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.
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: GCTGACTCCCTCAGGTG; reverse: TGGTTTGGATAGGTGATG- CCGC. PCR products were 293 bp, and digestion with Bsr 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: CTGTCATGCCATAGTGCC- CCTAGA; reverse: CTTGAAATGGCACAGATTCTC- CCT TA. 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: ATATTATGCCCACATGT- GTGTACCCTCAGGTG; reverse: TGTTTTGATAAGGTGATT- CTACCCG. PCR products were 293 bp, and digestion with BsrI (3 days, 37°C) generated 2 additional fragments (90 bp and 202 bp in the presence of the C allele and a band of 336 bp in the presence of the T allele). 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.

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 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.4 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, Hegele8 recently discussed the
Table 2. Thrombospondin Genotypes in CAD Patients, MI Patients, and Control Subjects

<table>
<thead>
<tr>
<th>THBS-1</th>
<th>Controls n (%)</th>
<th>CAD n (%)</th>
<th>MI n (%)</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
<th>CAD n (%)</th>
<th>MI n (%)</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>15 (1.4)</td>
<td>3 (0.6)</td>
<td>1 (0.3)</td>
<td>0.47 (0.13–1.65)</td>
<td>0.24</td>
<td>0.39 (0.09–1.70)</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>196 (18.9)</td>
<td>90 (18.3)</td>
<td>57 (18.2)</td>
<td>1.03 (0.77–1.37)</td>
<td>0.86</td>
<td>0.93 (0.68–1.26)</td>
<td>0.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>828 (79.7)</td>
<td>399 (81.1)</td>
<td>255 (81.4)</td>
<td>0.98 (0.74–1.31)</td>
<td>0.93</td>
<td>0.89 (0.66–1.24)</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1039</td>
<td>492</td>
<td>313</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>THBS-2</th>
<th>Controls n (%)</th>
<th>CAD n (%)</th>
<th>MI n (%)</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
<th>CAD n (%)</th>
<th>MI n (%)</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>69 (6.7)</td>
<td>22 (4.5)</td>
<td>10 (3.1)</td>
<td>0.58 (0.33–0.99)</td>
<td>0.48</td>
<td>0.44 (0.24–0.84)</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GT</td>
<td>367 (35.7)</td>
<td>170 (34.8)</td>
<td>105 (33.4)</td>
<td>0.89 (0.70–1.13)</td>
<td>0.33</td>
<td>0.85 (0.66–1.10)</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>592 (57.2)</td>
<td>296 (60.7)</td>
<td>199 (63.3)</td>
<td>0.84 (0.67–1.06)</td>
<td>0.15</td>
<td>0.79 (0.62–1.01)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1028</td>
<td>488</td>
<td>314</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>THBS-4</th>
<th>Controls n (%)</th>
<th>CAD n (%)</th>
<th>MI n (%)</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
<th>CAD n (%)</th>
<th>MI n (%)</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>58 (5.9)</td>
<td>19 (3.9)</td>
<td>10 (3.2)</td>
<td>0.51 (0.28–0.94)</td>
<td>0.03</td>
<td>0.43 (0.22–0.85)</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>339 (34.6)</td>
<td>160 (32.6)</td>
<td>99 (31.2)</td>
<td>0.85 (0.67–1.09)</td>
<td>0.21</td>
<td>0.83 (0.64–1.07)</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>583 (59.5)</td>
<td>312 (63.5)</td>
<td>208 (65.6)</td>
<td>0.81 (0.64–1.02)</td>
<td>0.07</td>
<td>0.77 (0.60–0.99)</td>
<td>0.042</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>980</td>
<td>491</td>
<td>317</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ORs and associated 95% CIs were calculated to quantify the risk of premature CAD and premature MI for homozygotes of the variant allele, heterozygotes, and carriers of the variant allele compared with wild-type individuals. ORs were adjusted for sex, total cholesterol, hypertension, diabetes, and smoking.

pros and cons of genetic association studies and proposed several desirable attributes. Our study design incorporated several characteristics to minimize the effect of potential errors and confounders. Both the GeneQuest study and ours had strict criteria that were very similar although not identical. Inclusion in GeneQuest required that at least one sibling of the proband also fulfilled the criteria for premature CAD, a requirement not used in our study. However, in our sample, a positive family history for premature CAD was equally distributed among all genotypes. An important difference between the studies is that unlike GeneQuest, our control group was matched to the cases by age, sex, and ethnicity to avoid genetic admixture. Finally, our study included more patients and more controls than the GeneQuest study. However, several limitations remain. To investigate the functionality of this polymorphism, we attempted to determine THBS-2 plasma levels. We could not detect any THBS-2 in human plasma, which is consistent with the observation that THBS-2 is not present in platelets and is not synthesized by cultured mouse endothelial cells.

Thus, unlike THBS-1, THBS-2 is not present in platelets and is not synthesized by cultured mouse endothelial cells.

Thus, our report does not provide data on the potential functionality of the THBS-2 polymorphism. Conceivably, the polymorphism is associated with an altered local expression pattern 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 distributed among all genotypes. An important difference between the studies is that unlike GeneQuest, our control group was matched to the cases by age, sex, and ethnicity to avoid genetic admixture. Finally, our study included more patients and more controls than the GeneQuest study. However, several limitations remain. To investigate the functionality of this polymorphism, we attempted to determine THBS-2 plasma levels. We could not detect any THBS-2 in human plasma, which is consistent with the observation that THBS-1, THBS-2 is not present in platelets and is not synthesized by cultured mouse endothelial cells. Thus, our report does not provide data on the potential functionality of the THBS-2 polymorphism. Conceivably, the polymorphism is associated with an altered local expression pattern 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 distributed among all genotypes. An important difference between the studies is that unlike GeneQuest, our control group was matched to the cases by age, sex, and ethnicity to avoid genetic admixture. Finally, our study included more patients and more controls than the GeneQuest study. However, several limitations remain. To investigate the functionality of this polymorphism, we attempted to determine THBS-2 plasma levels. We could not detect any THBS-2 in human plasma, which is consistent with the observation that THBS-1, THBS-2 is not present in platelets and is not synthesized by cultured mouse endothelial cells. Thus, our report does not provide data on the potential functionality of the THBS-2 polymorphism. Conceivably, the polymorphism is associated with an altered local expression pattern 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.

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.

References


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

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
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/22/12/e24

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/