Association of Transforming Growth Factor-β1 Gene Polymorphisms With Myocardial Infarction in Patients With Angiographically Proven Coronary Heart Disease

Werner Koch, Petra Hoppmann, Jakob C. Mueller, Albert Schömig, Adnan Kastrati

Objective—Transforming growth factor (TGF)-β1 (TGF-β1) is a multifunctional cytokine that exhibits vasculoprotective properties. Production and plasma levels of TGF-β1 are influenced by polymorphisms in the TGF-β1 gene (TGFB1). We investigated whether the −509C/T (rs1800469), 868T/C (rs1982073), 913G/C (rs1800471), and 11929C/T (rs1800472) polymorphisms of TGFB1 are associated with myocardial infarction.

Methods and Results—The study population consisted of 3657 patients with myocardial infarction (885 women and 2772 men) and 1211 control individuals (598 women and 613 men) with angiographically normal coronary arteries and without signs or symptoms of myocardial infarction. Polymorphism-related genotypes were determined with TaqMan assays and haplotypes were estimated from the genotype data. The −509C/T polymorphism and −509C/868T/913G/11929C (CTGC) haplotype were associated with myocardial infarction in men, independently from the potentially confounding factors age, arterial hypertension, hypercholesterolemia, cigarette smoking, and diabetes mellitus. Lower risks of myocardial infarction were observed among the carriers of the −509CC genotype (adjusted odds ratio [OR], 0.49; 95% confidence interval [CI], 0.27 to 0.87; P=0.014) and homozygous carriers of the CTGC haplotype (adjusted OR, 0.61; 95% CI, 0.38 to 0.98; P=0.042) than among the noncarriers of this genotype or haplotype. None of the genotypes (P≥0.37) or haplotypes (P≥0.35) was associated with myocardial infarction in women.

Conclusions—Positive association findings in this study suggest that TGFB1 is a susceptibility locus for myocardial infarction. (Arterioscler Thromb Vasc Biol. 2006;26:1114-1119.)

Key Words: haplotype ■ myocardial infarction ■ TGFB1 ■ transforming growth factor-β1 ■ SNP

An important function in the maintenance of arterial wall integrity has been attributed to transforming growth factor-β1 (TGF-β1), a multifunctional cytokine that signals through a well-characterized pathway of transmembrane serine-threonine kinase receptors and intracellular signaling molecules.1–3 Biologic effects of TGF-β1 range from the regulation of target gene activity and cell function to the control of cell growth and differentiation. The vasculoprotective properties of TGF-β1 include the inhibition of the adhesion of neutrophils and T cells to the endothelium, the transmigration of neutrophils through the endothelium, and the production of proinflammatory adhesion molecules in endothelial cells, both in the presence and absence of proinflammatory cytokines.4–7 TGF-β1 is one of the primary differentiation factors for vascular smooth muscle cells and prevents their dedifferentiation, proliferation, and migration.1,8 Macrophage deactivation is mediated by TGF-β1, as indicated by its ability to suppress inducible nitric oxide synthase, interfere with the production of proinflammatory cytokines, and inhibit macrophage foam cell formation.9–12 In addition, TGF-β1 has inhibitory effects on T-cell differentiation and proliferation, either directly or through its suppressing function on antigen-presenting cells.2,11 The protective properties of TGF-β1 may be functional not only in the initial stages of atherosclerosis but also against disease progression, because plaque-stabilizing activities in advanced lesions have been attributed to TGF-β1.1,2,13–15

A number of studies have attempted to determine whether naturally occurring polymorphisms in the TGF-β1 gene (TGFB1; gene ID: 7040; gene map locus 19q13.1 to 13.3) affect TGFB1 expression and TGF-β1 production and are associated with coronary artery disease and myocardial infarction (MI).16–24 A C-to-T single nucleotide polymorphism (SNP) at position −509 relative to the first major transcription start site (−509C/T SNP; rs1800469) was found to be differentially related with transcription factor binding to the TGFB1 promoter, transcriptional activity of TGFB1, and TGF-β1 plasma concentration.21,24,25 Two SNPs, the 868T/C SNP (rs1982073) and the 913G/C SNP (rs1800471), are located at positions 29 and 74 of the translated sequence of

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**TGFB1** and give rise to amino acid substitutions at positions 10 (Leu10Pro) and 25 (Arg25Pro) in the signal peptide of TGFB1, respectively.16,17 The 868T/C SNP was reported to influence steady-state concentrations of **TGFB1** mRNA in peripheral blood mononuclear cells and serum levels of TGFB1, and the 913G/C SNP was found to be related to TGFB1 production in peripheral blood leukocytes.17,20,22,23 Another nonsynonymous SNP of **TGFB1**, the 11929C/T SNP (Thr263Ile; rs1800472), is located in exon 5.16,17 The 11929C/T SNP is located closely to the site where the latency-associated peptide is cleaved from the active part of the protein26 and, therefore, this SNP may be related to the activation process of TGFB1, as suggested previously.16,18 A high degree of linkage disequilibrium was observed between pairs of the −509C/T, 868T/C, 913G/C, and 11929C/T SNPs in white populations.16,18

Although no relationship of the −509C/T, 868T/C, 913G/C, and 11929C/T SNPs with coronary artery disease was found in previous studies, the 868T/C SNP was observed to be associated with MI in a Japanese population and the 913G/C SNP, but not the 868T/C SNP, was reported to be related with MI in some European populations.16,18,19,22 Among Japanese men, but not women, the carriers of the 868T allele (868TT and 868TC genotypes) were significantly more frequent in the patient group than in the control group.22 In the European populations in which an association between the 913G/C SNP and MI was found, the 913C allele was present more often among patients than among control subjects.16 Taken together, current evidence on the role of the **TGFB1** gene polymorphisms in cardiovascular disease shows conflicting results, probably related to the definition of the phenotype of interest.16,18,19,22 Positive associations have been found between **TGFB1** polymorphisms and MI in studies that included control individuals without angiographically verified presence of coronary artery disease.16,22 On the other side, no association has been found when angiographic coronary artery disease has been the phenotype of interest.16,19 The present study included patients with proven MI and control individuals with angiographically established absence of coronary artery disease.

To assess the role of **TGFB1** in MI, we conducted genotype and haplotype analyses of **TGFB1** SNPs (−509C/T, 868T/C, 913G/C, and 11929C/T SNPs) in a large white population.

**Methods**

**Patients and Controls**

The study population consisted of 3657 white patients with MI and 1211 white control individuals. All patients and control persons were examined with coronary angiography at Deutsches Herzzentrum München or 1. Medizinische Klinik rechts der Isar der Technischen Universität München. The diagnosis of MI was established in the presence of chest pain lasting >20 minutes combined with ST-segment elevation or pathologic Q waves on a surface ECG. Patients with MI had to show either an angiographically occluded infarct-related artery or regional wall motion abnormalities corresponding to the electrocardiographic infarct localization, or both. Individuals were considered disease-free and, therefore, eligible as controls when their coronary arteries were angiographically normal and when they had no history of MI, no symptoms suggestive of MI, no electrocardiographic signs of MI, and no regional wall motion abnormalities. Written informed consent was obtained from all study participants. The study protocol was approved by the Institutional Ethics Committee and the reported investigations were in accordance with the principles of the Declaration of Helsinki.

Systemic arterial hypertension was defined as a systolic blood pressure of ≥140 mm Hg, and/or a diastolic blood pressure of ≥90 mm Hg,27 at least on 2 separate occasions, or antihypertensive treatment. Hypercholesterolemia was defined as a documented total cholesterol value ≥240 mg/dL (≥6.2 mol/L) or current treatment with cholesterol-lowering medication. Persons reporting regular smoking in the previous 6 months were considered as current smokers. Diabetes mellitus was defined as the presence of an active treatment with insulin or an oral antidiabetic agent; for patients administered dietary treatment, documentation of an abnormal fasting blood glucose or glucose tolerance test based on the World Health Organization criteria28 was required for establishing this diagnosis.

**Genotyping of **TGFB1** SNPs**

TagMan assays (Applied Biosystems, Darmstadt, Germany) were established and used for genotyping of the **TGFB1** SNPs. The structures of primers and probes used in the TaqMan reactions are shown in Table 1. To verify the accuracy of the new TagMan systems, a limited number of samples (n=100) were reanalyzed using the allele-discriminating restriction enzymes Eco81I (MBI Fermentas, St. Leon-Rot, Germany; −509C/T SNP), BglII (MBI Fermentas; 868T/C and 913G/C SNPs), and BceI (New England BioLabs, Frankfurt a.M., Germany; 11929C/T SNP). At least 5 samples of each genotype were included in this test. The results obtained with TaqMan assays and restriction enzyme analyses were in full agreement. Repetition of genotyping of each SNP in 20% of the study participants provided confirmation of results without exception. The genetic analyses were performed without knowledge of the case or control status of the DNA samples.

Nucleotide sequencing was used to examine the probe-binding sections of the amplicons for the presence of one or more further polymorphisms, because such additional heterogeneities may result in mistyping. We used 100 randomly selected DNA samples to analyze each of the 4 different amplicons and did not find sequence variabilities except the SNPs that were to be investigated. Thus, the probability was relatively low that wrong genotype assignments were made because of possible further variations within the probe-binding sequences.

**Statistical Analysis**

We compared the distributions of genotypes, haplotypes, and haplotype-based diplotypes between the control group and the group of patients with MI. In addition, separate analyses were performed in the groups of men and women because a sex-specific association of the 868T/C SNP with MI was observed in a Japanese population.22 Genotype distributions in the control and case groups were compared with values predicted by Hardy-Weinberg equilibrium using the χ² test. Discrete variables are expressed as counts (%) and were compared by the χ² test. Continuous variables are expressed as mean±SD and were compared by means of the unpaired 2-sided t test. The PHASE software package was used to infer haplotype patterns from genotype data.29 We tested for the independent association of SNP-related genotypes and diplotypes in multiple logistic regression models of MI that included as covariates age, gender, history of arterial hypertension, history of hypercholesterolemia, current cigarette smoking, and diabetes mellitus. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated on the basis of the multiple logistic regression models.

**Results**

Baseline clinical characteristics of the control group and the group of patients with MI are shown in Table 2. The data suggested that there may be differences in the genotype distributions of the −509C/T and 868T/C SNPs between the control group and the patient group (P=0.996) (Table 3).
Analysis of the 913G/C and 11929C/T SNPs indicated similar genotype distributions between the groups (P≥0.33) (Table 3). In either group, the genotype distributions were consistent with those expected for samples in Hardy-Weinberg equilibrium.

Genotype distributions of the TGFB1 SNPs were compared separately for women and men. The distributions were not different between the women of the control and case groups (P≥0.37) (Table 4). Substantially different genotype distributions of the −509C/T and 868T/C SNPs were present among the men of the control and patient groups (P<0.0070) (Table 4). In contrast, genotype distributions of the 913G/C and 11929C/T SNPs were similar among the men of the study groups (P≥0.39) (Table 4). The genotype distributions among the women and men of the control and patient groups were in Hardy-Weinberg equilibrium.

To address the possibility of combined effects of the TGFB1 SNPs, we examined whether associations existed between SNP-based haplotypes and MI. The frequencies of the 4 most abundant haplotypes in the women and men of the study groups (rs1800471) 913GC 141 (11.6) 486 (13.3) 913G/C 913GG 1063 (87.8) 3149 (86.1) 0.33

868T/C 868TT 458 (37.8) 1235 (33.8) 0.035

TCGATAGTCTTGCAGGTGGATAGTC VIC-CGGCCGGCGGGGC-TAMRA (for 913C)

CCAGGCGTCAGCACCAGTA VIC-AGCAGCGGCAGCA-MGB-NFQ (for 868C)

GCCGGGACCTTTGCAAGVIC-TCATGGCCATCCCGCTGGA-TAMRA (for 11929T)

SNP Genotype Control Group (n=1211) MI Patients (n=3657) P

−509C/T −509CC 564 (46.6) 1581 (43.2) 0.096

(rs1800469) −509CT 508 (41.9) 1659 (45.4)

−509TT 139 (11.5) 417 (11.4)

868T/C 868TT 458 (37.8) 1235 (33.8) 0.035

(rs1982073) 868TC 565 (46.7) 1802 (49.3)

868CC 180 (15.5) 620 (17.0)

913G/C 913GG 1063 (87.8) 3149 (86.1) 0.33

(rs1800471) 913GC 141 (11.6) 486 (13.3)

913CC 7 (0.6) 22 (0.6)

11929C/T 11929CC 1138 (94.0) 3421 (93.5) 0.80

(rs1800472) 11929CT 72 (5.9) 231 (6.3)

11929TT 1 (0.1) 5 (0.1)

Variables are presented as number (%) of controls and patients.
tions, we tested for independent effects of the CTGC and TCGC haplotypes in multiple logistic regression models of MI. The risk of MI was lower in the homozygous carriers of the CTGC haplotype than in the non-carriers of this haplotype (adjusted OR, 0.61; 95% CI, 0.38 to 0.98; \( P = 0.042 \)). When the homozygous carriers and the non-carriers of the TCGC haplotype were compared, no independent effect of this haplotype on the risk of MI was detected (adjusted OR, 1.12; 95% CI, 0.67 to 1.86; \( P = 0.66 \)).

Discussion

In line with findings in univariate analyses, the results of multiple logistic regression analyses showed that the risk of MI was lower in the men homozygous for the −509C allele or CTGC haplotype than in those who were not homozygous for this allele or haplotype. Contrary to univariate data that pointed to associations with MI, the results of multivariate analyses indicated that the 868T/C SNP and TCGC haplotype were not related to MI risk in the men. The 913G/C and 11929C/T SNPs were apparently neutral because they did not contribute measurable effects. We do not know why associations are apparent in men only but not in women. However, such observations are not unusual because sex-specific relationships between SNPs and MI were found also in previous studies.\(^2\)\(^2\),\(^3\)

In this consecutive series of study participants, systematic examinations, including electrocardiography, left ventricular angiography, and coronary angiography, enabled us not only to determine safely the cases with MI but also to select individuals with a well-defined control status. The control individuals were examined for chest pain and did not constitute a typical control group of age-matched and sex-matched subjects that were recruited from the general population. However, the control group appeared not to be fundamentally different from normal control groups, because the genotype distributions were similar to those observed in other control groups or population-based samples that consisted of white individuals.\(^1\)\(^6\),\(^1\)\(^8\),\(^1\)\(^9\),\(^2\)\(^3\),\(^2\)\(^4\),\(^3\)\(^1\)–\(^3\)\(^3\)

The possibility exists that the positive association findings shown here were not based on real effects of SNPs or haplotypes, but rather reflect unknown differences in population ancestry between the case and control groups.\(^3\)\(^4\),\(^3\)\(^5\) We

| TABLE 4. Genotype Distributions of the \(TGFB1\) −509C/T, 868T/C, 913G/C, and 11929C/T SNPs in the Women and Men of the Control and Patient Groups |
|---|---|---|---|---|---|---|---|
| Genotype | Control Group (n=598) | MI Patients (n=885) | Control Group (n=613) | MI Patients (n=2772) |
| −509CC | 255 (42.6) | 377 (42.6) | 0.82 | 309 (50.4) | 1204 (43.4) | 0.0070 |
| −509CT | 266 (44.5) | 403 (45.5) | 242 (39.5) | 1256 (45.3) |
| −509TT | 77 (12.9) | 105 (11.9) | 62 (10.1) | 312 (11.3) |
| 868TT | 213 (35.6) | 297 (33.6) | 0.57 | 245 (40.0) | 938 (33.8) | 0.0061 |
| 868TC | 278 (46.5) | 436 (49.3) | 287 (46.8) | 1366 (49.3) |
| 868CC | 107 (17.9) | 152 (17.2) | 81 (13.2) | 468 (16.9) |
| 913GG | 528 (88.3) | 770 (87.0) | 0.47 | 535 (87.3) | 2379 (85.8) | 0.39 |
| 913GC | 68 (11.4) | 108 (12.2) | 73 (11.9) | 378 (13.6) |
| 913CC | 2 (0.3) | 7 (0.8) | 5 (0.8) | 15 (0.5) |
| 11929CC | 559 (93.5) | 820 (92.7) | 0.37 | 579 (94.5) | 2601 (93.8) | 0.52 |
| 11929CT | 38 (6.4) | 65 (7.3) | 34 (5.5) | 166 (6.0) |
| 11929TT | 1 (0.2) | 0 | 0 | 5 (0.2) |

Variables are presented as number (%) of women or men in the control and patient groups.

| TABLE 5. Haplotypes of the \(TGFB1\) −509C/T, 868T/C, 913G/C, and 11929C/T SNPs in the Women and Men of the Control and Patient Groups |
|---|---|---|---|---|---|---|---|
| Haplotype | Control Group (1196 haplotypes) | MI Patients (1770 haplotypes) | Control Group (1226 haplotypes) | MI Patients (5544 haplotypes) |
| CTGC | 701 (58.6) | 1029 (58.1) | 0.80 | 777 (63.4) | 3234 (58.3) | 0.0011 |
| TCGC | 379 (31.7) | 547 (30.9) | 0.65 | 332 (27.1) | 1703 (30.7) | 0.012 |
| CCC | 72 (6.0) | 122 (6.9) | 0.35 | 83 (6.8) | 406 (7.3) | 0.50 |
| TCCT | 39 (3.3) | 65 (3.7) | 0.55 | 34 (2.8) | 173 (3.1) | 0.52 |
| Other | 5 (0.4) | 7 (0.4) | 0.88 | 0 | 28 (0.5) |

Data are presented as number (%) of haplotypes in the women and men of the control and patient groups. Each haplotype is defined as a specific chromosome-based allele combination estimated from the genotypes of 4 SNPs in \(TGFB1\). The order of the alleles in the haplotypes is in accordance with the relative chromosomal positions of the SNPs (from left to right): −509C/T, 868T/C, 913G/C, and 11929C/T.
consider the probability of false-positive inference attributable to population stratification rather small, because the patients and control individuals were recruited from an ethnically homogeneous population with no indication of a significant amount of recent genetic admixture.

Cambien et al assessed the relationships of polymorphisms in TGFβ1, including the 4 SNPs examined here, with MI and other diseases in men (563 cases and 629 age-matched control individuals) from 4 regions in France and Northern Ireland.16 Their data suggested that one of the tested polymorphisms, the 913G/C SNP, and MI were significantly associated, with a higher prevalence of the 913C allele carriers in the patient group than in the control group (17.6% versus 13.2%).16 When the 4 regional samples were examined separately from each other, such an association was evident only in 2 of them.16 Essential results reported by Cambien et al16, positive with regard to the 913G/C SNP and negative with regard to the −509C/T SNP, were not in line with our findings on these SNPs. Differences in particular characteristics between the study samples may have accounted for the opposite results. The patients in the present study were older than the patients examined by Cambien et al.16 All participants of the present study underwent coronary angiography, whereas in the population from France and Northern Ireland, coronary angiography was incomplete in the patient group and not performed in the control group.16,36

Yokota et al investigated the association of the 868T/C SNP with MI in a Japanese population and they found a significantly higher frequency of the 868T allele in the men of the patient group (n = 234) than in the men of the control group (n = 289).22 The frequencies of the 868T allele were not significantly different between the women of the patient group (n = 81) and the women of the control group (n = 302).22 Although univariate analysis suggested an association of the 868T/C SNP with MI in the men of the present population, our data rather pointed to a protective effect of the 868T allele than to a risk effect as observed in the men of the Japanese sample.22 Different genetic backgrounds between whites and Japanese may explain, at least in part, the disparate findings of the studies. The possible existence of ethnicity-specific association profiles may be reflected by the significantly different allele frequencies and genotype distributions of the 868T/C SNP between the present white population and populations from Japan, including the sample examined by Yokota et al.20,22,37 Heterogeneities in the prevalences of conventional risk factors for MI, including arterial hypertension, hypercholesterolemia, cigarette smoking, and diabetes mellitus, may have contributed also to the divergent results between the study populations.

Our finding of an association of the CTGC haplotype with MI risk in men indicated that TGFβ1 is a susceptibility locus for MI. The present results require verifications in independent white samples and their validity in non-whites needs to be examined separately. Common complex human diseases, such as MI, are thought to be under the control of many genes that contribute modest individual effects, and TGFβ1 may act in concert with or independently from other MI susceptibility loci.

Limitations
This study has not examined the relationship of the TGFβ1 genotypes and haplotypes with coronary heart disease in patients without MI. Because 20% to 30% of patients who develop an acute coronary event die before arrival at the hospital, a considerable number of potentially eligible patients with acute MI were probably not included in the study. The ratio of cases and control individuals differed markedly between the groups of women and men, and this statistical power difference may underlie the apparent sex-specific effect.

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References


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