

Synergistic Effects of the Apolipoprotein E $\epsilon 3/\epsilon 2/\epsilon 4$, 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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INTRODUCTION

Coronary artery disease (CAD) is a multi-factorial disease. Lipid abnormalities (including high LDL cholesterol, high triglycerides, high Lp(a), small LDL particle size, and low 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 high density lipoprotein (HDL). There are three apoE isoforms (apoE3, E2, and E4), which are genetically determined by three alleles ($\epsilon 3$, $\epsilon 2$, and $\epsilon 4$). Compared to the most common $\epsilon 3/\epsilon 3$ genotype, genotypes including the $\epsilon 4$ allele have been associated with higher serum levels of total cholesterol, low density cholesterol (LDL), and triglycerides (TG) and with a smaller LDL particle size. Concordant with its association with these atherogenic lipid phenotypes, the $\epsilon 4$ allele has been shown to be a genetic risk factor for the development of cardiovascular disease (Song Y, et al. 2004). CETP drives the exchange of cholesteryl esters and triglycerides between HDL particles and TG-rich lipoproteins. 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 (Boekholdt SM, et al. 2005). 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. 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 (Li WW, et al. 1995), but significant associations between the APOC3 -482 C>T SNP and cardiovascular disease have not been reported yet. 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 $\epsilon 3/\epsilon 2/\epsilon 4$, 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 angiographed coronary patients.

MATERIAL AND METHODS

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. 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%. 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). To evaluate the association of individual and combined genotypes with the presence of angiographic CAD we applied logistic regression analyses. Statistical significance was defined as a two-tailed p value <0.05 . Statistical analyses were performed with the software package SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL). The ethics committee of the University of Innsbruck approved the present study, and all participants gave written informed consent.

RESULTS AND DISCUSSION

The demographic data of the 557 included patients were characteristic for a cohort undergoing coronary angiography for the evaluation of CAD, with a mean age of 62.1 ± 10.1 years, a preponderance of male gender ($n = 387$; 69.5%), and a high prevalence of type 2 diabetes mellitus ($n = 119$; 21.4%), hypertension ($n = 294$; 52.8%), and smoking ($n = 328$; 58.9%). Angiography revealed that 332 patients (59.6%) had significant coronary stenoses. Table 1 shows the distribution of the individual APOE $\epsilon 3\epsilon 4/\epsilon 4\epsilon 4$, CETP B1>B2, and APOC3 $-482C>T$ genotypes with respect to the presence of significant coronary stenoses. For further evaluations of the CAD risk conferred by these polymorphisms, the putative risk genotypes APOE $\epsilon 3\epsilon 4/\epsilon 4\epsilon 4$, CETP B1B1, and APOC3 $-482CT/TT$ were compared versus carriers of all remaining genotypes individually, and also in pair-wise as well as in triple combinations.

The associations of individual and combined genotypes with angiographically determined significant stenoses are presented in Figure 1. Of the individual genotypes only the APOE $\epsilon 3\epsilon 4/\epsilon 4\epsilon 4$ genotype was associated with significant stenoses in univariate analyses (OR = 1.77 [1.16-2.709]; $p = 0.008$). When genotype combinations were considered, patients who simultaneously carried the APOE $\epsilon 3\epsilon 4/\epsilon 4\epsilon 4$ and the CETP B1B1 genotype showed a significantly increased prevalence of significant stenoses, with an OR of 2.74 [1.29-5.83], $p = 0.009$. The prevalence of significant stenoses was also significantly increased in carriers of both the APOE $\epsilon 3\epsilon 4/\epsilon 4\epsilon 4$ genotype and the APOC3 $-482T$ allele (OR = 1.97 [1.06-3.66], $p = 0.031$). Despite the weak individual associations between the CETP B1B1 genotype and APOC3 $-482T$ alleles with CAD, the simultaneous presence of both genetic markers resulted in a highly significant association with significant coronary stenoses (OR = 2.12 [1.31-3.44]; $p = 0.002$). Patients who carried all three risk genotypes were at the highest risk of significant coronary stenoses (OR = 3.99 [1.57-13.79]; $p = 0.029$).

Our study demonstrates that there are strong synergistic effects of the apolipoprotein E $\epsilon 3/\epsilon 2/\epsilon 4$, the cholesteryl ester transfer protein TaqIB, and the apolipoprotein CIII -482 C>T polymorphisms on their association with CAD. The combination of DNA polymorphisms of these pathophysiologically interacting genes surpasses the effect of the individual DNA polymorphisms on CAD risk.

This is the first study investigating in combined genotype models the effect of the APOE $\epsilon 3/\epsilon 2/\epsilon 4$, the CETP TaqIB and the APOC3 C-482T gene polymorphisms on the susceptibility to CAD. Although numerous studies have investigated the associations of individual SNPs or haplotypes of single genes on cardiovascular risk, only few reports exist on a polygenic effect of DNA-polymorphisms on the susceptibility to CAD. Remarkably, during writing this manuscript, results from a large association-study using the strategy of combining various SNPs involved in lipid-transport were published (Kathiresan S, et al. 2008). According to our findings, the study demonstrated a significant influence of combined SNPs on cardiovascular risk classification.

CONCLUSION AND OUTLOOK

We conclude that there are strong synergistic effects of the apolipoprotein E $\epsilon 3/\epsilon 2/\epsilon 4$, the cholesteryl ester transfer protein TaqIB, and the apolipoprotein C3 -482 C>T polymorphisms on the risk of angiographically characterized CAD. Data now are required that confirm our findings in different patient populations. Finally, our results suggest that future studies addressing the impact of SNPs on cardiovascular disease should always consider possible risk modification by combinations of risky genotypes.

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TABLES AND FIGURES

Table 1: Genotype distributions in patients with significant coronary stenoses and in patients without such lesions

Gene	No significant stenoses n = 225	Significant stenoses n = 332	p value
CETP (%)			
B1B1	31.6	37.7	0.270
B1B2	51.6	48.8	
B2B2	16.9	13.6	
APOC3 (%)			
-482 CC	52.0	48.8	0.568
-482 CT	38.7	43.1	
482 TT	9.3	8.1	
APOE (%)			
$\epsilon 2\epsilon 2$	0.4	0.3	0.081
$\epsilon 2\epsilon 3$	15.6	12.7	
$\epsilon 3\epsilon 3$	65.8	59.1	
$\epsilon 3\epsilon 4$	17.3	25.3	
$\epsilon 4\epsilon 4$	0.0	1.8	
$\epsilon 2\epsilon 4$	0.9	0.9	

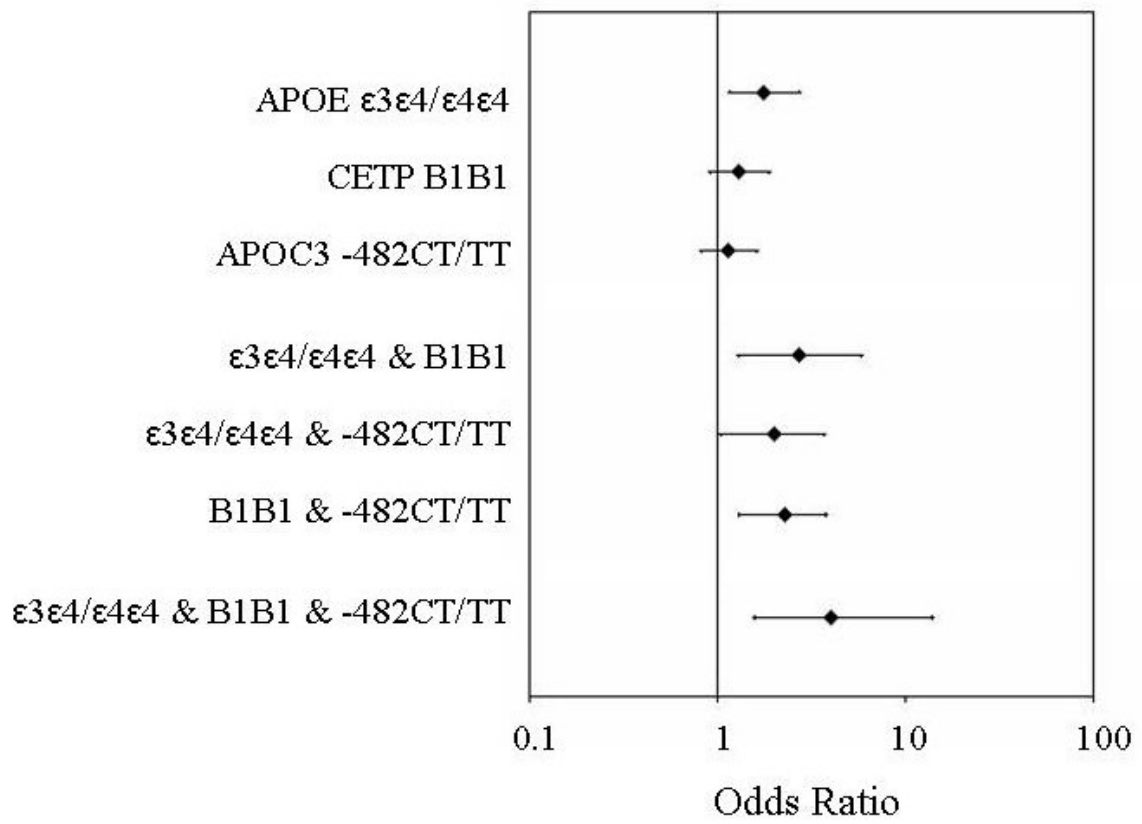


Figure 1: Associations of individual and combined genotypes with the presence of significant stenoses $\geq 50\%$.