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;23:660-665.)

Key Words: thrombospondin ▪ polymorphism ▪ premature coronary artery disease ▪ premature myocardial infarction
TABLE 1. Characteristics of Cases and Controls

<table>
<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, 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. BMI indicates body mass index. Percentages may not apply to all individuals because not all baseline characteristics were available for every individual.

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: GCATGGTGACCCTCAGGTG; reverse: TGTGGTGAAGTGGATGGGC. 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: CTGTGCATGCCAATGTCCCTAGA; reverse: TATCATATGGCTTTATGCACGTAATTCCTCCTACA. PCR products were 363 bp, and digestion with DdeI 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 C allele. For THBS-4, the following primers were used: forward: ATATTGCCACACTGTCCTTAG; reverse: CCTAGATTACCATTCTAGCCCG. PCR products were 310 bp, and digestion with Cac8I (12 hours, 37°C) generated 2 additional 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 biosciences. 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. 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 testing 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 387P allele was associated with a lower risk of premature CAD (OR 0.76, 0.58 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.
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 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, which contradicts the GeneQuest result. 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 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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References


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