Thrombospondins and Premature Coronary Artery Disease
Time to Go Beyond Genotype-Phenotype Association Studies

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The extracellular matrix (ECM) has been for several years a research focus in the field of vascular biology. In addition to providing structural support to the arterial wall, the ECM exerts several important biological functions. It serves as a substrate for cell adhesion, plays an active role in regulating cell migration and proliferation, influences cell-cell interactions, acts as a reservoir for growth factors, and constitutes a site for lipoprotein binding. The composition of the ECM has a strong impact on its function and is controlled by the synthesis and degradation of the various components, which interact through entanglement and cross-linking to form a biomechanically active polymer network. Whereas collagens and elastin ensure structure, strength, and elasticity, proteoglycans, hyaluronan, and glycoproteins interact with vascular cells, growth factors, and cytokines to modulate cell adhesion, migration and proliferation, arterial wall permeability, and lipoprotein metabolism and hemostasis.

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Thrombospondins are glycoproteins which constitute a family of at least five, structurally related, multidomain ECM proteins, of which three (THBS-1, -2 and, -5) have been detected in vascular tissue.1-3 Whereas a range of functional properties have been attributed to THBS-1,4 the diverse functions in the ECM of THBS-2 and -5 remain poorly understood. However, like THBS-1, THBS-2 is considered to play a role as an adaptor and modulator of cell-matrix interactions through interaction with cell-surface receptors, cytokines, growth factors, proteases, and structural proteins.4 THBS-3, on the other hand, may be involved in the adhesion and migration of vascular smooth muscle cells during atherogenesis.5 Of the remaining members of the thrombospondin family, THBS-3 is not very well characterized with respect to function, whereas THBS-4 appears to act as an adaptor protein in ECM assembly.5

Considering their potential significance for important cellular events in the arterial wall, thrombospondins may be regarded as candidate genes for atherothrombosis and as such further investigated by using the techniques of contemporary molecular genetics. Naturally occurring sequence variations with a frequency in the population of more than 1% which most often affect single nucleotides, so-called single nucleotide polymorphisms (SNPs), are interspersed throughout the human genome. SNPs are commonly used as tools to elucidate genes with unknown functions in man, applying the strategy of the genotype-phenotype association study. Genotype-phenotype association studies involve testing of whether one SNP, or a combination of several SNPs, occurs at a significantly different frequency in subjects with a defined disease manifestation compared with matched healthy controls, or whether a particular clinical phenotype is more common in patients carrying a certain allele. In a recent report from GeneQuest,6 an exploratory study with high-throughput genomics technology, SNPs in multiple thrombospondin genes were, indeed, found to be associated with familial myocardial infarction (MI). In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Boekholdt et al7 provide further evidence that homozygosity for the less common T-allele resulting from a thymidine-to-guanine substitution in the 3 untranslated region (3 UTR) of the THBS-2 gene is associated with reduced risk of premature MI. This latter case control study was prompted by the demonstration in the GeneQuest study that three polymorphisms in the THBS-1, -2, and -4 genes might influence the risk of premature coronary artery disease (CAD) or MI.8 However, whereas the indication first obtained in GeneQuest of a protective role of the less common THBS-2 variant was replicated, discrepant findings were obtained with respect to the missense variants studied in the THBS-1 and -4 gene loci. No association with premature CAD or MI was found for the THBS-1 N700S polymorphism, and homozygosity for the THBS-4 variant 387P allele was associated with a lower risk of premature MI, which is opposite to the almost 2-fold increase in risk of MI observed in GeneQuest. Of note, neither of the two studies provided significant associations between THBS genotypes and clinical phenotypes after correction for the number of independent hypotheses tested. It should also be emphasized that both studies were underpowered, not least in relation to the number of questions asked.

What are the implications of these two genotype-phenotype association studies for our understanding of the potential roles of thrombospondins in CAD? In our view, they are fairly limited. By definition, genotype-phenotype association studies of complex diseases are exploratory and at best hypothesis-generating, and require supplementation with functional studies before a given candidate gene can be suggested as a disease susceptibility gene. At this stage, THBS-2 therefore is primarily an interesting candidate for further genetic and functional studies, which need to be completed before a causative role can be assigned or refuted.
Thus, it remains to be determined whether the common variation in the 3′ UTR affects THBS-2 gene regulation or merely is a marker for functional variation located elsewhere in the THBS-2 gene or in a different gene locus. The molecular mechanisms by which THBS-2 would promote atherothrombosis also need to be defined in greater detail. So far, gene knockout studies in fibroblasts have indicated that THBS-2 might influence the regulation of gelatinase A (MMP-2), a matrix metalloproteinase that is overexpressed in vulnerable atherosclerotic plaques. Conversely, THBS-2 deficient mice have a phenotype which would be expected to reduce the risk of MI. It should also be realized in this context that the leap from genotypes at a few thrombospondin loci to complex clinical phenotypes such as CAD or MI is a gigantic one and that SNP association studies examine only one or at best a limited number of potential mechanistic pathways. Clearly, a consequence of the dynamic nature of biological systems is that individual genes should not be viewed in isolation.

How should we interpret the inconsistencies between the two studies? In general, the existing literature in the field of molecular genetic epidemiology suggests that significant between-study heterogeneity (diversity) is common, that the results of the first study of a particular gene locus correlate only fairly weakly with subsequent work on the same association, and that the first study often indicates the presence of a stronger genetic effect than is encountered in subsequent studies. This is most likely due to a combination of bias and genuine population diversity. Indeed, as elaborated on in recent overview commentaries, a number of hazards lurk and threaten to perturb the results of genetic association studies, and concerns have been expressed about their cost-effectiveness and scientific validity. Simultaneously, problems with the replication requirement itself have been highlighted. Nevertheless, a couple of differences in design features might have contributed to the discrepancies in this instance. These include differences in sample size, inclusion criteria (familial premature CAD in the GeneQuest study as opposed to premature CAD with no requirement for an affected sibling), matching strategies to avoid genetic admixture, and representativeness of the cases, with a distinct risk of a survivor bias in the GeneQuest study. Insufficient power of both studies represents an additional issue. Intrinsic differences (both genetic and environmental) might also exist between the population samples examined—in parameters not yet known to be of importance or in parameters not captured in the two studies. Furthermore, a total of 72 SNPs were assessed in GeneQuest. Accordingly, some associations might have resulted from chance alone.

In all, two genotype-phenotype association studies of limited power have implicated thrombospondins in premature CAD and added further support to the notion that composi-
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