Lack of *MEF2A* Mutations in Coronary Artery Disease

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Abstract

Mutations in *MEF2A* have been implicated in an autosomal dominant form of coronary artery disease (adCAD1). In this study we sought to determine whether severe mutations in *MEF2A* might also explain sporadic cases of coronary artery disease (CAD). To do this, we resequenced the coding sequence and splice sites of *MEF2A* in ~300 patients with premature CAD and failed to find causative mutations in the CAD cohort. However, we did identify the 21 base pair (bp) *MEF2A* coding sequence deletion originally implicated in adCAD1 in one of 300 elderly control subjects without CAD. Further screening of an additional ~1,500 non-CAD patients revealed two more subjects with the *MEF2A* 21 bp deletion. Genotyping of 19 family members of the three probands with the 21 bp deletion in *MEF2A* revealed that the mutation did not co-segregate with early CAD. These studies demonstrate that *MEF2A* mutations are not a common cause of CAD and cast serious doubt on the role of the *MEF2A* 21 bp deletion in adCAD1.

Coronary artery disease (CAD) is one of the leading causes of mortality in western societies (*1*). Like other common chronic diseases, CAD has a complex etiology that is postulated to involve both genetic and environmental factors. Several risk factors for CAD have been established, including family history, hypertension, dyslipidemia, obesity, smoking, diabetes, and diet (*2-5*). In addition, genetic association studies and genome-wide linkage scans have uncovered several susceptibility loci and candidate genes that might contribute to the pathogenesis of CAD (*6-13*), although many of these studies remain controversial (*14, 15*).

Recently, a mutation in the human *MEF2A* gene, a member of the myocyte enhancer family of transcription factors, has been implicated in a familial form of CAD (adCAD) (*16*). Genetic linkage analysis of a single large Caucasian family with an autosomal dominant pattern of premature CAD indicated positive linkage (lod=4.19) to a single locus on chromosome 15q26 that included ~90 annotated genes. Resequencing of *MEF2A,* a prime candidate gene in the linked locus (*17-19*), revealed a 21 base pair (bp) coding sequence deletion in all affected family members. The predicted 7 amino acid deletion was found to occur in a region conserved among MEF2A proteins in human, mouse, pig and spider monkey, and *in vitro* assays of *MEF2A* function suggested that the deletion disrupts nuclear localization of the mutant protein and reduces MEF2A-induced transcriptional activation. No *MEF2A* mutations were identified in three

additional large families with prevalent CAD or 50 additional sporadic patients with this common disease. However, since the completion of the original study, the authors have reported *MEF2A* missense mutations (N263S, P279L, and G283D) in 4 out of 207 sporadic cases of CAD and estimate that ~2% of CAD is due to loss of functional mutations in this gene (*20*).

To explore further the role of *MEF2A* mutations in the pathogenesis of non-familial cases of CAD, we resequenced the exons and flanking intron sequences of *MEF2A* in 300 Caucasians who developed symptomatic CAD before the age of 55 years (males) or 65 years (females), and in 300 elderly controls (>60 yr, males; > 70 yr, females) who did not have signs or symptoms of CAD (*21, 22*). Only one non-synonymous change (S360L) was identified in a single subject among the 300 CAD patients. Computational analysis using PolyPhen (http://tux.embl-heidelberg.de/ramensky/polyphen.cgi) predicted that this change is benign and additional examination of deep vertebrate protein alignments further revealed this is not a constrained position in MEF2A or other paralagous protein family members (*23, 24*). The absence of severe mutations in *MEF2A* in our 300 CAD patients suggest that mutations in *MEF2A* are not a major cause of sporadic forms of CAD in Caucasians, in contrast to the recent finding of Bhagavatula et al (*20*).

Whereas none of the CAD cases in this study had *MEF2A* mutations, the 21 bp deletion originally implicated in adCAD1 (*16*) was identified in one of the elderly control subjects (control 1) (Figure 1), a 71 year old with no symptoms of CAD. The deletion did not segregate with CAD in the subject's family (Table 1 & Figure 2). Notably in this kindred, 3 elderly subjects with the 21 bp deletion do not have evidence of premature CAD whereas the 2 subjects with premature CAD do not carry the deletion. This finding suggested that the 21 bp deletion may not be a disease-causing mutation.

To further explore this possibility, we screened for the 21 bp deletion in *MEF2A* in an additional 1,521 unrelated Caucasian individuals who did not have symptoms of CAD. Two additional subjects (controls 2 and 3) were found to carry the deletion (Figure 1) and in both cases the deletion did not segregate with CAD in their extended families. Overall, amongst the family members of controls 1, 2 and 3, we identified four subjects bearing the 21 bp deletion who were older than 60 years of age and had other major CAD risk factors, including dyslipidemia, hypertension, diabetes and a history of smoking. However, none of these subjects had evidence of CAD. Since the 21 bp deletion has been reported to have a dominant negative effect on the transcriptional activity of wild-type MEF2A (16) and since mice with inactivation of *Mef2a* display marked right ventricular hypertrophy (19), we also performed two-dimensional echocardiography on two controls bearing the 21 bp deletion. These included a

71 year old female (control 1) (II-6 in kindred #1, Table 1) and a 45 year old obese male with type 2 diabetes (control 2). Both individuals had normal right and left ventricular size and function.

Although we cannot completely rule out the possibility that the 21 bp deletion might affect susceptibility to CAD with variable penetrance, our observation of three families with this allele and its lack of association with CAD, despite the concomitant presence of other CAD risk factors strongly supports that this rare variant is not responsible for adCAD1. A likely alternative possibility is that other sequence changes linked to the 21 bp deletion are responsible for adCAD1. This large 15q26 linkage interval contains >90 additional genes including numerous candidates such as desmuslin (involved in heart muscle structure) (*25*), *NR2F2/ARP-1* (involved in lipid metabolism gene regulation) (*26*), and *IGF1R* (the receptor for IGF-1, an insulin-like growth factor which has recently been shown to be capable of rescuing cardiac defects in mice) (*27*). Taken together these results support that missense mutations in *MEF2A* do not contribute appreciably to non-familial CAD in the general population and that the 21bp deletion in this gene has an allele frequency of ~0.16% in Caucasians and is not associated with increased risk of premature CAD.

References and Notes

1. S. Yusuf, S. Reddy, S. Ounpuu, S. Anand, *Circulation* **104**, 2746 (2001).
2. P. Greenland *et al.*, *Jama* **290**, 891 (2003).
3. A. J. Lusis, *Nature* **407**, 233 (2000).
4. J. J. Nora, R. H. Lortscher, R. D. Spangler, A. H. Nora, W. J. Kimberling, *Circulation* **61**, 503 (1980).
5. J. M. Schildkraut, R. H. Myers, L. A. Cupples, D. K. Kiely, W. B. Kannel, *Am J Cardiol* **64**, 555 (1989).
6. U. Broeckel *et al.*, *Nat Genet* **30**, 210 (2002).
7. S. B. Harrap *et al.*, *Arterioscler Thromb Vasc Biol* **22**, 874 (2002).
8. S. Francke *et al.*, *Hum Mol Genet* **10**, 2751 (2001).
9. Q. Chen *et al.*, *Circulation* **107**, 3146 (2003).
10. Q. Wang *et al.*, *Am J Hum Genet* **74**, 262 (2004).
11. E. J. Topol *et al.*, *Circulation* **104**, 2641 (2001).
12. P. Pajukanta *et al.*, *Am J Hum Genet* **67**, 1481 (2000).
13. E. R. Hauser *et al.*, *Am J Hum Genet* **75**, 436 (2004).
14. M. T. Scheuner, *Genet Med* **5**, 269 (2003).
15. A. J. Lusis, *Trends Cardiovasc Med* **13**, 309 (2003).
16. L. Wang, C. Fan, S. E. Topol, E. J. Topol, Q. Wang, *Science* **302**, 1578 (2003).
17. B. L. Black, E. N. Olson, *Annu Rev Cell Dev Biol* **14**, 167 (1998).
18. T. A. McKinsey, C. L. Zhang, E. N. Olson, *Trends Biochem Sci* **27**, 40 (2002).
19. F. J. Naya *et al.*, *Nat Med* **8**, 1303 (2002).
20. M. R. Krishna Bhagavatula *et al.*, *Hum Mol Genet* (2004).
21. Primers were designed to give a maximum product size of 500 bp and a minimum of 40 bp flanking the splice sites using the Exon Locator & eXtractor for Resequencing program (EXLR) (http://mutation.swmed.edu/ex-lax/). An M13F tag (gttttcccagtcacgacgttgta) and M13R tag (aggaaacagctatgaccat) was added to forward and reverse primers respectively. 10ng of DNA from each sample was amplified in a 10ul PCR reaction using AmpliTaq Gold® (Applied Biosystems) and cleaned using Tetra-Ethylene Glycol (TET) (http://www.jgi.doe.gov/sequencing/protocols/BETcleanup.doc). Sequencing reactions were done using the M13 primers along with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) (http://www.jgi.doe.gov/sequencing/protocols/ BigDye3.1auto1.0.doc) and cleaned again with TET before running on a 3730xl DNA Analyzer (ABI) or a MegaBACE 4000 (GE Healthcare). Base calling, quality assessment and assembly was carried out using the Phred, Phrap, Polyphred, Consed software suite developed by Phil Green (www.phrap.org). All sequence variants identified were verified by manual inspection of the chromatograms by two individuals to ensure accuracy.
22. CAD cases were recruited from the patient population of the University of

Ottawa Heart Institute. Criteria for inclusion included age of onset of CAD (defined as documented myocardial infarction, angina with positive exercise stress test or nuclear perfusion scan, percutaneous coronary intervention or coronary artery bypass grafting) < 55y for men and < 65y for women, no history of diabetes, absence of monogenic lipid disorders or severe dyslipidemia (total cholesterol prior to treatment < 7.0 mmol/L or < 270 mg/dl). Elderly control subjects (n=300) consisting of men >60y and women >70y with no history or symptoms of CAD were recruited from the Ottawa area. A second cohort of 1,521 control subjects, mean age 46y, including both obese and normal weight subjects, without a history of premature CAD were recruited separately from the Ottawa region. Families of three unrelated subjects from the above cohorts who were found to bear the MEF2A 21bp deletion were also studied. These included 9 members of kindred #1, 9 subjects in kindred #2 and 4 individuals in kindred #3. This study was approved by the Institutional Review Board of the University of Ottawa Heart Institute and informed written consent was obtained from all participants. Genomic DNA was extracted from white blood cells by standard methods (Miller SA Nucleic Acids Res 1988;16:1215) and from saliva samples collected in Oragene Kits by the manufacturer's instructions (DNA Genotek, ON).

23. S. Sunyaev *et al.*, *Hum Mol Genet* **10**, 591 (2001).
24. V. Ramensky, P. Bork, S. Sunyaev, *Nucleic Acids Res* **30**, 3894 (2002).
25. Y. Mizuno, A. A. Puca, K. F. O'Brien, A. H. Beggs, L. M. Kunkel, *BMC Genet* **2**, 8 (2001).
26. S. Kang, N. J. Spann, T. Y. Hui, R. A. Davis, *J Biol Chem* **278**, 30478 (2003).
27. D. Fraidenraich *et al.*, *Science* **306**, 247 (2004).
28. We would like to thank Edward Rubin and James Bristow for critical feedback on this work as well as Robert Dent for assistance in recruitment of the control subjects. This work was supported in part by the Department of Energy Contract DE-AC0378SF00098 (LAP), administered through the University of California, and by CIHR #MOP-44360 & HSFO #NA-5413 (RM).

Figure Legends

Figure 1: Sequence of the 21 bp deletion region of *MEF2A*. The PCR product from common and deletion alleles were separated by 3% agarose gel, purified and cloned for sequencing analysis. The 21 bp deletion was identified in three independent control individuals. The seven deleted amino acids are boxed with dashed line.

Figure 2: *MEF2A* intragenic deletion does not co-segregate with CAD in kindred C117 (Table 1). Individuals with premature CAD are indicated by solid squares (males) or circles (females). Unaffected individuals are indicated by open squares or circles. Normal males under the age of 50 years or normal females under the age of 55 years are shown in light gray as uncertain phenotype. Deceased individuals are indicated by a slash (/). The proband is indicated by an arrow. Genetic status: **+** indicates the presence of the 21bp deletion of *MEF2A* (heterozygous); **-** indicates the absence of the deletion. Note that 3 elderly subjects with the 21 bp deletion do not have premature CAD whereas the 2 subjects with premature CAD do not carry the deletion.