Gender Dependent Association of Thrombospondin-4 A387P Polymorphism With Myocardial Infarction

To the Editor:
A recently identified novel missense variant of TSP-4, A389P (29926 G>C) was correlated with premature myocardial infarction (MI) by an exploratory genetic association study on 184 MI patients from the American population. In this study, the TSP-4 A387P allele showed the strongest association, with an adjusted odds ratio for MI of 1.89 (P = 0.002 adjusted for covariates) for individuals carrying the 387P allele. This was replicated by the same group using a larger number of patients. An in vitro functional study of the TSP-4 A387P substitution provided further evidence in support of a proatherogenic effect. The TSP-4 A387P substitution was shown to have a gain of function effect leading to suppression of endothelial cell adhesion and proliferation. The TSP-4 A387P variant also shows ethnic distribution differences. The TSP-4 387P allele showed a dramatically lower prevalence in Asian Chinese populations compared with whites (3.8% versus 19.6 to 23.2%) and failed to associate with coronary artery disease (CAD) or MI in the studied populations. Interestingly, in a more recent investigation, the TSP-4 variant 387P allele was significantly associated with reduced risk, rather than increased risk, for premature MI (OR = 0.43, 0.22 to 0.85) in Netherlands whites. Therefore, the true effect of TSP-4 A387P in MI remains unknown. We believe that the contradictory results may be caused by either population genetic mixture or the effects of different modifiers for the TSP-4 A387P allele among the different ethnic populations.

We examined the TSP-4 A387P variant as part of our study to explore potential gene variants as risk factors for MI and the effect of interaction between different variants on MI. So far 11 different variants have been analyzed, and some have been published. To test the hypothesis that the TSP-4 variant constitutes increased risk for MI we carried out a case control study by genotyping the TSP-4 A387P in a large number of patients with MI from the genetically isolated Newfoundland population. This approach reduces the effects of genetic admixture in the studied subjects, a common challenge facing case control studies. The population of the island of Newfoundland consists mainly of descendants of English and Irish settlers who arrived in the 17th and 18th Centuries. The geographical and social isolation of this island has ensured very little inward migration for several hundred years and thus has led to a small population (530,000 individuals; Statistics Canada 2001) with a relatively homogenous genetic background ideal for the study of complex multifactorial disease such as MI.

Blood samples were collected from 500 consecutive MI patients (306 males and 194 females) and 500 normal controls (214 males and 286 females) of the genetically isolated Newfoundland population. Patients categorized in the MI group represented those with symptoms and biochemical evidence suggestive of MI (Troponin I values >2.0 μg/L or >0.5 μg/L). Control subjects were selected from consecutive individuals without prior history of MI or thrombosis presenting to the emergency department for trauma, accidental injury, or other noncardiac and nonthrombotic related events. Ethics approval for this study was granted by the Human Investigations Committee of Memorial University and by the Health Care Corporation of St. John’s.

Genotyping of the TSP-4 A387P polymorphism was conducted by using Taq Man SNP genotyping technology on real-time PCR. The primers used were: forward, GCACCTAGGTGCTGACGACTTCG; and reverse, CCGGTGACCGTGGTTTTGATAAACAC. The probes used were: 387A allele, AAATGGACCGTGTT; which was labeled at the 5’ end with VIC; 387P allele, AAATGGACCGTGCGTT, which was labeled at the 5’ end with FAM. Both probes also had a quencher dye TAMRA at 3’.

Pearson x² statistical analysis was performed using SPSS v10.0 to test the association between genotypes and the prevalence of MI. Odds ratios (OR) were calculated as a measure of the relative risk for MI and were given with 95% CIs.

Our study showed that both patients and controls were in Hardy-Weinberg equilibrium for the A387P polymorphism. The gene frequency of TSP-4 A387P in the Newfoundland population was 23.1%, similar to other white populations. Slightly increased carrier frequency, allele frequency, and homozygous frequency of the 387P allele were observed in patients with MI compared with controls (43.6% versus 41.8%, 24.9% versus 23.1%, and 6.2% versus 4.4%, respectively), but the differences did not reach statistical significance.

The distribution of the TSP-4 A387P variant was further analyzed by subgrouping patients and controls according to age and sex (Table). MI patients were divided into those with an early age of onset (≤ 50 years) and those with a later age of onset (> 50 years). The control population was also divided into the 2 corresponding age groups.

The 387P allele showed a tendency toward higher prevalence in female patients with MI compared with the controls of same sex and gave an OR of 1.34 (0.93, 1.94), but the difference was not statistically significant (P = 0.132; Table). However, the prevalence of homozygotes for the 387P allele was significant higher in female patients compared with the male controls, giving an odds ratio of 2.96 (1.29, 6.78; P = 0.008). In contrast, there was a tendency toward reduced prevalence of the 387P allele and homozygosity in male patients with MI compared with the male controls (41.8% versus 45.3% and 4.6% versus 6.1%). These differences were not statistically significant.

Genetic predisposition in multifactorial disease, such as MI, results from the coeffect of multiple genes, gene–gene, and gene–environment interactions. These collectively produce an additive or synergistic effect which affects risk for disease. Individual genetic changes may produce small or insufficient effects to impart significant pathogenic risk. Therefore, it is not surprising to see that most of the genetic variants associated with MI are characterized as polymorphisms rather than mutations. Gene–gene interaction as a significant factor in genetic predisposition for MI has been clearly demonstrated in our previous studies.

In our present study, the 387P allele showed a tendency toward increased prevalence in the MI patients group compared with the controls. The carrier frequency of the 387P allele (GC+CC) was only slightly higher in patients with MI compared with the controls (43.6% versus 41.8%; OR 1.08). However, such increases become remarkable when comparing the prevalence of homozygosity for the 387P allele between patients and controls (6.2% versus 4.4%; OR 1.436). Further analysis of the 387P allele in subjects subgrouped according to sex showed that the higher prevalence of 387P alleles in MI patients only occurs in females. Female MI patients compared with control females gave odds ratios of 1.34 (P = 0.132) and 2.96(P = 0.008), respectively, when compared for prevalence of carriers (genotype of CC+GC) and homozygotes (genotype of CC). This indicates that the 387P allele may be a weak and dosage sensitive risk factor for MI. Other female-specific factors, genetic or otherwise, may have modifying effects on the relationship of 387P with MI risk.

Based on our investigation it is not clear whether the 387P allele has any effect on MI risk for male patients. We noticed a trend of reduced prevalence for 387P in carriers and homozygous males in the MI group compared with male controls (41.8% versus 45.3% and 4.6% versus 6.1%). However, the difference did not reach statistical significance. Further study on increased sample size will be useful.

Our results showed an increased prevalence of the 387P allele in patients with MI. This is in contrast with the results from the Netherlands study, in which the 387P alleles were associated with a
reduced risk for MI. Several differences are worth noting, however. The Netherlands study examined patients with early onset MI, a situation where one would expect strong predisposing factors to be most relevant. Our study examines a much older MI population where the etiology for MI may be significantly different. Secondly, the Netherlands study population had a high proportion of males (80%) compared with our study (50%).

Finally, we conclude that TSP-4 A387P polymorphism is associated with MI in females, especially in female homozygosity condition.

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