Novel 6-bp deletion in *MEF2A* linked to premature coronary artery disease in a large Chinese family

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Abstract. The aim of the present study was to identify the genetic defect responsible for familial coronary artery disease/myocardial infarction (CAD/MI), which exhibited an autosomal dominant pattern of inheritance, in an extended Chinese Han pedigree containing 34 members. Using exome and Sanger sequencing, a novel 6-base pair (bp) 'CAGCCG' deletion in exon 11 of the myocyte enhancer factor 2A (*MEF2A*) gene was identified, which cosegregated with CAD/MI cases in this family. This 6-bp deletion was not detected in 311 sporadic cases of premature CAD/MI or in 323 unrelated healthy controls. Determination of a genetic risk profile has a key role in understanding the pathogenesis of CAD and MI. Among the reported risk-conferring genes and their variants, mutations in *MEF2A* have been reported to segregate with CAD/MI in Caucasian families. Causative missense mutations have also been detected in sporadic CAD/MI cases. However, this suggested genetic linkage is controversial, since it could not be confirmed by ensuing studies. The discovery of a novel *MEF2A* mutation in a Chinese family with premature CAD/MI suggests that *MEF2A* may have a significant role in the pathogenesis of premature CAD/MI. To better understand this association, further *in vitro* and *in vivo* studies are required.

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Introduction

Coronary artery disease (CAD) is a leading cause of mortality (1). Similar to other common complex diseases, the pathogenesis of CAD and associated myocardial infarction (MI) is multifactorial, and is influenced by complex interactions between environmental and genetic factors (1). Several risk factors, including hypertension, dyslipidemia, obesity and smoking, have been established for CAD (2). Genome-wide association studies have uncovered several susceptibility loci and candidate genes that are associated with CAD, either by directly participating in the pathogenesis of CAD or by indirectly regulating the contributing risk factors (3-10). As a multifactorial complex disease, common sequence variants or mutations in numerous genes are commonly associated with CAD (4). In these cases, the genetic contribution of each gene is relatively small; however, it has previously been suggested that CAD/MI may manifest via autosomal dominant inheritance in some families (4).

A mutation in the human myocyte enhancer factor 2A (*MEF2A)* gene, which is a member of the myocyte enhancer family of transcription factors, has previously been detected in an autosomal dominant form of CAD (1). Genetic linkage analysis of a large Caucasian family exhibiting an autosomal dominant inheritance pattern of premature CAD indicated a positive linkage to a single locus on chromosome 15q26, which includes ~90 annotated genes. Resequencing of the *MEF2A* gene, which is a prime candidate gene in the linked locus, revealed a 21-base pair (bp) coding sequence deletion at exon 11 in all affected family members (1). Although this initial study suggested the involvement of *MEF2A* variants in the risk of CAD/MI, they have not been supported by more recent reports. Weng *et al* identified these variants in elderly Caucasian control subjects without CAD (11), whereas other studies found no evidence of any linkage or association between *MEF2A* and CAD in 1,700 patients with sporadic MI and multiple families with apparent Mendelian inheritance of the disease (12,13). These findings suggested that these

mutations may be rare and isolated only to families exhibiting premature CAD, or that the *MEF2A* gene is unrelated to CAD. The present study aimed to identify the genetic defect responsible for familial premature CAD/MI in an extended Chinese Han pedigree of 34 members exhibiting an autosomal dominant pattern.

Materials and methods

Participants and clinical evaluation. A four-generation, 34-member Chinese Han family with familial CAD was recruited from the Qilu Hospital, Shandong University (Jinan, China) through reviewing the records of patients displaying the clinical features of CAD/MI. CAD/MI in this family followed an autosomal dominant pattern of inheritance (Fig. 1A). Upon basic clinical examination, members of the family with preceding or existing indications of CAD/MI (based on the existence of at least two of the following criteria: Prolonged chest pain, electrocardiography patterns consistent with acute MI, or significant elevation of cardiac enzymes) underwent coronary computed tomography analysis. Coronary angiograms were subsequently carried out on all subjects to confirm a diagnosis of CAD (Fig. 1B). According to angiographic appearance, a vessel was regarded as diseased if it contained at least one stenosis involving >50% loss of lumen diameter. Seven living patients in this family were identified as having CAD (II-2, III-1, III-5, III-7, III-9, III-13 and IV-1) (Fig. 1A). In addition, all family members were subjected to a physical examination, blood testing, and a standardized interview that included questions related to medical history, physical activity, medication and personal habits (Table I). In addition, blood pressure was taken according to the MONICA guidelines (3), using the random-zero method and using standard mercury sphygmomanometers after the subjects had been resting in a seated position (Table I). The control group consisted of 311 patients (mean age, 46.3±10.12; male/female, 151/160) who had attended the Cardiology Departments of the Qilu Hospital between 2005 and 2008, and had suffered a first episode of MI, as defined according to World Health Organization criteria (14). The healthy control group consisted of 323 Chinese individuals (mean age: 46 years), including both obese and normal weight subjects, without a history of premature CAD, who were recruited separately from the Jinan region. All subjects provided written informed consent for the present study, which was approved by the ethics committee of Qilu Hospital, Shandong University.

Exome capture. Genomic DNA was extracted from peripheral blood using the standard phenol-chloroform extraction method (15). The genomic DNA of three patients in the Qilu hospital (III-1, III-5 and III-7) was sheared by sonication and was then hybridized to the Nimblegen SeqCap EZ Library (Roche Diagnostics, Basel, Switzerland), in order to enrich exonic DNA in each library, according to the manufacturer's protocol. Sequencing of the enriched library was performed using the Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA) to generate 90-bp paired-end reads (16). A mean exome coverage of 78.87x was obtained, allowing each selected region of the genome to be checked. Such coverage provided sufficient depth to accurately call variants at 99.34% of the targeted exome (17).

Read mapping and variant analysis. The human reference genome was obtained from the online University of California, Santa Cruz database (http://genome.ucsc.edu/), version hg19 (build 37.1). Alignment of patient sequences was performed using the Short Oligonucleotide Analysis Package (SOAP) aligner (soap2.21; http://soap.genomics.org.cn/soapsnp.html) and single nucleotide polymorphisms (SNPs) were called using the SOAP snp set with the default parameters, after the duplicated reads [obtained mainly in the polymerase chain reaction (PCR) step] were deleted. PCR was performed accoreding to the protocol of Illumina Paired-End Sample Prep Kit (Illumina). A reaction volume of 50 μl was used, containing 100 ng gDNA and 10 pmol of each primer, and PCR was performed in a 9700 Thermal Cycler system (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The reaction was performed for 35 cycles of denaturation at 95˚C for 30 sec, annealing at 58˚C for 35 sec and extension at 72˚C for 50 sec, and a final extension step at 72˚C for 5 min. Small insertions or deletions (indels) affecting coding sequence or splicing sites were detected (16,17). The thresholds for calling SNPs and short indels included the number of unique mapped reads supporting a SNP ≥4 and a consensus quality score ≥20. The quality score represents a Phred score, generated by the program SOAP snp 1.05, where quality = -10log (error rate). It is unlikely that causative variants are present in the general population. All candidate variations identified in the patient sequences were filtered against the SNP database (dbSNP build 137; http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), 1000 Genomes Project (1000genomes release_20100804; http://www.1000genomes.org/), HapMap project (2010-08_phase II+III; http://hapmap.ncbi.nlm.nih.gov/) and YanHuang project (http://yh.genomics.org.cn/). Sorting intolerant from tolerant prediction (http://sift.jcvi.org/) was performed to evaluate whether amino acid substitutions, amino acid indels and frameshifting indels could affect protein function (16).

Mutation validation. Locus-specific PCR and detection primers were designed (Boshang Biotechnology Company, Jinan, China). Sanger sequencing was performed to determine the presence and identity of potential disease-causing variants using the ABI3500 sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.). PCR amplification and Sanger sequencing were conducted as described previously (18). The primer sequences used to identify *MEF2A* disease-linked variants were as follows: forward, 5'-GCATCAAGTCCGAACCGATT-3', and reverse, 5'-GGAGCGACCCATTTCCTGTC-3'.

Results

Clinical characteristics of the family. The present study identified an extended Chinese family containing 34 members (five members deceased) with a history of CAD/MI (Fig. 1A). The family consisted of 20 females and 14 males distributed in four generations; nine members were diagnosed with CAD/MI (two of which were deceased). The proband (III-9) with CAD was identified at the Department of Cardiology, Qilu Hospital, Shandong University at 36 years of age, with right coronary artery stenosis with >80% severity (Table II). Subsequently, the patient's two elder sisters, one elder brother,

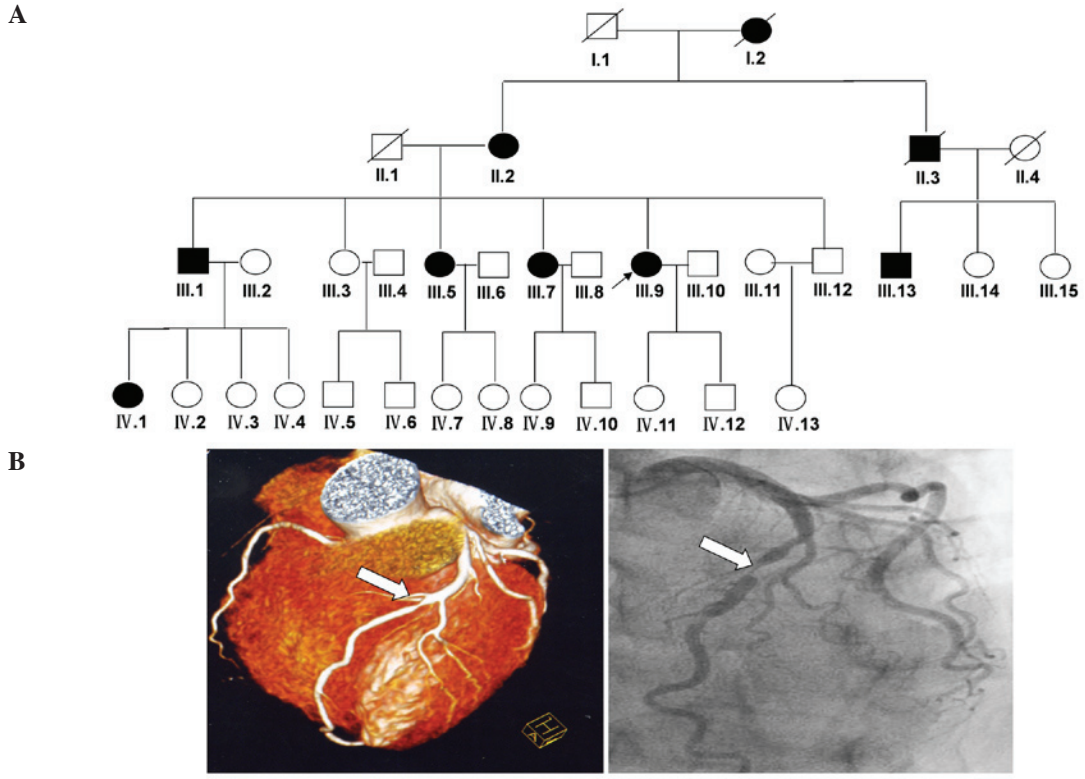

Figure 1. (A) Pedigree structure of a family demonstrating autosomal dominant inheritance of coronary artery disease (CAD). Individuals with CAD are indicated by solid squares (males) or solid circles (females). Unaffected individuals are indicated by open symbols. Deceased individuals are indicated by a slash (/). The proband is indicated by an arrow. (B) Coronary computed tomography image (left) and coronary angiogram image (right) from the proband, who experienced an inferior myocardial infarction (MI) caused by a significant stenotic lesion (arrow) in the distal right coronary artery. This lesion was at a bifurcation site typical of coronary atherosclerosis.

and male cousin developed symptoms of CAD. Subjects III-1 [left anterior descending coronary artery (LAD) angiogram >75% stenosis] and III-13 (RCA angiogram >80% stenosis) were diagnosed at the ages of 49 and 46, respectively; subjects III-5 (LAD angiogram >90% stenosis) and III-7 (LAD angiogram >90% stenosis) were diagnosed at the ages of 43 and 45, respectively. Subject III-1 was diagnosed 10 years ago, at the age of 49 years old. Subjects III-1 and III-5 suffered MI and stroke at the ages of 49 and 51 years old, respectively. The severity of the disease in female patients was greater than that in males (Table II). As shown in Table I, a few members of the family exhibited mildly elevated serum levels of total cholesterol and triglycerides, but all had normal serum levels of low-density and high-density lipoproteins, and none of the family members were cigarette smokers or had hypertension, diabetes or obesity. These clinical manifestations strongly suggested heritable CAD in this family. Pedigree analysis of the family suggested autosomal dominant inheritance of CAD (Fig. 1A).

Identification of a 6-bp deletion in the MEF2A gene. Using the filtering criteria as described previously (19), *MEF2A* was identified as a CAD-causing candidate gene after exome sequencing of genomic DNA. Following validation by Sanger sequencing, a 6-bp deletion (CAGCCG) was identified in exon 11 in all seven family members with CAD and in five non-CAD members (IV-3, IV-7, IV-8, IV-10 and IV-12). The 6-bp deletion was located at position 1671 to 1677 in the cDNA sequence of the *MEF2A* gene (Fig. 2A). The CAGCCG deletion also contained the first CAG repeat of the (CAG)n repeats polymorphism in exon 11, which resulted in variable expression and was associated with CAD (5). The 6-bp deletion was identified in family members with a normal phenotype (IV-3, IV-7, IV-8, IV-10 and IV-12) and segregated with CAD in the family, thus suggesting that this variant is the pathogenic mutation (Fig. 2B). The five family members (IV-3, IV-7, IV-8, IV-10 and IV-12) with the *MEF2A* deletion but without CAD likely have not yet developed the phenotype due to their younger ages (all <40 years old). In order to determine whether the mutation is present in sporadic CAD cases, the exon 11 coding sequence of *MEF2A* was sequenced in 311 unrelated subjects with an established diagnosis of CAD and in 323 healthy subjects. The 6-bp deletion was not detected in these subjects. The entire coding region of *MEF2A* and the intron-exon boundaries were also screened for mutations in all members of the family. No mutations were identified in any other exons (data not shown).

Discussion

The present study identified a novel mutation in *MEF2A* in a Chinese family with inherited CAD. To the best of our knowledge, the present study is the first to report a causative association between a *MEF2A* mutation and CAD in the Chinese population, and a novel mutation of *MEF2A* due to a 6-bp deletion in exon 11.

Table I. Clinical characteristics of family members.

ID no.	Current age (years)	Gender	Premature CAD	TC	TG	LDL-C	HDL-C	TC/HDL-C	HTN	Smoker	BMI	FBG
I-1	-	M	-	-	-	-	-	-	-	-	-	-
I-2	-	F	-	-	-	-	-	-	-	-	-	-
II-1	-	M	-	-	-	-	-	-	-	-	-	-
II-2	84	F	Yes	183.6	83.7	115.6	65.73	2.79	No	No	22.2	5.8
II-3	-	M	-	-	-	-	-	-	-	-	-	-
II-4	-	F	-	-	-	-	-	-	-	-	-	-
III-1	59	M	Yes	179.1	71.5	131.2	69.4	2.58	No	No	24.1	4.7
III-2	60	F	No	221.8	88.5	148.2	79.7	2.78	No	No	24.7	4.9
III-3	58	F	No	200.4	76.1	124.2	83.9	2.38	No	No	22.3	5.1
III-4	59	M	No	213.5	89.3	138.9	83.9	2.54	No	No	21.1	5.5
III-5	55	F	Yes	121.9	77.9	65.4	43.9	2.77	No	No	23.4	4.2
III-6	56	M	No	235.7	101.7	143.5	99.8	2.36	No	No	21.6	3.9
III-7	53	F	Yes	143.6	46.9	79.3	91.6	1.57	No	No	22.7	5.3
III-8	51	M	No	223.5	101.2	131.5	86.4	2.59	No	No	23	5.6
III-9	49	F	Yes	145.9	86.7	90.8	53.5	2.73	No	No	26.8	5.1
III-10	49	M	No	201.6	90.4	126.3	81.4	2.47	No	No	22.8	3.8
III-11	46	F	UK	173.8	61.9	115.3	59.3	2.93	No	No	24.1	4.6
III-12	41	M	No	207.2	74.8	121.3	81.7	2.54	No	No	22.6	5.3
III-13	57	M	Yes	167.7	88.5	101.2	71.7	2.34	No	No	24.5	4.3
III-14	49	F	No	146.2	94.8	111.1	61.5	2.38	No	No	23.9	5.8
III-15	51	F	No	168.6	90.6	131.1	73.4	2.3	No	No	22.8	5.2
IV-1	41	F	Yes	143.9	47.5	76.9	93.9	1.53	No	No	20.6	4.4
IV-2	38	F	No	220.9	124.8	137.4	78.5	2.81	No	No	23.8	3.7
IV-3	35	F	UK	216.7	36.3	144.7	97.8	2.21	No	No	22.4	4.8
IV-4	32	F	No	142.4	56.6	89.4	65.5	2.18	No	No	26.6	5.5
IV-5	36	M	No	226	312.4	127.3	44.7	5.06	No	No	22.8	3.9
IV-6	33	M	No	153.6	109.7	93.7	48.5	3.17	No	No	19.9	4.2
IV-7	30	F	UK	193.5	84.9	118.8	66.9	2.89	No	No	23.1	4.7
IV-8	28	F	UK	179.7	67.4	113.5	61.5	2.92	No	No	25.9	5.1
IV-9	28	F	No	172.6	46.2	92.9	102	1.69	No	No	19.8	4.7
IV-10	10	M	UK	177.3	65.9	87.6	103.7	1.71	No	No	21.4	5
IV-11	27	F	UK	178.8	90.3	77.8	106	1.68	No	No	22.3	4.1
IV-12	26	M	No	159.8	37.2	93.7	78.93	2.02	No	No	24.3	5.3
IV-13	22	F	No	166.8	62.8	99.1	81.62	2.04	No	No	22.1	4.7

The data for TC, TG, LDL-C and HDL-C are provided as mg/dl; the data for FBG are provided as mmol/l. M, male; F, female; UK, status of premature CAD was unknown; CAD, coronary artery disease; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HTN, hypertension; BMI, body mass index; FBG, fasting blood sugar.

Wang *et al* reported a possible role for *MEF2A* variants in the pathogenesis of CAD, describing a 21-bp deletion as the disease-causing genetic mutation for Caucasian familial CAD/MI without common risk factors (1). A subsequent functional study revealed that the 21-bp *MEF2A* gene mutation resulted in the deletion of seven amino acids in exon 11 of *MEF2A*, thus disrupting the transcriptional activity and blocking nuclear localization of the *MEF2A* protein (1). In a previous study, three genetic variants of the *MEF2A* gene (N263S, P279L and G283D) were detected in four out of 207 unrelated Caucasian patients (1.9%) with CAD (7). Furthermore, a (CAG)n repeat in exon 11 has been reported to be associated with CAD in a small Chinese case-control study (5). These data suggested that *MEF2A* may have a significant role in the pathogenesis of CAD in non-familial (sporadic) cases. However, this hypothesis was not supported by subsequent studies. Weng *et al* did not detect an MI causative *MEF2A* mutation in 300 cases of sporadic CAD in Caucasian patients (11). Furthermore, Lieb *et al* failed to detect the 21-bp deletion in the *MEF2A* gene in 1,481 individuals with a positive family history of CAD (3).

The present study identified a *MEF2A* gene mutation in a family with CAD. To the best of our knowledge, this is only the second report to identify a *MEF2A* mutation in a family

Table II. Characteristics of family members with coronary artery disease and myocardial infarction (MI).

Individual ID No.	Current age (years)	Age at time of diagnosis (years)	Clinical diagnosis
II-2	84	51	MI, stroke
III-1	59	49	LAD angiogram >75% stenosis
III-5	55	43	LAD angiogram >90% stenosis
III-7	53	45	LAD angiogram >90% stenosis
III-9	49	36	RCA angiogram >80% stenosis
III-13	57	46	RCA angiogram >80% stenosis
IV-1	41	40	LAD angiogram >50% stenosis

LAD, left anterior descending coronary artery; RCA, right coronary artery.

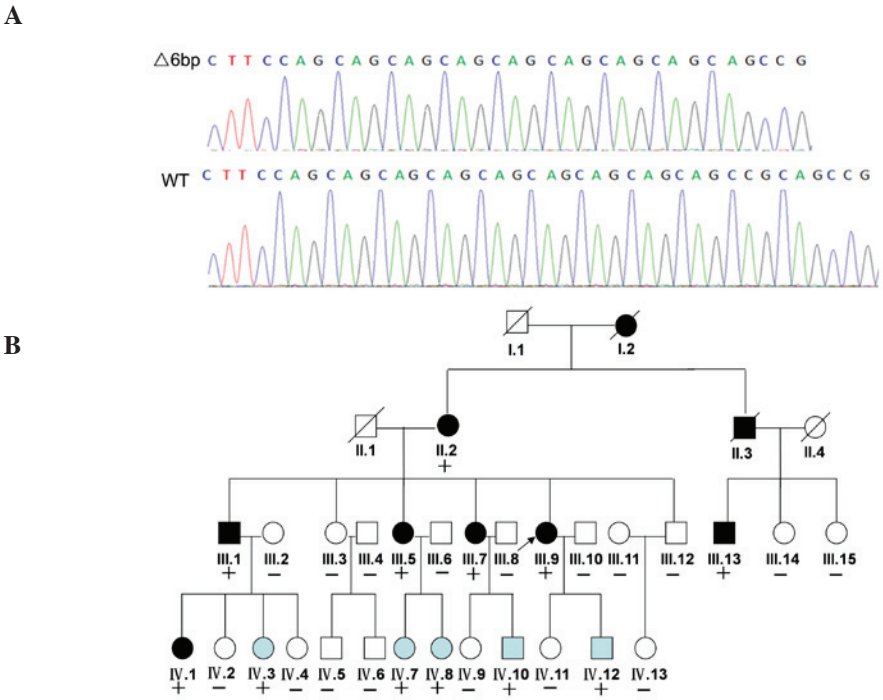

Figure 2. (A) DNA sequence analysis of the wild-type (WT) allele and the 6-base pair (bp) deletion allele (Δ6 bp) of myocyte enhancer factor 2A (*MEF2A*). Sequence analysis of exon 11 of *MEF2A* in the proband (III.9) revealed the presence of a deletion. The WT and deletion alleles were separated by 2% agarose gel electrophoresis, purified, and cloned for sequencing analysis. The location of Δ6 bp was indicated. (B) *MEF2A* intragenic Δ6 bp deletion cosegregated with coronary artery disease (CAD) in the family. The family pedigree indicated genetic status: + indicates the presence of the 6-bp *MEF2A* deletion (heterozygous); - indicates the absence of the deletion. Individuals with CAD are indicated by solid squares (males) or solid circles (females). Unaffected individuals are indicated by open symbols. Normal, healthy males under the age of 50 or normal females under the age of 55 with the Δ6 bp are shown in light gray, which indicates an uncertain phenotype. Deceased individuals are indicated by a slash (/). The proband is indicated by an arrow.

with CAD (4). These results strongly supported a causative role of *MEF2A* gene mutations in the pathogenesis of CAD. High *MEF2A* expression in the endothelium of coronary arteries suggests that an early step, or triggering event, in the development of CAD may involve the dysregulation of specific *MEF2A* transcriptional pathways in the endothelium, which is expected to result in endothelial dysfunction (5,10). Endothelial dysfunction is associated with atherosclerotic plaque formation and rupture, and subsequent thrombosis, which are common causes of MI and sudden cardiac death (6,7).

The present study demonstrated that the effects of *MEF2A* and its mutation on the pathogenesis of CAD are not confined to a single ethnic group, since it was originally reported in a Caucasian family (1). It has been well established that CAD is a multifactorial disease that is associated with an array of genes and their variants (8). Previous studies have clearly demonstrated that CAD is a multifactorial disease that is affected by multiple genes. It is therefore uncommon to observe a multifactorial common disease manifesting in a dominant Mendelian inheritance unless the mutation has a predominant effect on the pathogenesis of CAD/MI (9). In these situations, the functional effects of the mutation on the target gene are significant and dominant, and therefore may override other risk factors and induce pathological outcomes

in subjects with the mutation. The lack of *MEF2A* mutations in sporadic CAD cases may be attributed to two factors. Firstly, the mutation may be too rare to be detectable in 311 subjects with CAD; therefore, a much larger number of subjects may be needed in order to detect the mutation. Secondly, the mutation may be confined to autosomal dominant CAD cases and may not contribute in a significant way to common, sporadic CAD cases. The mutational effect observed in the present study was so large that, when it occurred, it affected family members in a Mendelian fashion. This hypothesis is not contradictory to the established relationship between common sporadic CAD cases and multiple genes with small effects. Additional functional studies of the *MEF2A* gene and mutation in both *in vitro* and *in vivo* models are required to further elucidate the functional implications. Considered alongside a growing body of evidence, the findings of the present study strongly indicated that the *MEF2A* gene, and its possible causal relationship with the pathogenesis of CAD, is too important to ignore.

In conclusion, the discovery of a novel mutation in the *MEF2A* gene in a Chinese family with autosomal dominant CAD suggests that *MEF2A* may have a significant role in the pathogenesis of CAD.

Acknowledgements

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