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Synergistic effects of the apolipoprotein E ε3/ε2/ε4, the cholesteryl ester transfer protein TaqIB, and the apolipoprotein C3 −482 C > T polymorphisms on their association with coronary artery disease

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Abstract

The single nucleotide polymorphisms (SNPs) apolipoprotein E (APOE) ε3/ε2/ε4, cholesteryl ester transfer protein (CETP) TaqIB, and apolipoprotein C3 (APOC3) −482 C > T have been associated with an atherogenic lipid profile and, in some studies, with increased cardiovascular risk. However, no data exist on their combined impact on atherosclerotic disease. We therefore aimed at investigating the combined impact of these SNPs on the presence of angiographically determined coronary artery disease (CAD). Genotyping was performed in 557 consecutive Caucasian patients undergoing coronary angiography for the evaluation of CAD.

From the individual SNPs, only the APOE ε3ε4/ε4ε4 genotype was significantly associated with an increased risk of significant coronary stenoses with lumen narrowing ≥50% (odds ratio (OR) = 1.77 [1.16–2.71]; $p = 0.008$). However, the risk of CAD strongly increased when more than one of the analysed genetic variants was present: ORs were 2.74 [1.29–5.83]; $p = 0.009$ for patients with both the APOE ε3ε4/ε4ε4 and the CETP B1B1 genotype, 1.97 [1.06–3.66]; $p = 0.031$ for patients with both the APOE ε3ε4/ε4ε4 genotype and the APOC3 −482T allele, 2.12 [1.31–3.44]; $p = 0.002$ for patients with both the CETP B1B1 genotype and the APOC3 −482T allele, and 3.99 [1.57–13.79]; $p = 0.029$ for patients with all three variants. Multivariate analyses confirmed these results.

We conclude that there are strong synergistic effects of the APOE ε3/ε2/ε4, the CETP TaqIB, and the APOC3 −482 C > T polymorphisms on their association with CAD.

Keywords: Coronary disease; Angiography; Dyslipidemias; Single nucleotide polymorphism; Multifactorial inheritance

1. Introduction

Coronary artery disease (CAD) is a multi-factorial disease. Lipid abnormalities (including high low density lipoprotein (LDL) cholesterol, high triglycerides, high Lp(a), small LDL particle size, and low high density lipoprotein (HDL) cholesterol) have been identified as major risk factors for the susceptibility to CAD. Serum lipid levels are partly genetically determined. In particular, the polymorph genes coding for apolipoprotein E (apoE), cholesteryl ester transfer protein (CETP), and apolipoprotein CIII (apoCIII) were found to play key roles in cholesterol transport and triglyceride metabolism. Indeed, single nucleotide polymorphisms (SNPs) of these genes have been linked to dyslipidemia and thus have been assumed to influence cardiovascular risk.

ApoE is a protein component of triglyceride-rich lipoproteins and of HDL. There are three apoE isoforms (apoE3, E2, and E4), which are genetically determined by three

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alleles (ε3, ε2, and ε4) [1,2]. Compared to the most common ε3/ε3 genotype, genotypes including the ε4 allele have been associated with higher serum levels of total cholesterol, LDL cholesterol (LDL-C), and triglycerides (TG) and with a smaller LDL particle size [3–5]. Concordant with its association with these atherogenic lipid phenotypes, the ε4 allele has been shown to be a genetic risk factor for the development of cardiovascular disease [6–8].

CETP drives the exchange of cholesteryl esters and triglycerides between HDL particles and TG-rich lipoproteins [9,10]. The B2 allele of the CETP TaqIB polymorphism has been associated with lower plasma CETP concentrations, and consequently, with increased HDL-cholesterol (HDL-C) levels. In several (but not in all) studies the B2 allele was associated with decreased cardiovascular risk [11–14].

ApoCIII is a protein component of triglyceride-rich lipoproteins and an inhibitor of lipoprotein lipase. Overexpression of the gene encoding for apoCIII, APOC3, has been associated with elevated serum triglyceride levels [15,16]. A −482 C > T polymorphism is located within the promoter region of the APOC3 gene, which potentially affects its gene expression. Associations of this SNP with high triglycerides and low HDL cholesterol have been found [17–19], but significant associations between the APOC3 −482 C > T SNP and cardiovascular disease have not been reported yet [20].

Previous studies have addressed the influence of DNA-polymorphisms or haplotypes of individual genes. However, genetic factors typically drive cardiovascular disease in a polygenic manner [21]. Potential synergistic effects of the APOE ε3/ε2/ε4, the CETP TaqIB, and the APOC3 −482 C > T polymorphisms on the risk of CAD have not been investigated until now. In the present study we therefore aimed at investigating the combined impact of these three SNPs on angiographically determined CAD in a large cohort of angiographied coronary patients.

2. Methods

2.1. Study subjects

The design of this investigation has been described in detail previously [22]. In brief, we obtained EDTA blood samples for DNA preparation from 560 consecutive Caucasian patients referred to coronary angiography for routine evaluation of established or suspected CAD. Information on conventional cardiovascular risk factors (history of smoking, hypertension, and established diabetes mellitus) was obtained by a standardized interview. Systolic/diastolic blood pressure was measured by the Riva-Rocci method under resting conditions. Diabetes mellitus was diagnosed according to WHO criteria [23]. Height and weight were recorded and body mass index (BMI) was calculated as body weight (kg)/height $(m)^2$. Patients with diabetes mellitus type 1 ($n = 3$) were excluded from the analyses. Coronary angiography was performed with the Judkins technique, and significant CAD was diagnosed in the presence of significant coronary stenoses with lumen narrowing of at least 50%, as described previously [24,25]. The ethics committee of the University of Innsbruck approved the present study, and all participants gave written informed consent.

2.2. Measurement of biochemical variables

LDL-C was measured directly (QuantolipLDL, Immuno, Austria). The serum levels of triglycerides, total cholesterol, and HDL cholesterol were determined by using enzymatic hydrolysis and precipitation techniques (Triglycerides GPO-PAP, CHOD/PAP, Roche, Switzerland and QuantolipHDL, Immuno, Austria) on a Hitachi-Analyzer 717 or 911.

2.3. Genotyping

Genomic DNA was extracted from EDTA blood using the peqGOLD® Blood DNA Mini kit (PEQLAB Biotechnologie Ltd., Erlangen, Germany). Genotyping of all investigated SNPs was carried out by the 5′ nuclease assay using TaqMan® MGB probes on an ABI Prism® 7000 Sequence Detection System (Applied Biosystems, Forster City, CA). TaqMan® MGB probes were allele-specifically labelled with FAM™ and VIC™ reporter dyes, respectively. TaqMan® MGB probes for determination of the Cys112Arg and Arg158Cys polymorphisms of the APOE gene and of the TaqIB polymorphism of the CETP gene were provided together with corresponding PCR primers by the Assay-on-demand™ service (Applied Biosystems) as a 20× primer/probe mix. For the determination of the APOC3 C −482T SNP corresponding allele specific probes and PCR primers were designed using the Primer Express Software v.2.0 (Applied Biosystems). The PCR primers and MGB-probes used for the assay were the following: GAGCTCAGCCCTGTAACCAG (forward primer), AACACAGCCTGGAGTAGAGGG (reverse primer), VIC-TGATGCCCGGTCTT (C-allele probe), FAM-TGATGCCTGGTCTT (T-allele probe).

The 5′ nuclease assay was performed in a 15 μl volume, comprising 30–100 ng genomic DNA, 1× TaqMan® Universal PCR Master Mix (Applied Biosystems), and 1× primer/probe mix under the following amplification conditions: 2 min at 50 °C, 10 min at 95 °C and 40 cycles at 92 °C for 15 s and 60 °C for 1 min. After measurement of the allele-specific fluorescence, SDS (version 1.1) software was used for allelic discrimination.

2.4. Statistical analysis

Differences in baseline characteristics were tested for statistical significance with the Chi-squared test and the Student's unpaired *t*-test for categorical and continuous variables, respectively. Values which were not normally distributed (i.e. BMI, HDL-C, and TG serum levels) were log-transformed prior to statistical analysis. The distribution of continuous variables is given as mean ± S.D. (of non log-