Is There Any Association Between the MEF2A Gene Changes and Coronary Artery Disease?

Soodeh Omidi1, Serwa Ghasemi2, Samira Kalayinia3

1 Department of Genetic, Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran
2 Department of Biology, School of Basic Sciences, Islamic Azad University Research Tehran Branch, Tehran, Iran
3 Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran

Abstract- Coronary artery disease (CAD) is a common multifactorial disease with a high rate of morbidity and mortality worldwide. The MEF2A gene transcription factor belongs to the myocyte enhancer factor-2 (MEF2) family and is involved in critical processes such as calcium-dependent signaling pathways and cardiac development. Although the variants of the MEF2A gene were studied in different CAD and myocardial infarction (MI) populations, the reality of this gene association with CAD is still unclear. This study reports the first in silico investigation on MEF2A variants. All reported variants in CAD/MI patients were collected from eleven countries. Their pathogenicity and variant position conservation were surveyed by online prediction tools, including Mutation- Taster, Polyphen-2, PROVEAN, SIFT, CADD, and GERP. In silico analysis did not confirm the pathogenic effect of 21-bp deletion, which was introduced as a monogenic cause of CAD. c.704C>A (p.S235Y), c.812C>G (p.P271R), c.836C>T (p.P279L) and c.848G>A (p.G283D) missenses, c.1315C>T (p.R439X) nonsense, and seven out-of-frame deletions were predicted as disease-causing variants. Although some variants of the MEF2A gene affect protein structure, the MEF2A variation studies in CAD/MI patients and in silico analysis do not approve the association and pathogenicity of MEF2A variants in the familial/sporadic CAD.

Keywords: Coronary artery disease; Myocyte enhancer factor-2 (MEF2A); In silico analysis

Introduction

According to recent World Health Organization (WHO) reports, cardiovascular diseases (CVD) with 17.9 million deaths per year, is the primary cause of death worldwide. Coronary artery disease (CAD) is one of the CVDs with defects in heart vessels (1). CAD is the cause of one-third of deaths in people older than 35 years (2). Various modifiable risk factors can result in this condition, including hypertension, high blood cholesterol levels, smoking, diabetes, overweight or obesity, lack of physical activity, unhealthy diet, stress, and conventional risk factors such as age, gender, family history, and race (3). Several genes have been considered to have a role in the monogenic form of CAD, comprising LDLR, ApoB-100, ARH and PCSK9, ApoA1, ABCA1, and LCAT. LPL, ApoC-II, and ABCG5/8 genes which are involved in lipid metabolism. Moreover, some other genes that have no direct effects on plasma lipid levels include LRP6, CYP27A1, ST6GALNAC5, and MEF2A (4).

The myocyte enhancer factor-2 (MEF2) transcription factors family with four proteins, i.e., MEF2-A, -B, -C, and -D, mediates calcium-dependent signaling pathways in the various process such as division, differentiation, and cell death (5). The MEF2A gene, which is located in the 99,565,417 to 99,716,466 bp interval on chromosome 15q26.3, has thirteen transcripts, nine of which are protein-coding. The longest transcript with 5824 bp and 11 coding exons encodes a protein with 499 amino acids (Ensemble accession number: ENSG00000068305).

MEF2A, in cooperation with other MEF2 transcription factors, regulates the differentiation of cardiac muscle cells (6), whereas MEF2A plays a critical role in the postnatal heart without supporting of other MEF2 isoforms. As Naya et al., indicated, mice with a deficiency in the MEF2A gene died in the first week of life (7). The first report of the role of MEF2A in CAD was from a family with thirteen patients. In this family, seven amino acid
deletions in exon 11 resulted in CAD with an autosomal dominant pattern (8). In another study, the sequencing of the MEF2A gene in 300 premature CAD patients and a healthy group revealed only one missense variant in one of the CAD patients and the specific seven amino acid deletions in one of 300 unaffected individuals. Further screening of 1500 additional individuals without CAD by this team indicated two cases with seven amino acid deletions and no association with CAD in their family analysis (9). Given the contradictory results of studies in different populations, the conformity of this transcription factor role in CAD/MI requires more investigation. This study is the first to analyze by in silico analysis all MEF2A reported variants, including substitutions, deletions, and insertions.

Materials and Methods

Collection of MEF2A gene variations

Due to what has been concluded in some publications about the MEF2A role in CAD etiology, we selected this gene for more investigation of its variations. “MEF2A” and “MEF2A variations” terms were used for our search. Variations which had been reported in different countries were collected from PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), Google Scholar, and Google in general.

Investigation of variants frequency

The frequency of all collected MEF2A variants was achieved from the 1000 Genomes Project (10) (IGSR; http://www.internationalgenome.org/), and Exome Aggregation Consortium (11) (ExAC; http://exac.broadinstitute.org/).

Variants evaluation

All reported variants in CAD/MI patients were analyzed to determine any possible effects of them, by computational prediction tools including Mutation Taster (http://www.mutationtaster.org/) (12), Polymorphism Phenotyping (PolyPhen-2: http://genetics.bwh.harvard.edu/pph/) (13), Sorting Intolerant From Tolerant (SIFT; http://sift.biastar.edu.sg/) (14), Protein Variation Effect Analyzer (PROVEAN; http://provean.jcvi.org/index.php) (15), Combined Annotation Dependent Depletion (CADD; https://cadd.gs.washington.edu/) (16), Rare Exome Variant Ensemble Learner (REVEL) (17); and the conservation scores of variant position was calculated by Genomic Evolutionary Rate Profiling (GERP; http://mendel.stanford.edu/SidowLab/downloads/gerp/)

online (18).

Mutation Taster has the highest accuracy in comparison of other tools (SIFT, PROVEAN, PolyPhen-2) in effect prediction of the insertion/deletion, intronic, and intron-exon splice site changes (12).

PolyPhen-2 predicts the effects of amino acid changes on protein stability and function based on structural properties and conservation profiles. The PolyPhen-2 score ranges from 0.0 to 1.0; variants with scores in the range between 0.95-1 are damaging (13).

SIFT classifies substitutions as intolerant and tolerant changes in genomic and protein levels based on sequence homology with similar sequences. Moreover, SIFT updated for insertions/deletions effect prediction recently. The SIFT score ranges from 0.0 to 1.0; substitutions with scores <0.05 have been considered deleterious (14).

PROVEAN measures the similarity of the query protein sequence to its homologous protein sequence with and without variation. PROVEAN provides a prediction of variants in genomic and protein levels for single/multiple substitutions and in-frame insertions/deletions. The default score threshold is set at 2.5; substitutions with probabilities <0.05 are deleterious (15).

CADD reports a single measure as the C-score (ranging from 1 to 99) due to multiple annotations. CADD is a useful tool for the prediction of single-nucleotide and short insertions/deletion variants (16).

REVEL is a method for predicting of missense variant pathogenicity. This method integrates scores from 13 prediction tools. REVEL scores range from 0 to 1, and higher scores are predicted more likely pathogenic (17).

GERP calculates a conservation score for any nucleotide positions by using the multiple alignments of 35 mammal’s genome sequences. It ranges from -12.3 to 6.17, i.e., 6.17 is the most conservation score (18).

It is designed to predict the functional consequences of not only amino acid substitutions but also in intronic and synonymous alterations, short insertion and/or deletion (indel) mutations, and variants spanning intron-exon borders

It is designed to predict the functional consequences of not only amino acid substitutions but also in intronic and synonymous alterations, short insertion and/or deletion (indel) mutations, and variants spanning intron-exon borders

It is designed to predict the functional consequences of not only amino acid substitutions but also in intronic and synonymous alterations, short insertion and/or deletion (indel) mutations, and variants spanning intron-exon borders
MEF2A variants in coronary artery disease

MEF2A gene-gene interactions

Interactions of the MEF2A gene were analyzed by the Multiple Association Network Integration Algorithm (GeneMANIA; https://genemania.org/). GeneMANIA finds related genes to the query gene based on various biological databases, and its output is categorized according to Protein-protein interaction (Physical Interaction), similar gene expression levels (co-expression), same domains in the protein structure (shared protein domains), the association of genes when perturbed genes affect each other (genetic interaction), genes products are participating in the same pathway (pathway), gene products are found in the same cellular location or tissue (co-localization), prediction of functional association between gene products especially through orthology (predicted) and other relationships such as phenotype correlations, disease information, and chemical genomics data (other) (19).

Results

Variants reports in various populations

Investigation of different populations from twelve countries, including American, Scandinavian, and Japanese, Chinese, Spanish, Italian, Iranian, Sicilian, Saudi, Turkish, Irish, and German CAD and/or MI patients indicate twenty-four variations in the coding region of the MEF2A gene. The greatest diversity of substitution variants, i.e., eight different ones, have been observed in Chinese and Italian populations (Table 1). The contribution of missense, synonym, and nonsense variants are eleven, eight, and one substitution respectively, as well as one deletion and insertion variants (Table 2). In the coding sequence of this gene, two polymorphic regions were identified as the poly-glutamine and poly-proline regions (Figure 1).

Table 1. Distribution of reported variants in various populations.

| No. | Mutation     | Protein change | America | Scandinavia | Japan | China | Spain | Italy | Iran | Italy | Saudi | Turkey | Ireland | Germany | Disease |
|-----|--------------|----------------|---------|--------------|-------|-------|-------|-------|------|-------|-------|--------|---------|---------|---------|
| 1   | c.704C>A     | p.S235Y        | 10 (29) |              | 10    |       |       |       |      |       |       |        |         | MI       |
| 2   | c.736A>G     | p.S246G        | 10 (29) |              |       |       |       |       |      |       |       |        |         | MI       |
| 3   | c.788A>G     | p.N263S        | 3 (29)  | 207 (30)     | 207   | 483   |       |       |      |       |       |        |         | CAD/MI   |
| 4   | c.812C>G     | p.P271R        | 10 (29) |              |       |       |       |       |      |       |       |        |         | MI       |
| 5   | c.835C>G     | p.P279A        | 11 (29) |              |       |       |       |       |      |       |       |        |         | MI       |
| 6   | c.836C>T     | p.P279L        | 207     | 3 (29)       | 207   |       | 483   |       |      |       |       |        |         | CAD/MI   |
| 7   | c.848G>A     | p.G283D        | 1 (29)  |              |       |       |       |       |      |       |       |        |         | MI       |
| 8   | c.860C>G     | p.P287R        |         |              |       |       |       |       |      |       |       |        |         | MI       |
| 9   | c.867T>C     | p.N289N        | 379     | 257 (20)     |       |       |       | 52    | 52   | 52    |       |        |         | MI       |
| 10  | c.873G>A     | p.Q291Q        |         |              |       |       |       |       |      |       |       |        |         | CAD/MI   |
| 11  | c.1055C>T    | p.S352L        | 300     | 52 (25)      |       |       |       | 52    | 52   | 52    |       |        |         | CAD      |
| 12  | c.1224A>G    | p.P408P        |         |              |       |       |       |       |      |       |       |        |         | CAD      |
| 13  | c.1227G>A    | p.S409S        |         |              |       |       |       |       |      |       |       |        |         | CAD/MI   |
| 14  | c.1266_1267insCCGCAG |            | 257 (20) |              |       |       |       |       |      |       |       |        |         | CAD      |
| 15  | c.1268C>A    | p.P423Q        | 156     | 37 (37)      |       |       |       |       |      |       |       |        |         | CAD      |
| 16  | c.1275A>G    | p.P425P        | 1045    | 36 (36)      |       |       |       |       |      |       |       |        |         | CAD      |
| 17  | c.1279C>T    | p.P427S        | 257     | 20 (30)      |       |       |       |       |      |       |       |        |         | CAD      |

368 Acta Medica Iranica, Vol. 58, No. 8 (2020)
Cont Table 1.

| Country | No. of different mutations in each country | No. of people were studied in each country |
|---------|------------------------------------------|------------------------------------------|
|          |                                          |                                          |
| 18      |                                          |                                          |
| 19      |                                          |                                          |
| 20      |                                          |                                          |
| 21      | p.G443G                                  |                                          |
| 22      |                                          |                                          |

The number of different mutations in each country: 5 1 5 8 3 4 1 4 - - -

The number of people were studied in each country: 520 13 379 1779 483 2008 352 1079 1186 69 1494 >1700 & 23 family

All MEF2A coding variants are reported according to NCBI nucleotide (NM_005587.3) and protein (NP_005578.2) sequences. CAD: coronary artery disease; MI: myocardial infarction

**Figure 1.** Nucleotide and amino acid sequence of two polymorphic regions, including poly-glutamine, poly-proline, and 21-bp deletion, according to NCBI nucleotide (NM_005587.3) and protein (NP_005578.2) sequences.

**MEF2A variants pathogenicity**

**Missense variants**

Among missense variants, eight substitutions were predicted as the disease-causing, seven damages, five deleterious, and eight had an effect on protein function by Mutation Taster, PolyPhen-2, PROVEAN, and SIFT, respectively. The c.704C>A (p.S235Y), c.812C>G (p.P271R), c.836C>T (p.P279L), and c.848G>A (p.G283D) variants were considered as the damaging changes in protein structure by all prediction tools. CADD scores of these four variants are reported as such 28.8, 29.2, 31, and 32, respectively. In addition, the conservation score of S235Y has been predicted 5.55, and the last three variants, i.e., p.P271R, p.P279L, and p.G283D, 5.85 by GERP (Table 2).

**Nonsense variant**

A nonsense variant in the MEF2A gene, c.1315C>T (p.R439X), changes the amino acid to stop codon, was predicted as a pathogenic mutation by Mutation taster and SIFT. It had a CADD score 44, which is the highest pathogenicity score among all MEF2A reported variants, and a conservation GERP score of 5.05 (Table 2).
# MEF2A variants in coronary artery disease

## Table 2. In silico analysis of reported MEF2A variants

| No. | Mutation | Protein change | dSNP | Mutation type | HGMD | Mutation taster | Polyphen-2 | SIFT | GERP | CADD | REVEL | Chaoy | EXAC Het/Hom | 1000G Het/Hom |
|-----|----------|----------------|------|---------------|-------|----------------|------------|-------|-------|-------|--------|--------|---------------|---------------|
| 1   | c.704C>A | p.S235Y | rs751251460 | M      | -      | DC       | PD       | De    | AFP   | 5.55  | 28.8   | 0.56   | CAD, MI   | 1/0           | 0/0           |
| 2   | c.736A>G | p.S246G | rs755896449 | M      | -      | P        | B        | N     | T     | 5.55  | 22.6   | 0.21   | -         | 3/0           | 0/0           |
| 3   | c.788A>G | p.N263S | rs121918530 | M      | -      | DC       | B        | N     | T     | -9.35 | 8.9    | 0.13   | -         | 11/0          | 2/0           |
| 4   | c.812C>G | p.P271R | rs776085239 | M      | -      | DC       | PD       | De    | AFP   | 5.85  | 29.2   | 0.45   | CAD, MI   | 1/0           | 0/0           |
| 5   | c.835C>G | p.P279A | -          | M      | -      | DC       | B        | De    | AFP   | 4.94  | 21.8   | 0.08   | CAD, MI   | 3/0           | 0/0           |
| 6   | c.836C>T | p.P279L | rs121918529 | M      | -      | DC       | PD       | De    | AFP   | 5.85  | 31.0   | 0.28   | -         | 85/0          | 4/0           |
| 7   | c.848G>A | p.G283D | rs121918531 | M      | -      | DC       | PD       | De    | AFP   | 5.85  | 32.0   | 0.47   | -         | 2/0           | 0/0           |
| 8   | c.860C>G | p.P287R | rs751751585 | M      | -      | DC       | PD       | N     | AFP   | 5.85  | 25.4   | 0.36   | -         | 1/0           | 0/0           |
| 9   | c.867T>C | p.N289N | rs325408   | S      | -      | P        | -        | N     | T     | -11.1 | -      | -      | -         | -             | 851/1108      |
| 10  | c.873G>A | p.Q291Q | rs325407   | S      | -      | P        | -        | N     | T     | -8.6  | -      | -      | -         | 315/2063      |
| 11  | c.1055C>T | p.S352L | -          | M      | -      | DC       | PD       | N     | T     | 5.65  | 24.6   | 0.15   | -         | 5/0           | -             |
| 12  | c.1224A>G | p.P408P | rs144461661 | S      | -      | DC       | -        | N     | T     | 14.7  | -      | -      | 4/0       | 4/0           |               |
| 13  | c.1227G>A | p.S409S | rs3730059  | S      | -      | DC       | -        | N     | T     | 9.6   | -      | -      | 335/9     | 53/1          |               |
| 14  | c.1266_126 | 71msCCGC | AGCAAG | p.A422PQ | Q ins  | -      | I        | -     | P     | N     | N     | -12.1  | -        | -             | -             |
| 15  | c.1268C>A | p.P423Q | -          | M      | -      | P        | B        | N     | T     | -5.5  | 0.4    | 0.07   | -         | 2/0           | -             |
| 16  | c.1275A>G | p.P425P | -          | S      | -      | P        | -        | N     | T     | 0.2   | -      | -      | 1/0       | -             |               |
| 17  | c.1279C>T | p.P427S | -          | M      | -      | P        | PD       | N     | AFP   | 3.65  | 8.6    | 0.11   | CAD       | 1/0           | -             |
| 18  | c.1281G>A | p.P427P | rs367780642 | S      | -      | DC       | -        | N     | T     | 0.02  | -      | -      | 21/0      | 30/0          |               |
| 19  | c.1294_131 | 4delCAAC | AGCCGCAGCC | p.432-  | -      | D        | CG035245 | P     | N     | N     | N     | 21.4   | -        | -             | -             |
| 20  | c.1315C>T | p.R439X | -          | N      | CM056644 | DC       | -       | NA    | D     | 5.05  | 44     | -      | -         | -             | -             |
| 21  | c.1329G>T | p.G443G | rs325400   | S      | -      | P        | -        | N     | T     | 16.4  | -      | -      | 525/20    | 997/736      |               |
| 22  | c.1416A>G | p.P472P | rs34851361 | S      | -      | P        | -        | N     | T     | 6.4   | -      | -      | 2124/0    | 139/9        |               |
| 23  | CCG/CCA del  | 4P or 5P | D      | CD068109 | -      | -        | -      | -     | -     | -     | -     | CAD, MI | -         | -             |               |
| 24  | (CAG)n Poly Q | rs3138597 | RV     | CE077839 | -      | -        | -      | -     | -     | -     | -     | -      | -         | -             |               |

All MEF2A coding variants are reported according to NCBI nucleotide (NM_005587.3) and protein (NP_005578.2) sequences. Variants were not classified into disease-causing and benign by CADD, GERP, and REVEL programs; the cutoff of 20, 5, and 0.5 was used for CADD, GERP, and REVEL scores respectively so that variants with CADD score ≥20 were grouped as harmful variants, GERP score ≥5 were grouped as conserved and REVEL score above 0.5 were grouped as likely disease-causing variants. M: Missense; S: synonymous; D: Deletion; I: Insertion; N: Nonsense; RV: Repeat variations; DC: Disease-causing; P: Polymorphism; PD: Probably damaging; B: Benign; De: Deleterious; N: Neutral; NA: Not available; AFP: Affect protein function; T: Tolerated

### Synonymous variants

Although three of eight synonymous variants were predicted as the disease-causing by Mutation Taster, but all of them were Neutral and Tolerated by the last tools, i.e., PROVEAN and SIFT. The CADD scores of all synonymous variants were <20 (Table 2).

### Deletion and insertion variants

In the coding region of the MEF2A gene, one 21-bp deletion was reported for the first time as a CAD causal mutation by autosomal dominant pattern (8). Moreover, one insertion variant, including 9-bp nucleotides, was concluded in the Chinese population (20). These variants were predicted Polymorphism and Neutral by Mutation.
Taster, PROVEAN, and SIFT. They had CADD scores 21.4 and 12.1, respectively. 7-amino acid deletion and 3-amino acid insertion were other variants that were located in the polymorphic areas, including 4-15 (Q)n and subsequently, 4-5 (P)n (Table 2).

There were nine out-of-frame deletions in which seven of them were frame-shift, disease-causing, and damaging by Mutation-Taster and SIFT. In addition, the CADD scores of them were >20 (Table 3).

**Gene-gene interactions**

*MEF2A* gene has been present in the complex network with nineteen genes. In this network, most of the *MEF2A* gene interactions were by *MEF2D, MEF2C, MEF2B, SMAD2, HDAC9* genes (Table 4). Among them, *MEF2A, MEF2C, MEF2D, MEF2B, HDAC9, HDAC5* involve in muscle/heart development and differentiation (Figure 2).

![Gene's interactions and their functions are predicted by GeneMANIA based on the MEF2A query. Networks are represented as colored lines between genes, and functions are represented as colored circles for each gene](image)

**Table 3. Out-of-frame deletions were reported in the MEF2A gene.**

| No. | Mutation       | Protein change | Mutation type | HGMD     | Mutation taster | Polyphen_2 | PROVEAN | SIFT | CADD | Phys. location              |
|-----|----------------|----------------|---------------|----------|-----------------|------------|---------|------|------|-----------------------------|
| 1   | c.1265_1266delAG | frameshift     | Deletion      | CD101281 | Disease causing | -          | NA      | Damaging | 24.2 | chr15:100252741_100252742delAG |
| 2   | c.1268_1268delC  | frameshift     | Deletion      | CD101282 | Disease causing | -          | NA      | Damaging | 22.7 | chr15:100252744_100252744delC |
| 3   | c.1269_1269delG  | frameshift     | Deletion      | CD101283 | Disease causing | -          | NA      | Damaging | 23.9 | chr15:100252745_100252745delG |
| 4   | c.1270_1271delCC | frameshift     | Deletion      | CD101284 | Disease causing | -          | NA      | Damaging | 21.4 | chr15:100252746_100252747delCC |
| 5   | c.1272_1272delG  | frameshift     | Deletion      | CD101285 | Disease causing | -          | NA      | Damaging | 23.8 | chr15:100252748_100252748delG |
| 6   | c.1273_1274delCC | frameshift     | Deletion      | CD101286 | Disease causing | -          | NA      | Damaging | 21.3 | chr15:100252749_100252750delCC |
| 7   | c.1275_1275delA  | frameshift     | Deletion      | CD101287 | Disease causing | -          | NA      | Damaging | 23.9 | chr15:100252751_100252751delA |

All MEF2A coding variants are reported according to NCBI nucleotide (NM_005587.3) and protein (NP_005578.2) sequences. Variants were not classified into disease-causing and benign by the CADD program; the cutoff of 20 was used for CADD scores, so variants with score ≥20 were grouped as harmful variants.
**MEF2A variants in coronary artery disease**

Table 4. Classification of related genes with MEF2A according to GeneMANIA network categories

| Physical interactions | Co-expression | Predicted | Co-localization | Pathway | Genetic interaction | Shared protein domains |
|-----------------------|---------------|-----------|-----------------|---------|---------------------|------------------------|
| HDAC9                 | MEF2C         | MEF2D     | MEF2D           | HDAC9   |                     | MEF2D                  |
| MEF2D                 |               | MEF2B     | SMAD2           | SMA2    |                     | MEF2B                  |
| MEF2B                 |               | SMAD2     |                 | ESRA    |                     | MEF2C                  |
| SMAD2                 | MEF2D         | MEF2C     | CABIN1          |         |                     | HJURP                  |
| MEF2C                 |               | SERBP1    |                 |         |                     |                        |
| CABIN1                | ENO3          |           |                 | PPARC1A |                     |                        |
| HDAC5                 | CKM           |           |                 | GRIP1   |                     |                        |
| VGLL4                 | SLC25A32      |           |                 | CAMK2G  |                     |                        |
| NFIX                  | VGLL4         |           |                 |         |                     |                        |
| HDAC7                 |               | NFIX      |                 |         |                     |                        |
| UBE2I                 |               |           |                 |         |                     |                        |

**CABIN1**: Calcineurin Binding Protein 1, **CAMK2G**: Calcium/CaMmodulin Dependent Protein Kinase II Gamma, **CKM**: Creatine Kinase, **ENO3**: Enolase 3, **ESRRA**: Estrogen Related Receptor Alpha, **GRIP1**: Glutamate Receptor Interacting Protein 1, **HDAC5**: Histone Deacetylase 5, **HDAC7**: Histone Deacetylase 7, **HJURP**: Holliday Junction Recognition Protein, **MEF2B**: Myocyte Enhancer Factor 2B, **MEF2C**: Myocyte Enhancer Factor 2C, **MEF2D**: Myocyte Enhancer Factor 2D, **PPARGC1A**: PPARG Coactivator 1 Alpha, **SEREP1**: SERPINE1, **VGLL4**: VGLL4, **UBE2I**: Ubiquitin Conjugating Enzyme E2 I, **WMKL**: Vestigial Like Family Member 4

**Discussion**

CAD is a multifactorial disease in which genetics plays a main role with approximately 50-60% heritability. Some genes have been identified as CAD monogenic causes, such as genes involved in high LDL, TG, and low HDL and genes with no effect on plasma lipid levels, such as the MEF2A gene (4).

The MEF2A gene was introduced as an associated gene with CAD by Wang et al., in 2003, in a large family with an autosomal dominant pattern. In this study, they reported the 15q26 region, which contains 93 genes as an associated locus with CAD. Because of the MEF2A expression in blood vessels during early embryogenesis of mouse, Wang et al., analyzed only the MEF2A gene and identified 21-bp deletion as a causal mutation of CAD (8). Although 21-bp deletion was introduced as a pathogenic mutation for CAD due to the conformational change in the MEF2A structure, its association with CAD was not confirmed in other populations (21-28). Functional studies by Guella et al., also showed that the 21-bp deletion did not alter the nuclear localization and transactivating properties of the MEF2A protein (29). Though the CADD score of this deletion was >20, its pathogenicity was not corroborated by in silico analysis. It is concluded that the assumption of a single-gene inheritance pattern for 21-bp deletion results from a high probability of common diseases such as CAD in individuals in a big family (9).

In 2004, the sequencing and single-strand conformation polymorphism (SSCP) analysis of all MEF2A exons in 207 CAD/MI patients and 191 control by Bhagavatula et al., found three missense mutations (N263S, P279L, and G283D) in only four patients (30). These three variants were reported by the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) free database, which interprets relationships among variants and phenotypes (31). P279L and G283D were pathogenic mutations for the CAD/MI condition; however, N263S was a likely benign variant. Moreover, according to in silico analysis, P279L and G283D were pathogenic changes in the MEF2A gene. Although P279L and N263S were reported in Spanish and Italian CAD/MI patients (23,29), these two variants did not impair the transactivation activity of the protein (29). S235Y and P271R were other variants that were reported in Italian CAD/MI patients, and in silico analysis confirmed their pathogenicity, similar to other variants identified in this population that did not change the transactivation activity of MEF2A by transcription activation assays (29). R439X, a nonsense variant with a pathogenic effect according to our in silico analysis, was reported in a 70-year-old Japanese patient with MI who had two risk factors, diabetes mellitus, and smoking, but no family history of ischemic heart disease (21). Polymorphic variations of the polyQ and polyP regions of MEF2A were reported in various populations, some as pathogenic (32-34), and others with no association with CAD/MI (20-27,29,35-39). Deletion of one proline and changes in glutamine length in CAD patients were reported as
pathogenic variations in the Chinese population by Yuan Hong et al., but they did not detect any other variations in CAD patients (32). Moreover, glutamine repeat extension, especially (CAG)\textsuperscript{N} variant, was reported as a highly frequent variant associated with CAD in the Chinese population (33). In a 2016 study, a 6-bp deletion “CAGCCG” in polyQ and polyP regions was introduced for the first time in a large Chinese family with some suspected CAD/MI individuals. Interestingly, this deletion was detected in all family members including patients and healthy individuals. More investigation of the 6-bp deletion in unrelated CAD and control groups did not confirm this deletion in sporadic CAD patients (34). Several deletions were reported in a Saudi population with frame-shift consensuses. Except two of these deletions, i.e., c.1265_1266delAG and c.1270_1271delCC, others were not associated with CAD, although they were predicted to be disease-causing variants due to >20 CADD scores (35).

MEF2A is one of the essential players in the transcriptional factor network, including GATA4, NKK2.5, and Srf genes. This network has critical roles in cardiac transcriptome regulation in cooperation with histones modifications (40). MEF2A interacts with HDAC5 and HDAC9 in the cardiac development pathway (41). Despite the significant function of the MEF2A gene in heart development, the results of MEF2A studies and in silico analysis did not confirm its association with familial and sporadic CAD. Furthermore, the 15q26 region was reported as a risk region for CAD patients by genome-wide association studies (GWAS) (42,43); however, the MEF2A gene was not introduced as an associated gene with CAD in this region by GWAS (44). Given that CAD is a common and multifactorial disease and the difficulty in pathogenic variants determination, the current researchers believe the role of MEF2A in CAD/MI can be further surveyed by additional functional studies.

The in silico analysis of MEF2A variations indicated that some variants have pathogenic effects on protein structures; however, the evaluation of case-control studies in MEF2A association with CAD/MI and in silico analysis did not detect an association between this gene and familial/sporadic CAD.

Acknowledgments

This research was provided by Rajaie Cardiovascular, Medical and Research Center (RCMRC), Tehran, Iran, Golestan University of Medical Science (GUMS), Gorgan, Iran, and Islamic Azad University Research Tehran Branch, Tehran, Iran.

References

1. Organization. WH. Cardiovascular diseases (CVDs). 2016.
2. Sanchis-Gomar F, Perez-Quilis C, Leischik R, Lucia A. Epidemiology of coronary heart disease and acute coronary syndrome. Ann Transl Med 2016;4:256.
3. Hajar R. Risk factors for coronary artery disease: historical perspectives. Heart Views 2017;18:109-14.
4. Dai X, Wiernek S, Evans JP, Runge M. Genetics of coronary artery disease and myocardial infarction. World J Cardiol 2016;8:1-23.
5. McKinsey TA, Zhang CL, Olson EN. MEF2: a calcium-dependent regulator of cell division, differentiation and death. Trends Biochem Sci 2002;27:40-7.
6. Pon JR, Marra MA. MEF2 transcription factors: developmental regulators and emerging cancer genes. Oncotarget 2016;7:2297-312.
7. Naya FJ, Black BL, Wu H, Bassel-Duby R, Richardson JA, Hill JA, et al. Mitochondrial deficiency and cardiac sudden death in mice lacking the MEF2A transcription factor. Nat Med 2002;8:1303-9.
8. Wang L, Fan C, Topol SE, Topol EJ, Wang QJS. Mutation of MEF2A in an inherited disorder with features of coronary artery disease. Science 2003;302:1578-81.
9. Altshuler D, Hirschhorn JN. MEF2A sequence variants and coronary artery disease: a change of heart? J Clin Invest 2005;115:831-3.
10. 1000 Genomes Project Consortium; Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM et al. A global reference for human genetic variation. Nature 2015;526:68-74.
11. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285-91.
12. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods 2014;11:361-2.
13. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet 2013;7:7-20.
14. Sim N-L, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res 2012;40:W452-7.
15. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics 2015;31:2745-7.
16. Kircher M, Witten DM, Jain P, O’Roak BJ, Cooper GM, Shendure J. A general framework for estimating the

Acta Medica Iranica, Vol. 58, No. 8 (2020) 373
MEF2A variants in coronary artery disease

relative pathogenicity of human genetic variants. Nat Genet 2014;46:310-5.
17. Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. Am J Hum Genet 2016;99:877-85.
18. Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, Batzoglou S. Identifying a high fraction of the human genome to be under selective constraint using GERP++. PLoS Comput Biol 2010;6:e1000125.
19. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badravi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 2010;38:W214-20.
20. Dai DP, Zhou XY, Xiao Y, Xu F, Sun FC, Ji FS, et al. Structural changes in exon 11 of MEF2A are not related to sporadic coronary artery disease in Han Chinese population. Eur J Clin Invest 2010;40:669-77.
21. Kajimoto K, Shioji K, Tago N, Tomoike H, Nonogi H, Goto Y, et al. Assessment of MEF2A mutations in myocardial infarction in Japanese patients. Circ J 2005;69:1192-5.
22. Weng L, Kavaslar N, Ustaszewska A, Doelle H, Schackwitz W, Hébert S, et al. Lack of MEF2A mutations in coronary artery disease. J Clin Invest 2005;115:1016-20.
23. González P, García-Castro M, Reguero JR, Batalla A, Ordoñez AG, Palop RL, et al. The Pro279Leu variant in the transcription factor MEF2A is associated with myocardial infarction. J Med Genet 2006;43:167-9.
24. Gulec S, Ruchan Akar A, Akar N. MEF2A sequence variants in Turkish population. 2008;14:465-7.
25. Rahatloo KI, Davaran S, Elahi E. Lack of association between the MEF2A gene and coronary artery disease in Iranian families. Iran J Basic Med Sci 2013;16:950-4.
26. Horan PG, Allen AR, Hughes AE, Patterson CC, Spence M, McGlinchey PG, et al. Lack of MEF2A Δ7aa mutation in Irish families with early onset ischaemic heart disease, a family based study. BMC Med Gen 2006;7:65.
27. Lieb W, Mayer B, Konig IR, Borwitzky I, Gotz A, Kain S, et al. Lack of association between the MEF2A gene and myocardial infarction. Circulation 2008;117:185-91.
28. Maiolino G, Colonna S, Zanchetta M, Pedon L, Seccia TM, Cesari M, et al. Exon 11 deletion in the myocyte enhancer factor (MEF) 2A and early onset coronary artery disease gene in a Sicilian family. Eur J Cardiovasc Prev Rehabil 2011;18:557-60.
29. Guella I, Rimoldi V, Asselta R, Ardissino D, Franchinini M, Martinelli N, et al. Association and functional analyses of MEF2A as a susceptibility gene for premature myocardial infarction and coronary artery disease. Circ Cardiovasc Genet 2009;2:165-72.
30. Bhagavatula MK, Fan C, Shen G-Q, Cassano J, Plow EF, Topol EJ, et al. Transcription factor MEF2A mutations in patients with coronary artery disease. Hum Mol Genet 2004;13:3181-8.
31. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, et al. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res 2015;44:D862-8.
32. Yuan H, Liu H-W, Hu J, Chen S-H, Yang G-P, Huang ZJ. MEF2A gene and susceptibility to coronary artery disease in the Chinese people. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2006;31:453-7.
33. Han Y, Yang Y, Zhang X, Yan C, Xi S, Kang J. Relationship of the CAG repeat polymorphism of the MEF2A gene and coronary artery disease in a Chinese population. Clin Chem Lab Med 2007;45(8):987-92.
34. Xu DL, Tian HL, Cai WL, Zheng J, Gao M, Zhang MX, et al. Novel 6-bp deletion in MEF2A linked to premature coronary artery disease in a large Chinese family. Mol Med Rep 2016;14:649-54.
35. Elhawari S, Al-Boudari O, Muiya P, Khalak H, Andres E, Al-Shahid M, et al. A study of the role of the myocyte-specific enhancer factor-2A gene in coronary artery disease. Atherosclerosis 2010;209:152-4.
36. Liu Y, Niu W, Wu Z, Su X, Chen Q, Lu L, et al. Variants in exon 11 of MEF2A gene and coronary artery disease: evidence from a case-control study, systematic review, and meta-analysis. PLOSE ONE 2012;7:e31406.
37. Li J, Yang J, Li W, Du R, Gui L, Tian L, et al. Study on novel mutations of MEF2A gene in Chinese patients with coronary artery disease. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2006;23:265-8.
38. Dai Y, Zhang S, Wu W. Analysis of MEF2A mutations in a Chinese population with premature coronary artery disease. Genet Test Mol Biomarkers 2013;17:352-5.
39. Li J, Chen H, Yang J, Li W, Du R, Tian L. MEF2A gene mutations and susceptibility to coronary artery disease in the Chinese population. Genet Mol Res 2014;13:8396-402.
40. Schlesinger J, Schuler M, Gronutt M, Fischer JJ, Zhang Q, Krueger T, et al. The cardiac transcription network regulated by Gata4, Mef2a, Nkx2. 5, Srf, histone modifications, and microRNAs. PLoS Genet 2011;7:e1001313.
41. Chang S, McKinsey TA, Zhang CL, Richardson JA, Hill JA, Olson EN. Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. Mol Cell Biol 2004;24:8467-76.
42. van der Harst P, Verweij NJCr. Identification of 64 novel genetic loci provides an expanded view on the genetic
architecture of coronary artery disease. Circ Res 2018;122:433-43.

43. Nikpay M, Goel A, Won H-H, Hall LM, Willenberg C, Kanoni S, et al. A comprehensive 1000 Genomes–based genome-wide association meta-analysis of coronary artery disease. Nat Genet 2015;47:1121-30.

44. Omidi S, Ebrahimzadeh F, Kalayinia S. 9P21.3 locus; an important region in coronary artery disease: a panel approach to investigation of the etiology of coronary artery disease. Int J Cardiovasc Pract 2019;4:21-35.

45. Foroughmand AM, Shahbazi Z, Galehdari H, Borujeni MP, Dinarvand P, Golabgirkhademi K. Association of MEF2A gene polymorphisms with coronary artery disease. Iran Red Crescent Med J 2014;16: e13533.