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MEF2A gene and susceptibility to coronary artery disease in the Chinese people

YUAN Hong[1], LÜ Hong-wei[1,*], HU Jing[1], CHEN Shu-hua[2], YANG Guo-ping[1], HUANG Zhi-jun[1]

(1. *Center for Experimental Medical Research, Third Xiangya Hospital, Central South University, Changsha* 410013;
2. *Department of Biochemistry, College of Biological Science and Technology, Central South University, Changsha* 410078, *China*)

Abstract: Objective To explore *MEF2A* gene and susceptibility to coronary artery disease in the Chinese. **Methods** One hundred seventy-five coronary artery disease (CAD) patients and 228 normal subjects were recruited and their blood samples were amplified to detect sequences of all 11 exons of *MEF2A* gene by PCR. Single-strand conformational polymorphism (SSCP) analysis was used to detect the mutation. The amplified products were purified and sequenced. **Results** The tri-nucleotide (CAG) length polymorphism in the last coding exon of *MEF2A* in the Chinese was revealed and 4 of the 175 (2.3%) CAD samples containing 4 prolines were due to one proline deletion in *MEF2A* gene. But all the 228 normal subjects contained 5 prolines. The mutation in both 175 CAD samples and 228 normal subjects was not found in other exons. **Conclusion** The deletion mutation in exon 11 in *MEF2A* gene may be related to CAD susceptibility in the Chinese population.

Key words: *MEF2A*; gene; coronary artery diseases; tri-nucleotide length polymorphism; mutations

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中国人群 *MEF2A* 基因与冠状动脉疾病的易感性

袁 洪[1],闾宏伟[1,*],胡 靖[1],陈淑华[2],阳国平[1],黄志军[1]

(中南大学 1.湘雅三医院医学实验中心,长沙 410013; 2.生物科学与技术学院生化教研室,长沙 410013)

[**摘要**] 目的:探讨中国人群 *MEF2A* 基因与 CAD 的易感性关系。方法:对 175 例冠状动脉疾病(CAD)患者和 228 例正常对照的血标本进行 PCR 扩增 *MEF2A* 基因的 11 个外显子,然后采用 SSCP 方法检测外显子的突变并对扩增产物进行纯化和测序分析。结果:*MEF2A* 基因的第 11 外显子存在三核苷酸(CAG)重复多态性,CAD 患者和正常对照之间无统计学差异($P>0.05$);另发现 4 例 CAD 患者在第 11 外显子存在 1 个 CCG 的缺失突变,突变率约为 2.3%,而正常对照未见此突变;CAD 患者和正常对照组 *MEF2A* 基因的其它外显子未发现突变。结论:中国人群 *MEF2A* 基因第 11 外显子存在 1 个 CCG 的缺失突变可能与冠状动脉疾病患者易感性有关。

[**关键词**] 肌细胞增强因子 2A; 基因; 冠状动脉疾病; 三核苷酸重复多态性; 突变

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Coronary artery diseases (CAD) is the most common form of heart diseases. CAD and its complications, including myocardial infarction (MI) and stroke, are the leading causes of disability and death in many countries. Relatively little is known about the genetic basis of CAD and MI because of the genetic heterogeneity and multifactorial etiology of these diseases. But a recent report by Wang et al.[1] showed that the myocyte enhancer factor 2A (*MEF2A*) gene was the most likely positional candidate responsible for CAD.

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* Corresponding Author, E-mail: hwlv2226@163.com

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MEF2A gene encodes a transcription factor that functions in the development of the fetal cardiovascular system. A 21-nucleotide deletion that eliminates seven amino acids from C terminus of *MEF2A* caused CAD. Since the completion of the original study, the authors have reported *MEF2A* missense mutations (N263S, P279L, and G283D) in 4 out of 207 cases of CAD and estimate that approximately 2% of CAD are due to mutations in this gene[2]. Is there any different molecular mechanism in different ethnic groups? We conducted a study to determine the relation between *MEF2A* gene and CAD susceptibility in Chinese population.

1 MATERIALS AND METHODS

1.1 Study subjects and isolation of genomic DNA

The study participants were identified at the Third Xiangya Hospital, Central South University. CAD patients ($n=175$) were defined as any previous or current evidence of significant atherosclerotic CAD [defined as MI, percutaneous coronary angioplasty (PTCA), coronary artery bypass surgery (CABG), or coronary angioplasty with >50% stenosis].

The normal controls ($n=228$) were defined as individuals at age ≥55 years whose coronary angioplasty showed no luminal stenosis.

Genomic DNA was prepared from the whole blood of each subject with the DNA isolation kit for mammalian blood (Gentra System, Inc).

1.2 Mutation analysis

PCR primers were designed based on the flanking intronic sequences of the all 11 exons (Table 1). The 11 exons' complete coding region and the intron splice sites were amplified by PCR. Single-strand conformational polymorphism (SSCP) analysis was used to detect the mutation.

1.3 Purification and sequencing of amplified products

The PCR products from common and deletion alleles were separated by 3% agarose gel, purified using the QIAQuick PCR purification kit and cloned for sequencing analysis with forward and reverse primers by an ABI377 Genetic Analyzer.

Table 1 PCR primers for amplification of *MEF2A* exons and mutational analysis

Exon	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Annealing temperature (℃)
1	AGAAGCTGTGTACGATGCATTAG	ACCCAACCATTCTGTCCTATGTT	64
2	AGATTCATCTTCAG ATAGCCCATA	ACAAGTCATTCTGACAGTTAATGC	64
3	AGTTCATTCCGTCTGTGCTCTCT	AAGTAGAGGTAAAGTAAAAGTACTTA	66
4	TAAGTACTTTTACTTTACCTCTACTT	GCAACAAGATGTTTGGTCAATCTCT	66
5	AGTAACTTGAGTTACCTTGCCA	GAACCTGCTTATGTTAACCAATGA	50
6	TCTCTATTCAGTTCACGT TCAGTTA	TGTATTAGTGAAAGTACCCTTCAG	50
7	GATACTCAAACCTGTAGTGAGT	GGAAGCTACAGATTGACTATGT	55
8	TGTGAGTACCAACAGTCTTAGTA	GGTTAGATAACAACACGTAAGAG	60
9	TCACATCATCAGTGCTTCAGAA	CACAGAAGCACACGTTGATCA	64
10	ATAGATTCCGTATGGACCTTCCA	AAGACAGTGTGTAGGCCAGGAGTG	66
11	TGCAGAGGTACTTGCAAGCCAT	AGATATGTAGGGCAGGTCACT	64

1.4 Statistical analysis

Data were denoted as means ± SD ($\bar{x} \pm s$), calculated and analyzed by the linear correlation and t test using the statistical software SPSS11.0. The significance level was set at $P<0.05$.

2 RESULTS

2.1 Sequencing of amplified products

Amplified products were sequenced and revealed a complex coding sequence length polymorphism in the last coding exon of *MEF2A* that resulted from tri-nucleotide length variants within a region of polyglutamine repeats (Figure 1). Analysis of the repeat region in 175 CAD samples and 228 control subjects revealed 5 length-variant alleles overlapping the polyglutamine repeat in our sample set. All 5 alleles occurred at similar frequencies in CAD patients and control subjects (Table 2 and Figure 2).

Table 2 *Analysis of the allele distribution of the polyglutamine repeat length polymorphism in the CAD and control subjects*

Alleles	$(CAG)_{11}$	$(CAG)_{10}$	$(CAG)_9$	$(CAG)_{8,7 \text{ or } 4}$	Total	*P*
N_{CAD}	185	66	85	14	350	
N_{CON}	246	85	106	19	456	0.985
Total	431	151	191	33	806	

N_{CAD}, number of alleles in CAD patients; N_{CON}, number of alleles in control subjects

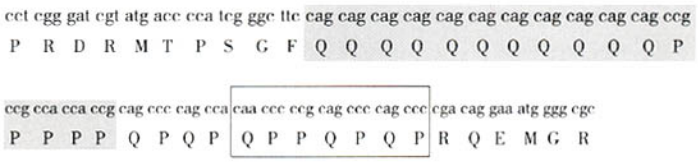

Fig. 1 Nucleotide and amino acid sequences of the repeat region in *MEF2A*. The shaded box marks the polyglutamine and polyproline tandem repeats; the 21-bp deletion is boxed with dashed lines.

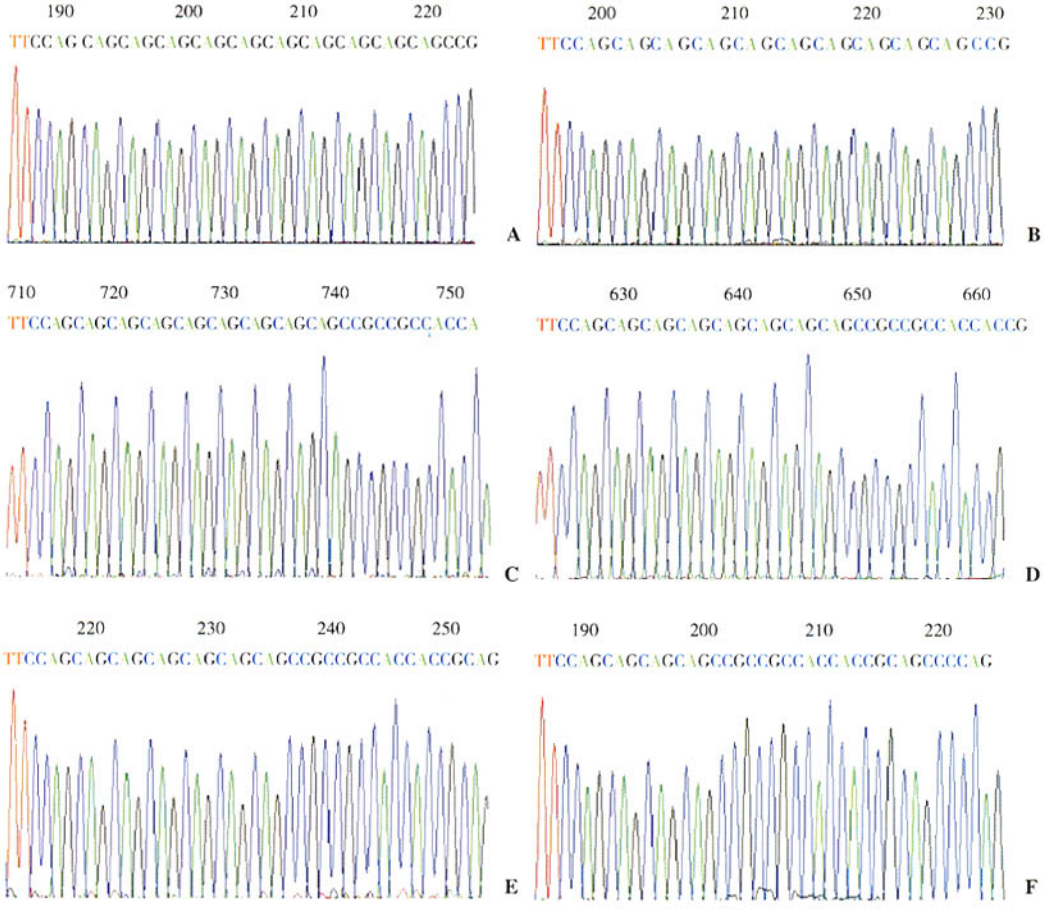

Fig. 2 Repeat numbers of tri-nucleotide (CAG) length polymorphism A: $(CAG)_{11}$; B: $(CAG)_{10}$; C: $(CAG)_9$; D: $(CAG)_8$; E: $(CAG)_7$; F: $(CAG)_4$

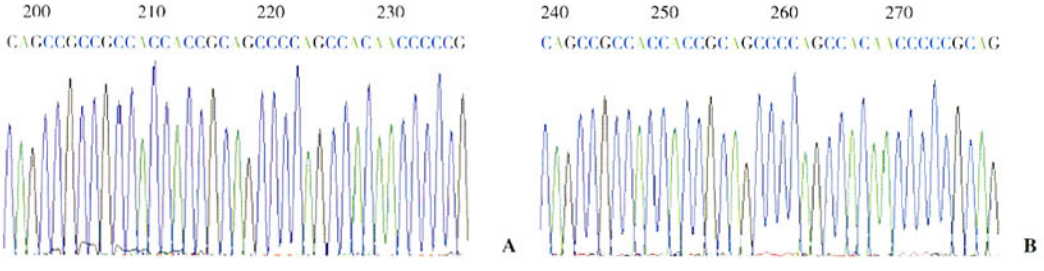

Fig. 3 Sequence deletion of the polyproline tandem repeats of *MEF2A* Deletion was identified in 4 independent CAD patients

2.2 **Sequence deletion of the polyproline tandem repeats of *MEF2A*** We also identified the deletion mutation of one proline within a region of polyproline repeats in the 11th exon of *MEF2A* (5 versus 4 prolines). Four of 175 CAD samples (approximately 2.3%) contained 4 prolines were due to one proline deletion in *MEF2A* gene. But all 228 control samples contained 5 prolines (Figure 3).

2.3 **Mutation screening in exons from 1 to 10** Mutational analysis did not detect any *MEF2A* mutations in exons from 1 to 10 in both 175 CAD patients and 228 control subjects. The results of SSCP analysis in the first exon of *MEF2A* are shown in Figure 4.

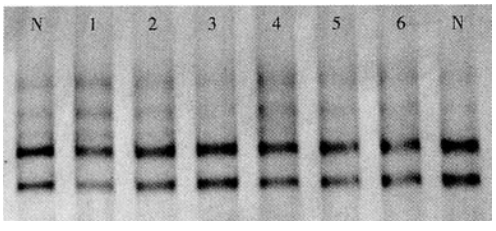

Fig. 4 Results of SSCP analysis in the first exon of *MEF2A* N: Normal; 1~6: CAD patients

3 DISCUSSION

The minute-to-minute function of the cardiovascular system requires seamless connections between endothelial cells, which line the lumens of arteries and veins, and intimate interactions between endothelial and smooth muscle cells and the extracellular matrix. Lesions within the arterial wall caused by lipid accumulation or other insults can result in occlusion of the vessel and the CAD, a primary cause of myocardial infarction (MI) and stroke[3].

MEF2A belongs to a family of 4 closely related transcription factors (*MEF2A*, -B, -C, and -D) that are conserved from yeast to humans. *MEF2A* gene is localized at 15q26. It has 11 exons and codes for 499 amino-acid[4]. The N terminus of *MEF2A* contain MADS and *MEF2A* 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[5,6]. 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[1]. A hallmark of the MEF2 proteins is their propensity to associate with cell-specific and signal-dependent cofactors[7,8]. *MEF2A* has been implicated in directing early angiogenesis and controlling vascular morphogenesis[9].

There is a region of polyglutamine and polyproline repeats in exon 11 in *MEF2A* gene. The sequence is $(CAG)_{11}$ $(CCG)_2$ $(CCA)_2$ CCGCAGCCCCAGCCA CAACCC CCGCAGCCCCAGCCC, containing microsatellite DNA sequence CAG as a unit[10,11]. Wang et al[1]. had identified 21 base-pair deletion (see the marked sequence underlined) in the MEF2A gene, resulting in a seven-amino acid deletion, which is responsible for a large family with CAD and MI. The findings of Wang raise a series of interesting questions. The seven-amino acid deletion possiblely perturbs *MEF2A* function, abolishes nuclear localization and creates a dominant negative protein. The results defined a genetic pathway and provided a molecular mechanism for the pathogenesis of familial CAD and MI[12~15].

In the present study, we identified a complex coding sequence length polymorphism that results from tri-nucleotide length variants within a region of polyglutamine repeats, but yielded no statistically significant difference between the 2 groups. We also identified that 4 (2.3%) CAD patients contained 4 prolines and were due to one proline deletion in *MEF2A* gene. Whereas all the controls contained 5 prolines. There may be relation between *MEF2A* gene and CAD susceptibility in Chinese population. Our results provide a potential novel molecular mechanism for the pathogenesis of CAD, which may be revealed by future studies with large sample sizes.

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