Thrombospondin-2 Polymorphism Is Associated With a Reduced Risk of Premature Myocardial Infarction


Objective—Recently, polymorphisms in thrombospondin (THBS) genes coding for THBS-1 (N700S), THBS-2 (T>G substitution in 3′-untranslated region), and THBS-4 (A387P) genes were proposed to modulate the risk of premature coronary artery disease (CAD) or myocardial infarction (MI). It was our objective to verify this hypothesis in an independent cohort.

Methods and Results—We performed a case-control study among patients (n=503) referred to our institution for symptomatic CAD that occurred before the age of 50 years and a group of age- and sex-matched population-based controls free of CAD (n=1071). The THBS-1 variant allele was not associated with an altered risk of premature CAD or MI. Homozygosity for the THBS-2 variant allele and the THBS-4 variant (387P) allele was significantly associated with a reduced risk of premature MI compared with wild-type individuals (OR=0.44, 0.24 to 0.84 and OR=0.43, 0.22 to 0.85, respectively). The latter observation is in contrast with a previous report, although confidence intervals overlap.

Conclusions—We conclude that a relationship between the THBS-1 N700S polymorphism and premature CAD is unlikely. For the THBS-4 A387P polymorphism, additional studies are required to elucidate its role in premature CAD. Finally, we conclude that the THBS-2 polymorphism is associated with a reduced risk of premature MI. (Arterioscler Thromb Vasc Biol. 2002;22:e24-e27.)

Key Words: thrombospondin ▪ polymorphism ▪ premature coronary artery disease ▪ premature myocardial infarction

Thrombospondins form a family of multidomain extracellular matrix proteins with related sequences but diverse tissue distributions. They are involved in a wide range of processes in the vessel wall, including smooth muscle cell proliferation,1 endothelial cell proliferation, and migration,2 and they bind to various extracellular matrix proteins.3 Therefore, variations in the genes coding for these proteins are potential risk factors for premature coronary artery disease (CAD). Recently, an exploratory genetic association study called GeneQuest tested 72 single nucleotide polymorphisms and identified 3 polymorphisms in thrombospondin-1, -2, and -4 (THBS-1, -2, and -4, respectively) to potentially modulate the risk of premature CAD or myocardial infarction (MI). These polymorphisms were an A>G substitution at position 8831 of the THBS-1 gene (located on chromosome 15q15), predicting an asparagine to serine substitution at position 700 (N700S); a T>G substitution in the 3′-untranslated region (3′ UTR) of the THBS-2 gene, which is located on chromosome 6q27; and a G>C substitution at position 29926 of the THBS-4 gene (located on chromosome 5q13), predicting an alanine to proline substitution at position 387 (A387P).4 However, this exploratory study design testing numerous hypotheses is prone to false-positive findings. Therefore, the findings require independent replication in other population samples. We tested the hypothesis in a larger group of individuals with CAD that occurred before the age of 50 years.

Methods

Design and Study Sample
Cases (n=503) were consecutive, unrelated individuals referred to the Academic Medical Center in Amsterdam for symptomatic CAD that occurred before the age of 50 years. Patients qualified for inclusion after an MI (according to World Health Organization criteria, n=320), surgical or percutaneous coronary revascularization, or a coronary angiogram with evidence of at least 70% stenosis in a major epicardial artery. The protocol was approved by our Institutional Review Board. Control subjects (n=1071) were selected from the participants of the Cardiovascular Disease Risk Factor Monitoring Project, a large screening project for cardiovascular risk factors.5 Approximately 2 control subjects per case were selected, group-matched for sex and age (within 5 years). All controls had the Dutch nationality and reported no history of CAD in a self-administered questionnaire. All patients and control subjects gave informed consent.

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Laboratory Procedures

Nonfasting blood samples were obtained in EDTA-coated Vacutainer tubes. Genomic DNA was extracted according to a standard protocol. Polymerase chain reaction (PCR) amplification was performed on 1 μL DNA in 10 μL ReddyMix (ABgene). For THBS-1, the following primers were used: forward: GCATTGTACCCCTAGGTT; reverse: TGTGTGTAGGGGTTGATGATGTTGCC. PCR products were 293 bp, and digestion with BsrI restriction enzyme (3 hours, 65°C) generated 2 additional fragments of 191 and 102 bp in the presence of the G allele. For THBS-2, the following primers were used: forward: CTGTGCATGCCATGTCCTAGA; reverse: TATCATATGCTATGCACATTCTCCTCA. PCR products were 363 bp, and digestion with Ddel restriction enzyme (12 hours, 37°C) generated 3 fragments of 27, 134, and 202 bp in the presence of the T allele and an additional 336-bp band in the presence of the G allele. For THBS-4, the following primers were used: forward: ATATTATGCCCACATGTCTTCT; reverse: TATCATAATGGCTTATGCACAGTTCA. PCR products were 336 bp, and digestion with DdeI restriction enzyme (12 hours, 37°C) generated 3 fragments of 191 and 102 bp in the presence of the G allele. For THBS-4, the following primers were used: forward: TATCATATGCTATGCACATTCTCCTCA; reverse: TATCATAATGGCTTATGCACAGTTCA. PCR products were 363 bp, and digestion with Ddel restriction enzyme (12 hours, 37°C) generated 3 fragments of 27, 134, and 202 bp in the presence of the T allele and an additional 336-bp band in the presence of the G allele. For THBS-4, the following primers were used: forward: ATATTATGCCCACATGTCTTCT; reverse: TATCATAATGGCTTATGCACAGTTCA. PCR products were 336 bp, and digestion with DdeI restriction enzyme (12 hours, 37°C) generated 3 fragments of 191 and 102 bp in the presence of the G allele. For THBS-4, the following primers were used: forward: ATATTATGCCCACATGTCTTCT; reverse: TATCATAATGGCTTATGCACAGTTCA. PCR products were 336 bp, and digestion with DdeI restriction enzyme (12 hours, 37°C) generated 3 fragments of 191 and 102 bp in the presence of the G allele.

Differences between groups were assessed with a Fisher exact test, Mann-Whitney test, or Kruskal-Wallis test, where appropriate. Odds ratios (ORs) and associated 95% confidence intervals (95% CI) were calculated to test the null hypothesis and to determine the risk of premature CAD and premature MI for carriers and homozygotes of the variant allele compared with wild-type individuals. ORs were adjusted for sex, total cholesterol, triglycerides, HDL, smoking, and diabetes.

Results

The average ages of cases and controls were 40 ± 6 and 39 ± 7 years, respectively, and percentages of males were 81% and 76%, respectively. Cases and controls differed significantly for the risk factors smoking, hypertension, diabetes, body mass index, total cholesterol, and HDL (P < 0.0001 for each) (Table 1). Genotyping for the THBS-1, THBS-2, and THBS-4 polymorphisms was successful in 97.2%, 96.3%, and 93.4%. The genotype distributions for the THBS polymorphisms are presented in Table 2. Both cases and controls were in Hardy-Weinberg equilibrium for all 3 polymorphisms.

The major risk factors were equally distributed in carriers (homozygotes + variant homozygotes) and wild-type individuals, except for a small difference in triglycerides between THBS-4 carriers and wild-type individuals. Compared with wild-type individuals, carriers of the THBS-1 variant allele were not at increased risk for having premature CAD (OR = 0.93, 0.74 to 1.31) or MI (OR = 0.89, 0.66 to 1.24). Homozygotes for the THBS-2 variant allele had a lower risk of premature CAD on the edge of significance (OR = 0.58, 0.33 to 0.99) but a strongly significantly lower risk of premature MI (OR = 0.44, 0.24 to 0.84). Similarly, homozgyosity for the THBS-4 variant allele had a weakly significant association with a lower risk of premature CAD (OR = 0.51, 0.28 to 0.94), but a strongly significant association was observed with a lower risk of premature MI (OR = 0.43, 0.22 to 0.85). Correcting for 6 independent hypotheses (3 genes tested for 2 outcomes) resulted in none of the associations reaching P < 0.05.

In an attempt to provide supporting evidence for the observed genotype-disease relationship for THBS-2, we set out to determine THBS-2 plasma levels as an intermediate phenotype. However, the detection of THBS-2 in human plasma has never been published. We performed Western blotting on stored human plasma samples of patients included in the study but could not detect THBS-2 in these samples.

Discussion

We found that the recently reported THBS-1 N700S polymorphism was not significantly associated with premature CAD or MI in our sample. Homozygosity for the THBS-2 variant allele was significantly associated with a lower risk of premature MI, which is in accordance with the GeneQuest findings. Homozygosity for the THBS-4 variant allele was also associated with a reduced risk of premature MI, which contradicts the GeneQuest result.

In recent years, numerous studies have proposed genetic variations as risk factors for cardiovascular disease but only few candidates have consistently passed the test of replication. In fact, the GeneQuest investigators report that they could not replicate their findings for the THBS-4 A387P polymorphism in 2 smaller samples. The discrepancy between the GeneQuest results and ours may be accounted for by intrinsic differences between the population samples. However, adjustment for traditional cardiovascular risk factors did not substantially affect the results. In addition, several biases and confounders can affect the results of genetic association studies and may account for the discrepant results. In this journal, Hegele recently discussed the

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<thead>
<tr>
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<th>Cases (n=503)</th>
<th>Controls (n=1071)</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>40 ± 6</td>
<td>39 ± 7</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>406 (81)</td>
<td>817 (76)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>338 (71)</td>
<td>398 (37)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>114 (23)</td>
<td>159 (15)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>37 (8)</td>
<td>6 (0.6)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.9 ± 4.1</td>
<td>25.3 ± 3.8</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.7 ± 1.5</td>
<td>5.4 ± 1.0</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.3</td>
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Values are mean ± SD or n (%). BMI indicates body mass index. Percentages may not apply to all individuals because not all baseline characteristics were available for every individual.
Thus, unlike THBS-1, THBS-2 is not present in platelets and is human plasma, which is consistent with the observation that THBS-2 plasma levels. We could not detect any THBS-2 in the vessel wall after injury. Because of the absence of a biochemical intermediate phenotype, research to test this hypothesis would require animal models. Second, the present study describes a single patient sample. The very strict inclusion criteria necessitate either an extensive multicenter design, as in the GeneQuest study, or a very long inclusion period, as in ours. Therefore, the number of large, similarly defined patient samples is most likely limited.

The mechanism by which THBS-2 affects atherosclerosis may involve the regulation of matrix metalloproteinase-2, a protein linked to the vulnerability of atherosclerotic plaque. THBS2-null fibroblasts produce a 2-fold quantity of this protein, which was shown to be lower in CAD patients than in controls. Alternatively, THBS-2–deficient mice have an increased vascular density and a bleeding tendency, which can both be hypothesized to reduce the risk of MI. We conclude that for the THBS-1 N700S and the THBS-4 A387P polymorphisms, a role as genetic risk factor for premature CAD is unlikely. In addition, we conclude that the THBS-2 3’UTR polymorphism is associated with a reduced risk of premature MI. Additional research into the functionality of this polymorphism is warranted.

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