19W) was associated with increased progression of atherogenesis [29]. In contrast, in 2578 subjects from the general population in the Framingham Heart Study, an increased risk of CVD was found only for the rare allele of the −1131T>C SNP (−1131C) [20,29]. The results for CVD in the Framingham Heart Study, however, conflicted with the results obtained for intima-media thickness (IMT), a surrogate marker of CVD. Differences in IMT were associated with the 19W allele, but an association of the −1131C allele was only present in obese subjects [25]. In FCH patients, who have an increased risk of CVD, the relationship between variants in the *APOA5* gene and CVD was not previously examined.

The aim of the present study was to investigate the association of *APOA5* gene variants (−1131T>C and S19W) with FCH and its associated phenotypes, including RLPc levels, using a family-based SNP and haplotype approach in well-characterized FCH families. Furthermore, the suggested increased risk of CVD associated with variants in the *APOA5* gene was investigated in our FCH families by taking both intima-media thickness and CVD prevalence into account.

2. Materials and methods

2.1. Study population

Back in 1994, we have recruited FCH families from the outpatient lipid clinic of the Radboud University Nijmegen Medical Centre, ascertained through probands, exhibiting a combined hyperlipidemia with both plasma TC and TG levels above the age- and gender-related 90th percentile [30], during several periods in which they were not treated with lipid-lowering drugs, and despite dietary advice. Additionally, a first-degree relative possessed elevated levels of TC and/or TG above the 90th percentile and the proband, or a first-degree relative, suffered from premature (before the age of 60 years) cardiovascular disease. Families were excluded when probands were diagnosed with underlying diseases causing hyperlipidemia (i.e., diabetes mellitus type 1 and 2, hypothyroidism, and hepatic or renal impairment), a first-degree relative had tendon xanthomata or probands were homozygous for the *APOE2* allele. All included subjects were Caucasian and above the age of 10 years. The ascertained families had a mean size of 24 members from multiple (between 2 and 4) generations. The present FCH study population include the 5-year follow-up data of our original FCH families, consisting of 36 Dutch families, comprised of 611 subjects with known genealogic, phenotypic, and genotypic data, of whom 157 individuals were diagnosed as FCH patients [31]. In 1999, the diagnosis of FCH was based on the nomogram [31]. Plasma TG and TC levels, adjusted for age and gender, and absolute apoB levels, were applied to the nomogram to calculate a probability of being affected with FCH. When this probability of being affected with FCH is greater than 60%, the diagnostic phenotype is present in at least one first degree relative, and premature CVD (before the age of 60 years) is present in at least one individual in the family, the individual is defined as affected by FCH. Also included in the family-based analyses were the normolipidemic relatives (n=390), the unaffected spouses of both FCH patients and normolipidemic relatives (n=64), and subjects without known phenotypic and/or genotypic data (n=230) to complete the pedigree structure. After the withdrawal of lipid-lowering medication for 4 weeks and an overnight fast, blood was drawn by venipuncture. Body mass index (BMI) was calculated as body weight (kilograms) divided by the square of height (meters).

Information concerning CVD was gathered through personal interviews and physical examinations performed by the clinical investigator. When the clinical investigator suspected the presence of CVD, further details and confirmation of the diagnosis were sought from the participant's general practitioner and hospital records. CVD was defined by angina pectoris (AP), myocardial infarction (MI), stroke, peripheral vascular disease or vascular surgery. In our study population (n=611), 56 subjects were identified with CVD, including 26 subjects with AP, 25 with previous MI, 10 with peripheral vascular disease, seven with stroke and 23 who underwent vascular surgery. In total, 45% (n=25) of these individuals were diagnosed with CVD based on the presence of two or more manifestations of CVD. The ethical committee of the Radboud University Nijmegen Medical Centre approved the study protocol and all procedures were in accordance with institutional guidelines. All subjects provided written informed consent.

2.2. Biochemical analyses

Biochemical analyses were performed as previously described for this population [31]. In short, plasma TC and TG were determined by enzymatic, commercially available reagents (Boehringer-Mannheim, Germany, catalog No. 237574 and Sera Pak, Miles, Belgium, catalog No. 6639, respectively). Total plasma apoB concentrations were measured by immunonephelometry. HDLc was quantified by the polyethylene glycol 6000 method. LDL subfractions were separated by single spin density gradient ultracentrifugation. A continuous variable, K, represented the LDL subfraction profile of each individual. A negative K-value ($K \leq -0.1$) reflected the presence of small dense LDL [31]. RLPc levels were measured using an immunoseparation technique as described elsewhere [32]. Glucose concentrations were analyzed using the oxidation technique (Beckman®, Glucose Analyser2, Beckman Instruments Inc., Fullerton, CA 92634, USA). Plasma insulin concentrations were ascertained by a double antibody method. Insulin resistance was assessed by the homeostasis model assessment (HOMA) method.

DNA was extracted from peripheral blood lymphocytes using a standard technique [33]. Genotyping for the S19W and the −1131T>C SNPs was carried out by PCR and restriction enzyme digestion, as previously described [34]. Genotyping of the S19W and −1131T>C SNPs failed in 5% and 3% of the subjects, respectively.