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

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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.

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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 post-natal 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

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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.bii.a-star.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 intronic and synonymous alterations, short insertion and/or deletion (indel) mutations, and variants spanning intron-exon borders

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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 there, are 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	Cecil	Saudi	Turkey	Ireland	Germany	Disease
1	c.704C>A	p.S235Y						10 (29)							MI
2	c.736A>G	p.S246G						10 (29)							MI
3	c.788A>G	p.N263S	207 (30)					3 (29)							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 (30)				483 (23)	3 (29)							CAD / MI
7	c.848G>A	p.G283D	207 (30)												CAD / MI
8	c.860C>G	p.P287R						1 (29)							MI
9	c.867T>C	p.N289N			379 (21)	257 (20)			52 (25)						CAD/ MI
10	c.873G>A	p.Q291Q							52 (25)						CAD
11	c.1055C>T	p.S352L	300 (9)												CAD
12	c.1224A>G	p.P408P									1186 (35)				CAD
13	c.1227G>A	p.S409S					483 (23)				1186 (35)				CAD / MI
14	c.1266_1267insCCGCAGCAG	p.422PQQins				257 (20)									CAD
15	c.1268C>A	p.P423Q				156 (37)									CAD
16	c.1275A>G	p.P425P				1045 (36)									CAD
17	c.1279C>T	p.P427S				1045 (36) 257 (20) 111 (38)									CAD

Cont Table 1.

18	c.1281G>A	p.P427P			379 (21)	1045 (36) 257 (20) 111 (38) 210 (39)									CAD/ MI
19	c.1294_1314delCAACCCCGCAGCCCCAGCCC	p.432-438QPPQPQP del	300 (9)	13 (8)	379 (21)	257 (20) 1045 (36)		5 (29)		1079 (28)					CAD / MI
20	c.1315C>T	p.R439X			379 (21)										MI
21	c.1329G>T	p.G443G			379 (21)	257 (20) 1045 (36) 111 (38) 156 (37) 210 (39)	483 (23)		300 (45) 52 (25)		1186 (35)				CAD/ MI
22	c.1416A>G	p.P472P							300 (45) 52 (25)		1186 (35)				CAD
The number of different mutations in each country			5	1	5	8	3	8	4	1	4	-	-	-	
The number of people were studied in each country			520	13	379	1779	483	2008	352	1079	1186	69 (24)	1494 (26)	>1700 & 23 family (27)	

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

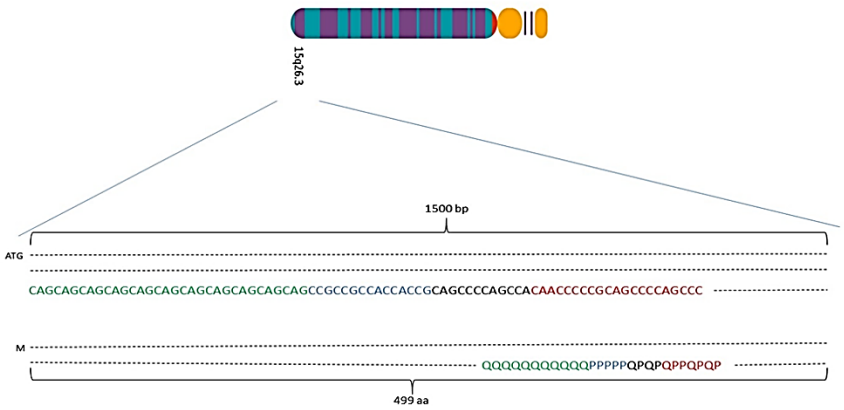

Figure 1. Nucleotide and amino acid sequence of two polymorphic regions, including poly-glutamine, poly-proline, and 21-bp deletion, according to NCBI nucleotide (NM_005587.3) and protein (NP_005578.2) sequences.

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).

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

No.	Mutation	Protein change	dbSNP	Mutation type	HGMD	Mutation taster	Polyphen_2	PROVEAN	SIFT	GERP	CADD	REVEL	ClinVar	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	CM043299	DC	B	N	T	-9.35	8.9	0.13	-	110/0	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	CM043300	DC	PD	De	AFP	5.85	31	0.28	-	85/0	4/0
7	c.848G>A	p.G283D	rs121918531	M	CM043301	DC	PD	De	AFP	5.85	32	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_1267insCCGCAGCAG	p.422PQQ ins	-	I	-	P	-	N	N	-	12.1	-	-	-	-
15	c.1268C>A	p.P423Q	-	M	-	P	B	N	AFP	-5.5	0.4	0.07	-	20/0	-
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_1314delCAACCCCCGCAGCCCCAGCCC	p.432-438QPPQPQPdel	-	D	CG035245	P	-	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	-	-	5252/0	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, GREP, 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).

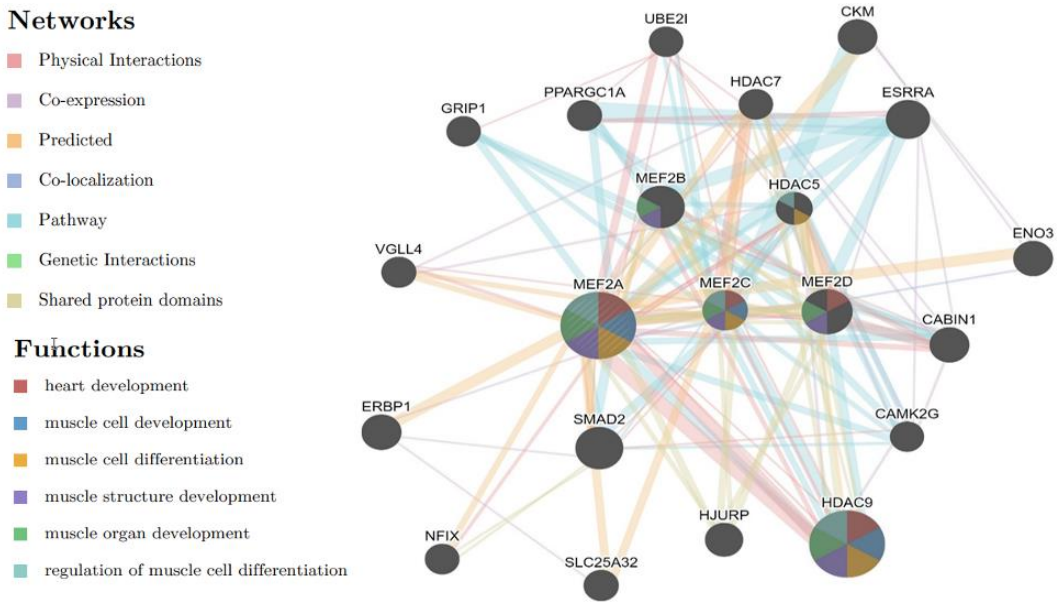

Figure 2. Gene's interactions and their functions are predicted by GeneMANIA based on the *MEF2A* query. Networks are represented as colored lines between genes, and functions are represented as colored circles for each gene

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

No.	Mutation	Protein change	Mutation type	HGMD	Mutation taster	Poly-phen_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.

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*		*MEF2B*
MEF2B		*SMAD2*		*ESRRA*		*MEF2C*
SMAD2		*MEF2C*		*CABIN1*		*HJURP*
MEF2C		*SERBP1*		*HDAC5*		
CABIN1		*ENO3*		*PPARGC1A*		
HDAC5		*CKM*		*GRIP1*		
VGLL4		*SLC25A32*		*CAMK2G*		
NFIX		*VGLL4*				
HDAC7		*NFIX*				
UBE2I		*HDAC7*				

CABIN1; Calcineurin Binding Protein 1, CAMK2G; Calcium/Calmodulin Dependent Protein Kinase II Gamma, CKM; Creatine Kinase, M-Type, ENO3; Enolase 3, ESRRA; Estrogen Related Receptor Alpha, GRIP1; Glutamate Receptor Interacting Protein 1, HDAC5; Histone Deacetylase 5, HDAC7; Histone Deacetylase 7, HDAC9; Histone Deacetylase 9, HJURP; Holliday Junction Recognition Protein, MEF2B; Myocyte Enhancer Factor 2B, MEF2C; Myocyte Enhancer Factor 2C, MEF2D; Myocyte Enhancer Factor 2D, NFIX; Nuclear Factor I X, PPARGC1A; PPARG Coactivator 1 Alpha, SERBP1; SERPINE1 MRNA Binding Protein 1, SLC25A32; Solute Carrier Family 25 Member 32, SMAD2; SMAD Family Member 2, UBE2I; Ubiquitin Conjugating Enzyme E2 I, VGLL4; 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 $(CAG)_9$ 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*, *NKX2.5*, 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.

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