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.

Received September 5, 2002; revision accepted November 17, 2002.

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Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org DOI: 10.1161/01.ATV.0000046235.22451.66
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: GCTGGTGTTACCTCAGGTTGTTAGTGGGCC. 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: CTGTCATGCCTATGGTCCTAGA; reverse: TATCATAGGCTTATGACAGATTTCCCTCA. 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: ATATTATGCCACATGTCTTAG; reverse: CCTCAGATTACCATTCCTTCCA. PCR products were 310 bp, and digestion with Cac8I (12 hours, 37°C) generated 2 fragments of 142 and 168 bp in the presence of the G allele and an additional band at 310 bp in the presence of a C allele. All restriction enzymes were obtained from New England Biolabs. The digest was analyzed by electrophoresis in a 2% agarose gel.

Plasma samples were obtained in citrate-coated Vacutainer tubes and stored at −80°C. Western blotting for the detection of THBS-2 in plasma was performed as previously described with minor modifications.6 The antibody was obtained from Becton Dickinson Biosciences.

Researchers and laboratory personnel had no access to identifiable information and could identify samples by a number only.

Statistical Analysis
Sample size calculations were based on the GeneQuest findings using the polymorphism with the strongest association (THBS-4). We expected similar allele frequencies in our subjects and aimed at including 500 patients and 1000 controls to have 80% power. For each polymorphism, risk factors were compared between wild-type individuals, heterozygotes, and homozygotes for the variant allele and also between noncarriers and carriers of the variant allele. These results did not differ importantly, and, thus, for the sake of brevity, only comparisons between carriers and noncarriers are presented. Differences between groups were assessed with a Fisher’s exact test, t test, Mann-Whitney test, or Kruskal-Wallis test, where appropriate. Odds ratios (ORs) and associated 95% confidence intervals (95% CI) were calculated to quantify 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, hypertension, diabetes, and smoking.

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 (heterozygotes + 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 (Table 2). However, adjustment for traditional cardiovascular risk factors did not substantially affect the results.

TABLE 1. Characteristics of Cases and Controls

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
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<tbody>
<tr>
<td>(n=503)</td>
<td>(n=1071)</td>
<td></td>
</tr>
<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>
</tr>
</tbody>
</table>

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.

(OR = 0.93, 0.74 to 1.31) or MI (OR = 0.89, 0.66 to 1.24). Homozygotes for the THBS-2 rare allele did have 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, homozygosity 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.7 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.8 In this journal, Hegele9 recently discussed the
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,11 which was shown to be lower in CAD patients than in controls.12 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.5 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.

Acknowledgments

Dr Boer was funded by the Netherlands Heart Foundation (No. 98.067). We gratefully acknowledge P. Bornstein, MD (University of Washington, Seattle, Wash), for his helpful suggestions.

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Arterioscler Thromb Vasc Biol. 2002;22:e24-e27; originally published online November 7, 2002;
doi: 10.1161/01.ATV.0000046235.22451.66
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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