Digenic Inheritance of Myocardial Infarction Risk Implicates Dysfunctional Nitric Oxide Signaling

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Study Hypothesis
The systematic identification of rare variants and novel pathways associated with myocardial infarction (MI) has introduced next-generation sequencing technology to large clinical cohorts. Although this population-wide approach to uncover the full spectrum of rare variation is promising, it has not replaced the smaller scale sequencing of well-phenotyped families with Mendelian disorders. Erdmann and colleagues2 recently presented a thorough evaluation of one such family in which they identified mutations in 2 functionally related genes that seem linked to disease. With careful genetic analysis, resequencing in independent cohorts, and functional validation experiments, they tested the hypothesis that 2 nitric oxide pathway-modifying genes can contribute to the pathogenesis of MI.

How Was the Hypothesis Tested?
Erdmann and colleagues2 began with the identification of a large family of 32 members with coronary artery disease, 22 of whom had an MI before the age of 60 years. After microsatellite-based linkage analysis failed to identify a causal locus, they sequenced 3 family members with early onset MI. Initially, 4 functional variants segregated with disease; however, only 2 gene mutations withstood testing with 2-locus linkage analysis. The nonsynonymous p.Leu163Phefs*24 and p.Ser525Leu mutations, in GUCY1A3 and CCT7, respectively, demonstrated insignificant logarithm of odds scores independently but with 2-locus linkage analysis had a maximum logarithm of odds score of 5.68. All 7 carriers of both mutations had early coronary artery disease.

Because evidence beyond sequencing in this single family is required to claim causality,3 the authors extensively validated their initial observation with gene resequencing in large numbers of control patients and other familial cohorts of early MI. Functional experiments in HEK 293 cells and in patient-derived platelets sought to validate a role for both genes in the soluble guanylyl cyclase (sGC) pathway. Finally, an in vivo murine model of thrombus formation provided the last link from genotype to phenotype for these potential disease-causing genes.

Principal Findings
To assess the linkage between GUCY1A3/CCT7 and MI beyond the index family, the authors first confirmed that both mutations were absent in large cohorts of control patients. However, their efforts to identify GUCY1A3 and CCT7 mutations in families other than the index family did not significantly strengthen the case for causality. A distinct GUCY1A3 mutation (p.Gly537Arg) was present in one of the 22 families, but no families had a digenic pattern of mutations in both genes. Whole-exome sequencing studies of many individuals with and without early MI found a statistically significant enrichment of missense mutations in GUCY1A3 in early MI cases (2% versus 0.37% in controls, P=0.023). The same did not hold true for CCT7, where 7 different missense mutations were found in disease cases (1.2%) versus 5 in control individuals (0.62%, P=0.12). Taken together, these data did not validate the digenic inheritance model because it was not clear that different mutations in both genes segregate with MI. Even so, the comprehensive effort that was undertaken represents the gold standard for validating a potential causal gene from a family-based sequencing study,3 and the authors should be commended for presenting the data in a straightforward manner.

The most compelling data for the digenic model of causality for GUCY1A3 and CCT7 instead came from the functional studies. In transfected 293 human embryonic kidney cells, both of the familial GUCY1A3 mutations and the one potentially causal CCT7 mutation resulted in reduction of α1-sGC protein expression and cGMP production. With all 3 mutations established as functional and of importance to cGMP production, the authors measured cGMP-forming activity in isolated platelets from members of the index family. The carriers of both GUCY1A3 and CCT7 mutations had reduced sGC protein levels by Western blot and reduced cGMP formation.

References
Noncarriers and single mutation carriers demonstrated normal sGC expression and function. The final functional link with MI was provided by a mouse thrombosis model. Given that both GUCY1A3 and CCT7 reduced sGC, it is notable that α1-sGC–deficient mice displayed enhanced thrombus formation.

Implications
Although human genetics is rife with examples of single gene mutations that account for disease in families, relatively few examples are convincingly explained by a 2-locus model.4 The first report of digenic inheritance in a human disease was in 1994 for retinitis pigmentosa.5 This report was convincing because it included data from multiple pedigrees, and the protein products of the 2 genes had a known interaction. The study performed by Erdmann and colleagues2 similarly demonstrated the synergistic role of functional mutations in a single pathway. However, they were unable to demonstrate the importance of these mutations in other pedigrees or in large-scale exome-sequencing studies. Although the case can be made that the GUCY1A3 and CCT7 mutations contribute to MI risk in the index family, without validation of digenic inheritance in independent cases, it remains possible that additional, unrecognized mutations in the family contributed to disease in a polygenic manner. Even so, the investigators’ data implicate the sGC system in thrombosis generation and further validate it as an emerging therapeutic target.6

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Disclosures
None.

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