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Lack of *MEF2A* mutations in coronary artery disease

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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 approximately 300 patients with premature CAD and failed to find causative mutations in the CAD cohort. However, we did identify the 21-bp *MEF2A* coding sequence deletion originally implicated in adCAD1 in 1 of 300 elderly control subjects without CAD. Further screening of approximately 1,500 additional individuals without CAD revealed 2 more subjects with the *MEF2A* 21-bp deletion. Genotyping of 19 family members of the 3 probands with the 21-bp deletion in *MEF2A* revealed that the mutation did not cosegregate with early CAD. These studies support that *MEF2A* mutations are not a common cause of CAD in white people and argue strongly against a role for the *MEF2A* 21-bp deletion in autosomal dominant CAD.

Introduction

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 an autosomal dominant form of CAD (adCAD1) (16). Genetic linkage analysis of a single large white family with an autosomal dominant pattern of premature CAD indicated positive linkage (log odds ratio [LOD], 4.19) to a single locus on chromosome 15q26 that included approximately 90 annotated genes. Resequencing of *MEF2A*, a prime candidate gene in the linked locus (17–19), revealed a 21-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 humans, mice, pigs, and spider monkeys, 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 3 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 approximately 2% of CAD is due to mutations in this gene (20).

Results

To further explore the role of *MEF2A* mutations in the pathogenesis of nonfamilial cases of CAD, we resequenced the exons and flanking intron sequences of *MEF2A* in 300 white individuals with documented CAD with onset before the age of 55 years (men) or 65 years (women). Missense changes observed in the CAD group were examined in 300 elderly control subjects (men, older than 60 years; women, older than 70 years) who did not have signs or symptoms of CAD, and only 1 isolated change (S360L) was found to be unique to the CAD cohort. Computational analysis using PolyPhen (21) predicted that this change is benign, and additional examination of deep vertebrate protein alignments further revealed that this is not a constrained position in *MEF2A* or other paralogous protein family members (22, 23). The absence of severe mutations in *MEF2A* in our 300 CAD patients suggests that mutations in *MEF2A* are not a major cause of sporadic forms of CAD in white people, in contrast to the recent finding of M.R.K. Bhagavatula et al. (20).

Resequencing did reveal a complex coding sequence length polymorphism in the last coding exon of *MEF2A* that results from tri-nucleotide length variants within a region of polyglutamine and -proline repeats (Figure 1). Analyses of the repeat region in 287 CAD samples and 296 controls revealed 9 length-variant alleles overlapping the polyglutamine repeat in our sample set. All 9 alleles occurred at similar frequencies in CAD patients and control subjects (Table 1). We also identified 2 length-variant alleles in an adjacent polyproline repeat (5 versus 4 prolines); however, more than 98% of individuals in both CAD and control classes contained 5 prolines, yielding no statistically significant difference between the 2 groups. These data support that there is no association between these length

Nonstandard abbreviations used: adCAD1, autosomal dominant form of coronary artery disease; CAD, coronary artery disease.

Conflict of interest: The authors have declared that no conflict of interest exists.

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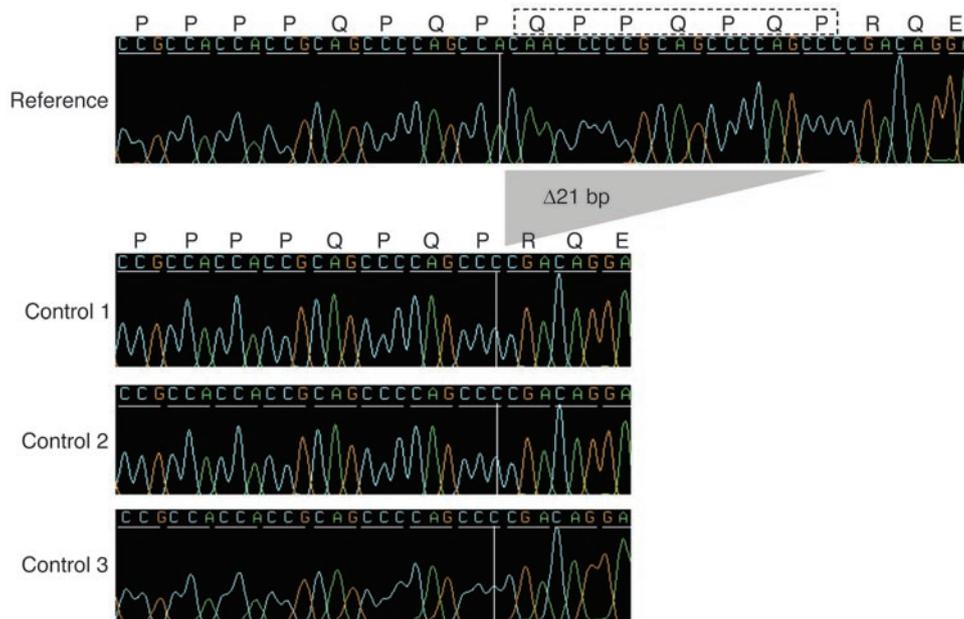


Figure 2

Sequence of the 21-bp deletion region of *MEF2A*. The PCR products from common and deletion alleles were separated by 3% agarose gel, purified, and cloned for sequencing analysis. The 21-bp deletion was identified in 3 independent control subjects. The 7 deleted amino acids are depicted with a dashed-line box. Haplotype analysis of all 3 subjects with the 21-bp deletion as well as approximately 370 control subjects indicates that this variant resides on a common haplotype, consistent with the deletion arising through a single ancestral founding event (data not shown).

Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) (27) and cleaned again with CleanSEQ before being run on a 3730xl DNA Analyzer (Applied Biosystems). Base calling, quality assessment, and assem-

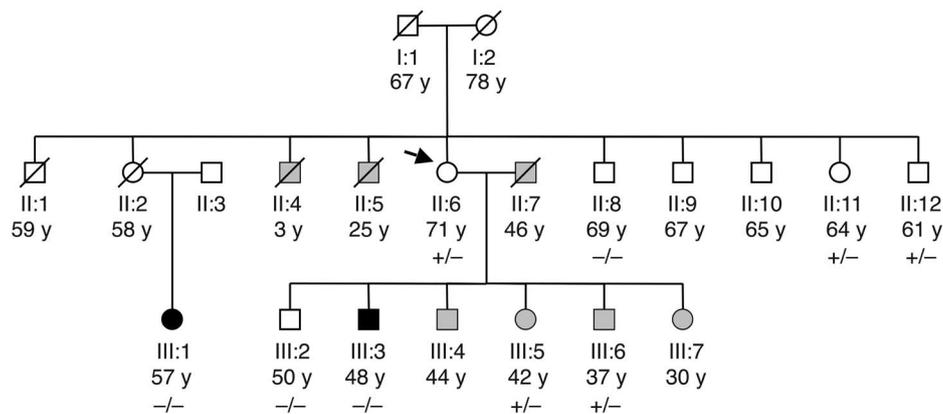
blly were carried out using the Phred, Phrap, PolyPhred, Consed software suite (28, 29). All sequence variants identified were verified by manual inspection of the chromatograms by 2 individuals to ensure accuracy.

Table 2

Clinical characteristics of family members in kindred no. 1

ID no.	Current age or age at death	Sex	Premature CAD ^A	21-bp deletion	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	TC/HDL-C ratio	HTN	Smoker	Diabetes	Other
I-1	67 y ^B (cancer)	M	No		–	–	–	–	–	–	–	–	–
I-2	78 y ^B (SD)	F	No		–	–	–	–	–	–	–	–	–
II-1	59 y ^B (cancer)	M	No		–	–	–	–	–	–	–	–	–
II-2	58 y ^B (cancer)	F	No		–	–	–	–	–	–	–	–	–
II-3		M	NK		–	–	–	–	–	–	–	–	–
II-4	3 y ^B (cancer)	M	NK		–	–	–	–	–	–	–	–	–
II-5	25 y ^B (cancer)	M	NK		–	–	–	–	–	–	–	–	–
II-6	71 y	F	No	+/-	201	249	120	32	6.3	Yes	No	T2DM	TIA at 69 y
II-7	46 y ^B (stroke)	M	NK		–	–	–	–	–	–	–	–	–
II-8	69 y	M	No	-/-	262	210	188	33	7.9	Yes	No	T2DM	–
II-9	67 y	M	No		–	–	–	–	–	–	–	T2DM	–
II-10	65 y	M	No		–	–	–	–	–	–	–	–	–
II-11	64 y	F	No	+/-	261	243	173	40	6.5	No	No	IFG	–
II-12	61 y	M	No	+/-	354	269	266	34	10.4	Yes	No	T2DM	NSE
III-1	57 y	F	Yes	-/-	170	289	87	26	6.5	No	Yes	–	–
III-2	50 y	M	No	-/-	229	166	167	29	7.9	Yes	Ex	–	–
III-3	48 y	M	Yes	-/-	217	116	155	39	5.6	Yes	Ex	–	–
III-4	44 y	M	NK		–	–	–	–	–	–	–	–	–
III-5	42 y	F	NK	+/-	252	87	194	41	6.1	No	Yes	–	NSE
III-6	37 y	M	NK	+/-	194	392	85	31	6.3	No	No	–	NSE
III-7	30 y	F	NK		–	–	–	–	–	–	–	–	–

^APremature CAD defined as onset before age 50 years (males) or before age 55 years (females). ^BDeceased. SD, sudden death; NK, early CAD status not known (male, younger than 50 years; female, younger than 55 years); TC, total cholesterol; TG, triglycerides; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; HTN, hypertension; T2DM, type 2 diabetes; IFG, impaired fasting glucose; TIA, transient ischemic attack; NSE, negative stress electrocardiogram.

**Figure 3**

MEF2A 21-bp deletion does not cosegregate with CAD in kindred no. 1 (Table 2). Individuals with premature CAD are indicated by filled squares (males) or circles (females). Unaffected individuals are indicated by open squares or circles. Normal males under the age of 50 years and normal females under the age of 55 years are shown in light gray, which indicates uncertain phenotype. Deceased individuals are indicated by a slash. The proband is indicated by an arrow. Genetic status: +/- indicates the presence of the 21-bp 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.

Fragment-size analysis. Fluorescently labeled PCR primers were designed to amplify approximately 180 bp of a poly-glutamine and -proline repeat region within the *MEF2A* gene (forward primer: FAM-ATCAAGTCC-GAACCGATTTCA; reverse primer: ACTAGAGCTGCTCAGACTGTCCA). Following PCR, a 1:100 dilution of the PCR product was performed with water. Aliquots of 1.5 μ l of the diluted PCR product were then mixed with 0.2 μ l of the 400HD ROX Size Standard (Applied Biosystems) and 8.3 μ l of Hi-Di formamide (Applied Biosystems) before loading onto an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Gel images were analyzed using GeneScan Analysis Software version 3.7 (Applied Biosystems), and the fragment sizes were called using Genotyper Software version 3.7 (Applied Biosystems). To independently evaluate the fragment size, we genotyped the same repeat region by direct sequencing.

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