Myocyte Enhancer Factor-2A Gene Mutation and Coronary Artery Disease

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Introduction

Coronary artery disease (CAD) and its clinical manifestations, including myocardial infarction (MI), are leading causes of death and infirmity worldwide. A variety of environmental and genetic risk factors are associated with CAD, including hypercholesterolemia, hypertension, obesity, diabetes, and a family history of early CAD. However, other than identifying mutations in genes whose products are involved in lipid metabolism and the handling of cholesterol and lipoproteins, relatively little progress has been made toward identifying genes that may predispose individuals to CAD and MI. As far as 2003, the mutant version of myocyte enhancer factor-2A (MEF2A) was named *adCAD/MI1* for the first autosomal dominant CAD and MI locus.[1] However, some studies negated the role of MEF2A in CAD while others confirmed its influential role. An important clue to understanding the etiology of CAD is its substantial heritability, which demonstrates that variation in DNA sequence influences risk.

In this article, we reviewed the current understanding of the MEF2A mutation and CAD, as well as MI. Further, we presented our viewpoint.

Myocyte Enhancer Factor-2A Gene

MEF2A belongs to a family of four closely related transcription factors (MEF2-A, -B, -C, and -D) that are conserved from yeast to humans.[2] The N termini of MEF2 proteins contain MADS and MEF2 domains. The MADS domain mediates protein dimerization and binding to AT-rich DNA sequences. The adjacent MEF2 domain is required for dimerization, high-affinity DNA binding, and interaction with cofactors. The C terminus functions as a transcriptional activation domain and also has a role in nuclear localization. The deleted residues in the familial

mutant of MEF2A, Gln-Pro-Pro-Gln-Pro-Gln-Pro, are conserved in MEF2A proteins from other species and in other MEF2 factors. They are contained in the region of the protein required for nuclear localization. Not surprisingly, this mutant MEF2A protein is sequestered in the cytoplasm of transfected cells and acts as a dominant negative mutant, presumably by forming heterodimers with wild-type MEF2A monomers or cofactors such as GATA factors.[3]

A hallmark of the MEF2 proteins is their propensity to associate with cell-specific and signal-dependent cofactors. Thus, the ultimate set of MEF2 target genes expressed by a cell is dependent on the cellular identity and environment. The MEF2 proteins have been shown to exert both prosurvival and proapoptotic functions and to activate genes involved in cell proliferation and genes involved in muscle differentiation, the expression of which is dependent on the termination of cell proliferation. Among the best-characterized MEF2 cofactors are muscle-specific transcription factors and chromatin remodeling enzymes, including histone acetyltransferases and deacetylases. Developmental and stress signals modulate MEF2 activity both positively and negatively by controlling its association with such cofactors and its phosphorylation state.[4,5]

Consistent with its potential involvement in vascular function, MEF2A is expressed at high concentrations in

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the endothelial and smooth muscle layers of the coronary arteries. MEF2A and other MEF2 factors also have been shown to be up-regulated in the smooth muscle cells within the vessel wall of balloon-injured rat carotid arteries.[6]

Myocyte Enhancer Factor-2A, adCAD/MI, the First Autosomal Dominant Coronary Artery Disease and Myocardial Infarction Locus

In the 28 November 2003 issue of science, Wang *et al*.[1] describe a human pedigree with an autosomal dominant predisposition to CAD and early-onset MI. Affected individuals had evidence of CAD with or without MI, often before the ages of 50 for men and 55 for women, without hypercholesterolemia. A genome-wide scan for the responsible gene (s) showed linkage to a region of chromosome 15q26 that contains ~93 genes (for the relevant marker [D15S120], the lod score [logarithm of the odds ratio for linkage] was 4.19). Among these 93 genes, MEF2A caught the authors' attention. This gene encodes a transcription factor that functions in the fetal development of the cardiovascular system and in calcium-dependent signaling pathways that control cell proliferation, differentiation, and death during fetal development and in the adult. Sequencing of the MEF2A locus revealed a 21-nucleotide deletion that eliminated seven amino acids from the C terminus of MEF2A, apparently perturbing its transcriptional activity. This mutant version of MEF2A was named *adCAD/MI1* for the first autosomal dominant CAD and MI locus.

The phenotype of individuals that harbor the MEF2A mutation identified by Wang *et al*. is clearly distinct from that of MEF2A null mice. The fact that coronary artery abnormalities have not been seen in heterozygous or homozygous MEF2A null mice supports the notion that the mutation in this affected pedigree creates a dominant negative version of the MEF2A protein that perturbs the activities of other MEF2 proteins, which might partially substitute for the lack of MEF2A in MEF2A null mice. However, it is also possible that mice are simply less sensitive than humans to the concentration of MEF2A protein or that the pathological consequences of MEF2A deficiency in humans are dependent on other genetic or environmental factors (diet, stress, age, and the like).

Identification of MEF2A mutations in families and patients with CAD and MI clearly links the MEF2 signaling pathway to an important human disease. Detection of a high level of expression of MEF2A in the endothelium of coronary arteries suggests that an early step or triggering event in the development of CAD and MI may involve deregulation of specific transcriptional programs in the endothelium, which is expected to cause abnormal development or function of the endothelium. The endothelium is a critical barrier between blood and arteries and plays a protective role against damages of coronary arteries by blood elements such as platelets and monocytes. Defective or malfunctioned endothelium will be more susceptible to inflammation and

the formation of an atherosclerotic plaque, which may result in thrombosis, MI, and sudden death.

Myocyte Enhancer Factor-2A Mutation of Coronary Artery Disease and Myocardial Infarction

Although a number of genome-wide linkage studies for MI and for coronary disease have been performed and have identified putative chromosomal loci related to disease, the utility of many of these studies remains in doubt. From 2005 to now, some data do not support a role for MEF2A in CAD patients from different country, included Iran, Japan, Italy, and Germany.[6-11] In Beijing of China, Dai *et al*.[12] revealed that structural changes of exon 11 in MEF2A are not involved in sporadic CAD in the Han population of China. After 2 years, Liu *et al*.'s findings failed to demonstrate a correlation between (CAG)(n) polymorphism with CAD in Shanghai of China.[13] They concluded that the rare 21-bp deletion might have a more compelling effect on CAD than the common (CAG)(n) polymorphism, and MEF2A genetic variant might be a rare but a specific cause of CAD/MI.

For example, the linkage study in a family with 13 members over three generations with coronary disease, nine of whom had suffered MI, narrowed a linkage signal to chromosome 15q26.[1] Of 93 genes in the locus, the MEF2A gene was subjected to deep resequencing in family members as a plausible candidate gene, given its expression in embryonic coronary vasculature. A 21-bp deletion in MEF2A, resulting in the excision of seven amino acids from the protein product, was detected in each of the affected family members for whom DNA was available and was absent from unaffected family members. *In vitro* studies documented that the 21-bp deletion impairs the nuclear localization of the MEF2A protein product in cells, suggesting that a functional defect in the protein might be responsible for the prevalent coronary disease in family members with the mutation.

However, a large follow-up study attempting to identify additional deleterious mutations in MEF2A in sporadic cases of premature MI did not find any definitive mutations, but did succeed in finding the 21-bp mutation in three individuals who had not suffered MI.[8] By genotyping family members of these individuals, researchers confirmed that the mutation exists at very low frequency in the general population (rather than being exclusive to one family); it did not segregate with MI or coronary disease outside of the family in which the mutation had originally been described. This study suggests that MEF2A does not cause autosomal dominant MI and that the original family's causal mutation may have resided in one of the other 92 genes in the mapped locus on chromosome 15q26. Moreover, in a separate study, investigators were unable to replicate the original observation that the 21-bp deletion led to defective nuclear localization of MEF2A.[9] This study illustrates the importance of replication studies in human genetics.

Another group reported that the mutations of MEF2A exon 12 are implicated in premature CAD, suggesting a strong genetic component in the pathogenesis of premature CAD in the Chinese population.[14] González *et al.*[15] has reported that subjects with a Pro279Leu variant of MEF2A in exon 7 has an odds ratio of 3.1 for MI. Recently, Li *et al.*[16] investigated mutations and polymorphisms of MEF2A gene in a Chinese population, six or seven amino acid deletions and synonymous mutations (147143G → A) may be correlated with susceptibility to CAD.

Unfortunately, no meta-analysis data was published.

Myocyte Enhancer Factor-2A Sequence Variants of Coronary Artery Disease

How can we reconcile these results, in these controversial studies,[6-17] the 21-bp deletion was absent in unrelated individuals with normal angiograms, and was found to alter the ability of the MEF2A protein to activate transcription *in vitro*. In 2010, Elhawari *et al.* studied the association of MEF2A gene single-nucleotide polymorphisms (SNPs), namely, rs325400 G>T and rs34851361 A>G, with CAD. These two SNPs are in 11th exon and are silence.[18] In 2014, rs325400 polymorphism was found in association with CAD; meanwhile, none of the rs34851361 genotypes was associated with CAD.[6] What do they tell us about the challenges in reliably implicating genetic variants in human disease?

What General Principles Can we Take Away?

If the available evidence is not adequate to conclude that mutations in MEF2A play a causal role in CAD and/or MI, what general principles can we take away?

Together these multiple variants should satisfy one or more of the following criteria. (a) Multiple different mutations exist, each of which is evidently functional and cosegregates with disease in human patients. (b) Enrichment of a particular allele in disease cases as compared with controls, with enough observations to establish statistical significance. (c) Enrichment in disease cases of different rare mutations, where the frequency of such mutations is ascertained with equal vigor in controls. The challenge here, is deciding which of the observed changes should be lumped into the "causal" category in the disease cases as compared with the controls. Unless functional studies are performed, and unless the available assays bear a validated relationship to the disease *in vivo*, it is difficult to know which observed changes might be causal and which are clinically neutral variants. (d) Observation of a *de novo* mutation that is present in an affected individual (but not in his or her biological parents), which is extraordinarily rare given the low spontaneous mutation rate in humans. (e) Compelling effects of a human mutation in a model system such as an *in vivo* mouse model with recapitulation of the human disease phenotype.

The genomic sequence of MEF2A gene is highly polymorphic. It is, thus, of added interest to detect which or how many

MEF2A genetic variants might have functional potential to affect the final bioavailability of MEF2A, and further the development of CAD. In fact, many case–control studies have attempted to investigate the unequivocal effects of MEF2A gene on CAD, especially its exon 11, claimed as the most polymorphic locus harboring various substitution and insertion/deletion (indel) polymorphisms such as a common variant (CAG)(n) polymorphism. However, the results have been inconsistent. With the improved genotyping technologies and the completion of the human HapMap project, Genome-wide Association Studies (GWASs) have been developed as an important approach in genetic research. Thus far, a large number of candidate loci conferring risk of or protection from common complex diseases such as CAD have been proposed.[19,20] Nonetheless, neither the MEF2A locus on chromosome 15q26 nor its adjacent region has been identified in any of the previous GWASs, thus generating debate over the nature of MEF2A genetic contribution to individual susceptibility to CAD.

Finally, it remains to be determined whether disruption of MEF2A function is unique to this particular pedigree or will prove to be more widely associated with CAD. In this regard, Wang *et al.* apparently looked at three other pedigrees, none of which were found to carry the MEF2A mutation.[17] Nevertheless, the findings of this study reveal a new function for an important transcription factor best known for its roles in muscle development, and show yet again the power of rare human mutations to illuminate basic mechanisms of human disease.

Future Direction

The significance of identification of MEF2A as the first disease-causing gene for CAD and MI is two-fold. (a) It makes genetic testing possible for many individuals with a very high risk for CAD and MI. Aggressive lifestyle modifications and pharmacologic strategies may be used to delay or prevent the development of MI in the gene carriers. (b) It provides a molecular mechanism for the pathogenesis of CAD and MI.

The results indicate that an early step in the development of CAD and MI may involve deregulation of specific transcriptional programs in the endothelium of coronary arteries. A immunostaining study with an antibody for MEF2A revealed that the MEF2A protein is highly expressed in the endothelium.[1] The endothelium plays a protective role for coronary arteries and prevents the arteries from damage by blood elements such as platelets and monocytes. Inflammation mediated by adhesion of monocytes to the endothelium is considered to be critical to the pathogenesis of CAD, and defective or abnormally developed vascular endothelium will be more susceptible to inflammation and the formation of an atherosclerotic plaque, which may result in thrombosis, MI, and sudden death.

It is important to point out, the recent discovery of MEF2A as a disease-causing gene for CAD and MI and its high

expression in the endothelium lead us to hypothesize that an early trigger for the pathogenesis of CAD and MI may be dysfunction or abnormal development of the endothelium, which increases susceptibility of the coronary arteries to inflammation, leading to the development of CAD and MI. It is important to note that the last view remains a hypothesis that needs further validation with more molecular biologic and genetic studies.

In conclusion, MEF2A may play an important role in cardiovascular biology, and rare variants inMEF2A may influence its activity as a transcription factor *in vitro*, but the genetic evidence available to date does not demonstrate that these mutations play a causal role in CAD and/or MI in humans. While further scrutiny of MEF2A may help sort out its role, these studies[6-17] are also valuable in reminding us that replication and multiplicity in the human genetic research are critically important; that linkage studies implicate a region, not a single gene, that there are limitations in extrapolating from a functional effect *in vitro* to a medical consequence *in vivo*. These principles will be increasingly important in the years to come.

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Conflicts of interest
There are no conflicts of interest.

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