Synergistic Effect of Thrombomodulin Promoter G-33A Mutation and Smoking on the Onset of Acute Myocardial Infarction

Running title: Thrombomodulin Gene Mutation and Myocardial Infarction

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Abstract

**Background** - Thrombomodulin plays an important role in the regulation of blood coagulation by decreasing thrombin enzymatic activity and activating protein C. Recently, we found that G-33A mutation in the promoter region of thrombomodulin gene significantly reduced thrombomodulin transcriptional activity. This study was to examine the possible association between the thrombomodulin G-33A mutation and acute myocardial infarction.

**Methods and Results** - In the case-control study, we recruited 278 patients (mean age 57.5 years, 241 men) with documented myocardial infarction and 450 age- and sex-matched control subjects. Polymerase chain reaction and single-strand conformation polymorphism was used to detect the thrombomodulin G-33A mutation. The frequency of the thrombomodulin G-33A mutation (G/A+A/A genotypes) among patients with myocardial infarction was higher than that in control subjects (22.7% vs. 16.2%, odds ratio [OR] 1.51, 95% confidence interval [CI] 1.04 to 2.21, p = 0.030). The mutation was significantly associated with myocardial infarction (OR 1.64, 95% CI 1.08 to 2.47) as was hypertension, diabetes mellitus and smoking. Among young myocardial infarction patients (age ≤ 45 years, n = 72), the mutation frequency was significantly higher than that in control subjects (29.2% vs. 16.2%, OR 2.13, 95% CI 1.21 to 3.75, p = 0.008). The thrombomodulin G-33A mutation (OR 2.28, 95% CI 1.26 to 4.13) and smoking (OR 4.45, 95% CI 2.51 to 7.88) were the only independent risk factors for young myocardial infarction. Furthermore, there was a significant interaction between the thrombomodulin G-33A mutation and smoking. Among patients who did not smoke, the mutation was not associated with a significantly increased risk of young myocardial infarction (OR 1.90, 95% CI 0.64 to 5.59); whereas, in
the presence of smoking, it was (OR 2.33, 95% CI 1.15 to 4.74). Smoking carriers of the mutation had a nearly 10-fold increased risk of young myocardial infarction (OR 9.76, 95% CI 4.25 to 22.40) when compared with the nonsmoking noncarriers.

**Conclusions.** There was a significant association between the G-33A mutation of thrombomodulin gene and acute myocardial infarction, especially in young patients. The clinical effect of this genetic factor was enhanced by smoking.

**Key Words:** Infarction; Genetics; Smoking
Condensed Abstract

We evaluated 278 patients with documented myocardial infarction and 450 age- and sex-matched control subjects for the presence of thrombomodulin G-33A mutation. We found that the frequency of the thrombomodulin G-33A mutation (G/A+A/A genotypes) among young myocardial infarction (age \( \leq 45 \) years, \( n = 72 \)) was significantly higher than that in control subjects (29.2% vs. 16.2%, OR 2.13, 95% CI 1.21 to 3.75, \( p = 0.008 \)). The mutation (OR 2.28, 95% CI 1.26 to 4.13) and smoking (OR 4.45, 95% CI 2.51 to 7.88) were the only independent risk factors for young myocardial infarction. The smoking carriers of the mutation had a nearly 10-fold increased risk of young myocardial infarction (OR 9.76, 95% CI 4.25 to 22.40) when compared with the nonsmoking noncarriers. We concluded that the G-33A mutation of thrombomodulin gene was a genetic risk factor for myocardial infarction and its clinical effect was enhanced by smoking.
Occlusive thrombus formation at the site of a ruptured atherosclerotic plaque in coronary artery is the most important pathogenetic mechanism for acute myocardial infarction.\textsuperscript{1-3} Although traditional coronary risk factors, such as hypertension, diabetes mellitus, smoking and hypercholesterolemia play critical roles in the development of myocardial infarction, there is an increased awareness of the contribution of inherited hemostatic disorders as risks for coronary thrombosis.\textsuperscript{4,5} Hemostasis depends on the balance between physiological procoagulant and anticoagulant factors. Genetic defects of any of these factors may cause clinical thrombotic disorders. Thrombomodulin is an endothelial cell surface receptor for thrombin. It suppresses blood coagulation by decreasing thrombin procoagulant activities, including fibrinogen clotting and platelet activation.\textsuperscript{6} The thrombomodulin-thrombin complex also activates protein C which catalyzes the proteolytic degradation of blood clotting factor V and VIII.\textsuperscript{7,8} Therefore, thrombomodulin acts as an important physiological anticoagulant, and a deficiency of this protein could result in excessive thrombosis formation.\textsuperscript{9}

Recently, a genetic variant (G-33A mutation) in the promoter region of human thrombomodulin gene was identified.\textsuperscript{10} Functional study of this mutation with reporter gene assays revealed that A allele had a significant effect in decreasing the thrombomodulin gene promoter activity.\textsuperscript{11} Furthermore, the G-33A mutation decreased the soluble thrombomodulin level in patients with coronary artery disease.\textsuperscript{11} We hypothesized that the subjects with thrombomodulin G-33A mutation carried a higher risk of thrombosis formation, leading to acute myocardial infarction. We performed a case-control study in patients with myocardial infarction and control subjects to test our hypothesis.
Methods

Study subjects. The study population was composed of 278 patients (mean age 57.5 years, 241 men) with documented myocardial infarction and 450 age- and sex-matched control subjects. Diagnosis of myocardial infarction was based on ischemic chest symptoms, typical electrocardiographic changes and elevation of serum creatine kinase and its MB isoenzyme more than twice the upper level of normal. Coronary angiography was performed using the Judkins method within 2 weeks after the onset of symptoms. Coronary stenosis was defined as ≥ 50% diameter narrowing. The coronary arteries were grouped as left anterior descending artery or its diagonal branches, left circumflex artery or its obtuse marginal branches, and right coronary artery or its posterior descending or posterolateral branches when single or multi-vessel coronary artery disease was defined. A subgroup of young myocardial infarction patients with the age at onset of myocardial infarction ≤ 45 years was further analyzed. Written informed consent was obtained from all patients and this study was in agreement with guidelines approved by the research committee of our hospital. The controls were recruited from consecutive subjects admitted to our hospital for routine health examination. They had not any clinical or electrocardiographic evidence of myocardial infarction or coronary artery disease. They also had no histories of cerebrovascular disease or peripheral arterial disease. All patients and controls included in this study were Han Chinese and came from the same geographic area. The demographic data and the presence of traditional coronary risk factors, including hypertension, diabetes mellitus, smoking and serum cholesterol, were collected from all study participants. For patients with myocardial infarction, these data were taken from the medical records at the time of admission for acute myocardial infarction; for control subjects, they were collected
at the time of hospital admission for routine health examination. They were considered to have hypertension if elevated blood pressure (>140/90 mmHg) was measured on 3 occasions or were already being treated with antihypertensive agents. They were defined as having diabetes mellitus if they had a fasting blood glucose level >140 mg/dl on at least 2 separate occasions or were already being under treatment for diabetes. All study participants were classified as smokers (including current or ex-smokers) or nonsmokers. The total cholesterol level was determined at the entry of the study.

**Polymerase chain reaction and single-strand conformation polymorphism.** Genomic deoxyribonucleic acid (DNA) was extracted from 3 ml peripheral blood in all study participants with Puregene DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, USA) according to the manufacturer’s instructions. Thrombomodulin promoter fragment containing –33 nucleotide was amplified from genomic DNA by polymerase chain reaction (PCR). The sequences of the primers were 5’-CAGCAATCCGAGTATGCGG-3’ (-112 to –94) and 5’-CTCCTGTCCGTCCCAGCC-3’ (+69 to +52), respectively. PCR were run for 35 cycles, with each cycle consisted of 60 seconds of denaturation at 94°C, 60 seconds of annealing at 63°C, 60 seconds of extension at 72°C, and a final extension at 72°C for 7 minutes. The reaction yielded a 181 base-pair DNA fragment. The thrombomodulin G-33A mutation was detected by single-strand conformation polymorphism. Five µL aliquots of PCR product was denatured by addition of 2 µL denaturing solution containing 95% formamide, 0.25% bromophenol blue, 0.05% xylene cyanol and 20 mM ethylene diamine tetraacetic acid and heating to 95°C for 5 minutes, followed by rapid cooling on ice before loading on gel. The electrophoresis was performed on commercialized polyacrylamide gels (GeneGel Excel Kit, Pharmacia Biotech, San
Francisco, USA) at 15°C, 400V for 1.5 hours. Then the gels were visualized by a silver-staining protocol using Hoefer Automated Gel Stainer (Pharmacia Biotech, San Francisco, USA). In single-strand conformation polymorphism electrophoresis, the single strand products with mutation demonstrated different band motility on the gel and could be detected clearly (Fig.1). The samples with G-33A mutation were further proved by direct DNA sequencing using ABI 377 automated sequencer (Applied Biosystems, Foster City, California, USA).

**Statistical analysis.** Data on age and serum cholesterol level were presented as mean value ± SD, and differences between the groups were analyzed by unpaired Student’s *t* test. The thrombomodulin genotypes and traditional coronary risk factors, including hypertension, diabetes mellitus, and smoking were presented as number (%) of patients with the condition, and the differences in frequencies were analyzed by chi-square test. To determine the independent risk factors for myocardial infarction, multiple logistic regression analysis was performed to evaluate the effect of thrombomodulin G-33A mutation and traditional coronary risk factors for myocardial infarction. Because there was no significant difference in serum cholesterol level between groups (all *p* > 0.50), the cholesterol level was not used in multivariate analysis. Other risk factors were coded as dummy variables: hypertension and diabetes mellitus: 0 for absence, 1 for presence; smoking: 0 for nonsmoker, 1 for smoker; thrombomodulin genotypes: 0 for GG genotype, 1 for GA+AA genotypes. All statistical analyses were performed using SPSS Advanced Statistics 8.0 for Windows. Statistical significance was defined as *p* < 0.05.

**Results**

**Distribution of thrombomodulin G-33A mutation.** Table 1 shows the distribution of
genotypes of thrombomodulin G-33A mutation in control subjects, all myocardial
infarction patients, and young myocardial infarction patients. The frequency of G-33A
mutation (GA+AA genotypes) was higher in myocardial infarction group than that in the
control group (22.7% vs. 16.2%, odds ratio [OR] 1.51, 95% confidence interval [CI] 1.04
to 2.21, p = 0.030). In patients with young myocardial infarction, the mutation frequency
was significantly higher when compared with the control group (29.2% vs. 16.2%, OR
2.13, 95% CI 1.21 to 3.75, p = 0.008). In control group, the G-33A mutation distributed
equally in young (age ≤ 45 years, n = 100) or old subjects (17.0% vs. 16.0%, p = 0.932).
According to the electrocardiographic diagnostic criteria, the site of infarction was
anterior in 133 patients, inferior in 131 patients, and at the other locations in 14 patients.
There was no significant difference in the mutation frequency between the anterior and
non-anterior myocardial infarction (24.9% vs. 20.7%, p = 0.499). In 278 patients with
myocardial infarction, coronary angiography was performed in 274 cases (98.6%). Seven
patients had no significant coronary stenosis, 50 patients had single-vessel disease, and 217
patients had multi-vessel disease. The young myocardial infarction patients had
significantly higher frequency of normal or single-vessel disease than that in old patients
(38.0% vs. 14.5%, OR 3.62, 95% CI 1.96 to 6.70, p < 0.001). In young myocardial
infarction group, the frequency of thrombomodulin G-33A mutation was higher in patients
with normal or single-vessel disease (n = 27) than those with multi-vessel disease (40.7%
vs. 22.8%, p = 0.106), but the difference was not statistically significant.

**Comparison of traditional coronary risk factors between myocardial infarction and
control groups.** We compared the control and myocardial infarction groups for traditional
coronary risk factors, including hypertension, diabetes mellitus, smoking and serum
cholesterol level (Table 2). There were significant differences in the frequencies of hypertension (p < 0.001), diabetes mellitus (p < 0.001) and smoking (p < 0.001) between the 2 groups. However, the total serum cholesterol level was similar (p = 0.820). When only young myocardial infarction patients were compared with the control subjects, we found that only the frequencies of smoking (p < 0.001) was significantly higher in young myocardial infarction patients, while the differences of hypertension (p = 0.121), diabetes mellitus (p = 0.459) and total serum cholesterol level (p = 0.939) was also similar between the 2 groups.

**Identification of independent risk factors of myocardial infarction.** Table 3 shows the results of multiple logistic regression analysis for identifying the independent risk factors of myocardial infarction. Hypertension, diabetes mellitus, smoking, and thrombomodulin genotype were used as the independent variables. We found the effect of thrombomodulin G-33A mutation (OR 1.64, 95% CI 1.08 to 2.47) was smaller than those of hypertension (OR 2.98, 95% CI 2.11 to 4.22), diabetes mellitus (OR 2.93, 95% CI 1.86 to 4.64), and smoking (OR 2.99, 95% CI 2.15 to 4.17); while all these factors were significant predictors of myocardial infarction (all p < 0.05). However, when young patients and controls were compared, only thrombomodulin G-33A mutation (OR 2.28, 95% CI 1.26 to 4.13) and smoking (OR 4.45, 95% CI 2.51 to 7.88) appeared to be independent risk factors for myocardial infarction. There was also an interaction between smoking and the presence of thrombomodulin G-33A mutation in the occurrence of young myocardial infarction. Among patients who did not smoke, the mutation was not associated with a significantly increased risk of young myocardial infarction (OR 1.90, 95% CI 0.64 to 5.59); whereas, in the presence of smoking, it was (OR 2.33, 95% CI 1.15 to 4.74). The smoking carriers of
the thrombomodulin G-33A mutation had a nearly 10-fold increased risk of young myocardial infarction (OR 9.76, 95% CI 4.25 to 22.40) when compared with the subjects who did not smoke and did not carry the mutation (Table 4).

Discussion

The present study is the first one to specifically evaluate the association between thrombomodulin G-33A mutation and myocardial infarction. We found a higher frequency of G-33A mutation in patients with myocardial infarction than that in control subjects. There was a significant association of the mutation with myocardial infarction, especially in young people. The influence of the mutation on young myocardial infarction was further enhanced by smoking.

Prevalence of thrombomodulin G-33A mutation. The G-33A mutation of human thrombomodulin gene was first identified by Ireland et al.\textsuperscript{10} Interestingly, there was a significant ethnic variation in the distribution of the mutation. The prevalence of the thrombomodulin G-33A mutation was extremely low (<1%) in Caucasians.\textsuperscript{10,12} However, we and others\textsuperscript{13} have found the mutation to be a common genetic variant in Oriental populations (Table 5). Previous studies\textsuperscript{14-17} in Caucasians have demonstrated that the G1691A mutation in the factor V gene and the G20210A mutation in the prothrombin gene are important genetic risk factors for vascular thrombotic disease. However, the prevalence of these mutations nears zero in our population.\textsuperscript{18,19} These data and the present study result supported the hypothesis that some genetic mutations are specific to particular ethnic groups. The ethnic background plays an important role in studying the clinical significance of genetic mutations.

Mechanism of the association between the thrombomodulin G-33A mutation and
myocardial infarction. The present study shows that the G-33A mutation occurs in 16.2% in healthy control subjects, and the frequency is quite similar to the previous study in Korea (14.9%). The mutation also distributed equally between the old and young subjects in our control group. Furthermore, the control subjects in this study were from the same general population as the myocardial infarction patients. They had similar geographic and ethnic background. This approach suggests that the genetic background of our control group is homogenous and represents the normal subjects of our general population. The frequency of the mutation in myocardial infarction patients was significantly higher than that in controls with similar age and sex. Furthermore, there was an independent association between the mutation and myocardial infarction in multivariate analysis. These results indicated that the G-33A mutation of thrombomodulin gene might be an important genetic marker for myocardial infarction in our population.

The G-33A mutation in the thrombomodulin proximal promoter region could be responsible for a reduced expression of thrombomodulin contributing to coronary thrombosis. Actually, expression of thrombomodulin is regulated primarily at the level of transcription. The G-33A mutation is located very near to the TATA box that is important for basal thrombomodulin gene transcriptional activity. As assessed by luciferase reporter gene assays, the promoter bearing the G-33A mutation showed a 36% decrease in transcriptional activity in comparison with the wild type promoter. The other evidence that the mutation may decrease thrombomodulin expression is the study results on soluble thrombomodulin. Generally, the soluble thrombomodulin level increased in patients with atherosclerosis. However, we found that the soluble thrombomodulin level was only increased in coronary artery disease patients with normal GG genotype, but not in patients
with the G-33A mutation. These experimental results demonstrated that the G-33A mutation was a functional genetic variant that decreased thrombomodulin gene transcription and reduced the thrombomodulin protein synthesis. In a murine model of thrombomodulin deficiency, Healy et al clearly showed that the decreased thrombomodulin expression increased intravascular coagulation activation and fibrin deposition. Therefore, the association of the G-33A mutation of thrombomodulin gene with myocardial infarction is most likely due to the impaired thrombomodulin expression and increased coronary thrombosis.

Synergistic effect of thrombomodulin G-33A mutation and smoking in young myocardial infarction. In current study, we also found that the association between thrombomodulin G-33A mutation and myocardial infarction was stronger in a selected group of young survivors of myocardial infarction. Previous studies have demonstrated that young patients with premature myocardial infarction tend to have less coronary atherosclerosis and higher prevalence of normal or near-normal coronary angiograms. The condition was similar in our young patients, and they also had less traditional coronary risk factors. Therefore, the role of hypercoagulability in the pathogenesis of myocardial infarction was more pronounced in this group of patients. The prothrombotic milieu provided by decreased thrombomodulin expression in patients with G-33A mutation may be an important mechanism of young myocardial infarction in our population. Our study also provides strong evidence for a gene-environment interaction between smoking and the thrombomodulin G-33A mutation. The presence of the mutation increased the risk of young myocardial infarction only in smokers. Smokers carrying the mutation had a nearly 10-fold increase in their risk of myocardial infarction when compared with the
nonsmoking noncarriers. The definite mechanism for such interaction is not clear. In fact, smoking itself is a potent risk factor for myocardial infarction in young age.\textsuperscript{26} Smoking may induce a hypercoagulable state by increasing platelet aggregability.\textsuperscript{27} The presence of thrombomodulin G-33A mutation in combination with environmental challenge of smoking may play a synergistic role in the coronary thrombosis formation leading to acute myocardial infarction.

**Limitations.** One of the limitations in the study is the screening method for G-33A mutation. Single-strand conformation polymorphism is an accurate, standard technique for detecting single-base mutation.\textsuperscript{28} However, its sensitivity varies with the size of the DNA fragment being analyzed. The optimal size for sensitive base substitution detection is around 150 base-pair in length.\textsuperscript{29} The detection rate is less sensitive is larger DNA fragment. Furthermore, although there were no clinical and electrocardiographic evidences of coronary artery disease in control group, we cannot exclude the presence of myocardial ischemia in some control subjects with traditional coronary risk factors. It is possible that some of them could develop myocardial infarction at a later stage. Therefore, further follow-up of these subjects is warranted, especially in those with thrombomodulin G-33A mutation.
References


**Figure legends**

Figure 1. Gel patterns of the single-strand conformation polymorphism demonstrating the GG, GA and AA genotypes of the G-33A mutation in the promoter region of thrombomodulin gene among 7 representative subjects. Lanes 1, 2, 5, 6 display the gel pattern of GG genotype. Lanes 3 and 7 display the gel pattern of GA genotype. Lane 4 displays the gel pattern of AA genotype.
Table 1. Frequency of Genotypes of Thrombomodulin Gene in Control Subjects and Patients With Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 450)</th>
<th>All MI Group (n = 278)</th>
<th>Young MI Group (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombomodulin G-33A Mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG genotype</td>
<td>377 (83.8%)</td>
<td>215 (77.3%)</td>
<td>51 (70.8%)</td>
</tr>
<tr>
<td>GA+AA genotypes</td>
<td>73 (16.2%)</td>
<td>63 (22.7%)*</td>
<td>21 (29.2%)†</td>
</tr>
<tr>
<td>GA genotype</td>
<td>71 (15.8%)</td>
<td>57 (20.5%)</td>
<td>19 (26.4%)</td>
</tr>
<tr>
<td>AA genotype</td>
<td>2 (0.4%)</td>
<td>6 (2.2%)</td>
<td>2 (2.8%)</td>
</tr>
</tbody>
</table>

GA+AA versus GG genotype: *p = 0.030, OR = 1.51, 95% CI = 1.04 – 2.21 vs. control group; †p = 0.008, OR = 2.13, 95% CI = 1.21 – 3.75 vs. control group.

Data are presented as number (%) of patients. AA = homozygous carriers of the G-33A mutation; CI = confidence interval; GA = heterozygous carriers of the G-33A mutation; GG = homozygous normal subjects; MI = myocardial infarction.
Table 2. Clinical Characteristics of Study Subjects

<table>
<thead>
<tr>
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<th>Control Group (n = 450)</th>
<th>All MI Group (n = 278)</th>
<th>Young MI Group (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>56.3±11.9</td>
<td>57.5±12.8</td>
<td>40.8±5.1*</td>
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<tr>
<td>Male</td>
<td>390 (86.7%)</td>
<td>241 (86.7%)</td>
<td>64 (88.9%)</td>
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<tr>
<td>Hypertension</td>
<td>100 (22.2%)</td>
<td>131 (47.1%)*</td>
<td>22 (30.6%)†</td>
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<tr>
<td>Diabetes mellitus</td>
<td>38 (8.4%)</td>
<td>68 (24.5%)*</td>
<td>8 (11.1%)‡</td>
</tr>
<tr>
<td>Smoking</td>
<td>183 (40.7%)</td>
<td>185 (66.5%)*</td>
<td>54 (75.0%)*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>201.8±40.9</td>
<td>202.5±40.9</td>
<td>202.2±43.6</td>
</tr>
</tbody>
</table>

* p< 0.001 versus control group. † p = 0.121 versus control group. ‡ p = 0.459 versus control group. Data are presented as mean value ± SD or number (%) of patients. MI = myocardial infarction.
Table 3. Risk Factors of Myocardial Infarction Identified by Multiple Logistic Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
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<tbody>
<tr>
<td><strong>All MI Group</strong></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>2.98</td>
<td>2.11 – 4.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.93</td>
<td>1.86 – 4.64</td>
<td>&lt;0.001</td>
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<tr>
<td>Smoking</td>
<td>2.99</td>
<td>2.15 – 4.17</td>
<td>&lt;0.001</td>
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<tr>
<td>Thrombomodulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA+AA genotypes</td>
<td>1.64</td>
<td>1.08 – 2.47</td>
<td>0.019</td>
</tr>
<tr>
<td><strong>Young MI group</strong></td>
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<td></td>
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<tr>
<td>Hypertension</td>
<td>1.64</td>
<td>0.92 – 2.94</td>
<td>0.092</td>
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<tr>
<td>Diabetes mellitus</td>
<td>1.25</td>
<td>0.54 – 2.90</td>
<td>0.598</td>
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<tr>
<td>Smoking</td>
<td>4.45</td>
<td>2.51 – 7.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thrombomodulin</td>
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<td></td>
</tr>
<tr>
<td>GA+AA genotypes</td>
<td>2.28</td>
<td>1.26 – 4.13</td>
<td>0.007</td>
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</table>

AA = homozygous carriers of the G-33A mutation; CI = confidence interval; GA = heterozygous carriers of the G-33A mutation; MI = myocardial infarction, OR = odds ratio.
Table 4. Interaction Between Smoking and Thrombomodulin G-33A Mutation

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Thrombomodulin Genotype</th>
<th>Young MI Patients</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
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<tr>
<td></td>
<td>(n = 72)</td>
<td>(n = 450)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>GG</td>
<td>13</td>
<td>222</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>GA+AA</td>
<td>5</td>
<td>45</td>
<td>1.90</td>
<td>0.64 – 5.59</td>
</tr>
<tr>
<td>Yes</td>
<td>GG</td>
<td>38</td>
<td>155</td>
<td>4.19</td>
<td>2.16 – 8.12</td>
</tr>
<tr>
<td>Yes</td>
<td>GA+AA</td>
<td>16</td>
<td>28</td>
<td>9.76</td>
<td>4.25 – 22.40</td>
</tr>
</tbody>
</table>

AA = homozygous carriers of the G-33A mutation; CI = confidence interval; GA = heterozygous carriers of the G-33A mutation; GG = homozygous normal subjects; MI = myocardial infarction, OR = odds ratio.
<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Frequency of GA+AA Genotypes</th>
<th>Reference No.</th>
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<tbody>
<tr>
<td>Taiwanese (n = 450)</td>
<td>16.2%</td>
<td>Present study</td>
</tr>
<tr>
<td>Korean (n = 114)</td>
<td>14.9%</td>
<td>13</td>
</tr>
<tr>
<td>British (n = 104)</td>
<td>0.96%</td>
<td>10</td>
</tr>
<tr>
<td>French (n = 394)</td>
<td>0.25%</td>
<td>12</td>
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</table>

AA = homozygous carriers of the G-33A mutation; GA = heterozygous carriers of the G-33A mutation.