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A Study of transcription factor MEF2A gene polymorphisms in patients with coronary artery disease

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Abstract

MEF2A (myocyte enhancer factor-2A) is a transcription factor of the MEF2 family. It has been recognized as the cause of coronary artery disease in the absence of any other clinical characteristic. It is involved in vascular development and is most commonly found in the coronary artery endothelium. The goal of this case-control study was to see if there was a link between polymorphisms in the MEF2A gene and coronary artery disease. A case-control study was carried out to look into the possible significance of MEF2A polymorphisms as a risk factor for coronary artery disease. This research included 225 patients and 225 healthy controls. A biochemical examination was carried out to evaluate the risk factors for developing this condition. The polymorphisms of Mef2A (1250 C > T in exon 8 and 452 G > T, 481 A > G in exon 11) were found using the PCR-RFLP technique. All identified risk variables, such as hypercholesterolemia, diabetes mellitus, and hypertriglyceridemia, were shown to be statistically significant in the current study for coronary artery disease occurrence. The most polymorphisms were found in MEF2A 1250 C > T, MEF2A 452 G > T, and MEF2A 481 A > G. The genotyping results for MEF2A 1250, MEF2A 452, and MEF2A 481 were ($X^2 = 2.985$; $P = 0.235$), ($X^2 = 4.371$; $P = 0.112$), and ($X^2 = 4.025$; $P = 0.134$), respectively. In conclusion, we identified a much higher incidence of MEF2A in people with coronary artery disease, and MEF2A may play a crucial role in cardiovascular pathophysiology. Patients and controls had considerably different genetics and frequency of alleles.

Keywords: Coronary artery disease, MEF2A gene polymorphism, Transcription factor, PCR-RFLP technique

1. Introduction

Coronary artery disease (CAD) is the leading cause of mortality globally. Coronary artery disease CAD is the most frequent kind of heart disease and is predicted to affect more than 13 million Americans, making it one of the leading causes of mortality in the country [1]. CAD is the most dangerous illness in the world, with the greatest death and disability rates. It is the world's biggest cause of illness and mortality, as well as the leading cause of sudden death [2].

Arrhythmia and heart failure have a negative impact on patients' quality of life and are quite expensive for society. Smoking, drinking alcohol, having diabetes, having high blood pressure, being obese, not exercising, being depressed, and other variables have all been associated to the onset of CAD. Currently, the majority of CAD treatments involve a combination of medicine and surgery, such as coronary artery bypass grafting, coronary artery dilation therapies, and anti-platelet aggregation medicine [3].

Myocyte-specific enhancer Factor 2A (MEF2A) is a transcription factor in the Mef2 family. It is mostly present in skeletal and cardiac muscle, smooth muscle, and brain cells. It is a member of the monocyte enhancer factor family. Using family-based linkage analysis and genome-wide association studies, several genetic variations associated with CAD have been found [4].

The MEF2A gene has been identified as a risk factor for coronary heart disease. Since MEF2A is crucial in growth and development, changes in its sequence may negatively impact the regulatory mechanisms that govern these processes, potentially leading to vascular diseases. Since then, the MEF2A gene has been associated with coronary artery disease. Using angiography, MEF2A gene variations were shown to be associated with CAD in a study of a Saudi Arabian patient cohort. They also found gene variants and a haplotype that increase risk and support MEF2A's independent status as a CAD susceptibility gene [5].

When intracellular Ca2+ levels increase, MEF2A participates in Ca signalling and controls the expression of the CAD gene by activating Calmodulin CaM kinases. MEF2A mutations and deletions alter the endothelial transcriptional profile, rendering the endothelium dysfunctional and more vulnerable to inflammatory particle invasion such as LDLs, monocytes, and macrophages. These macrophages are crucial for lipid accumulation, cytokine production, and growth factor release, which promote the proliferation of smooth muscle cells, and lead to the collapse of the fibrous cap, plaque development, and atherosclerosis [6].

MEF2A may be important in cardiovascular biology, and rare polymorphisms in MEF2A may alter its function

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as a gene transcription factor in vitro, although genetic information does not suggest a causative role for these mutations in CAD in humans. As a precaution, people who are at high risk of CAD should undergo genetic testing. To postpone or prevent the development of CAD in gene carriers, significant changes in habits and pharmacologic treatment may be used [7].

There have been not many published studies in the medical literature that link Mef2A gene variants to CAD, particularly in the Iraqi population. As a result, this study was conducted to evaluate the function of the MEF2A polymorphism gene in CAD in an Iraqi patient cohort in order to give protection against this illness.

2. Material and methods

1.1. Study design and participants

The current comparative case-control study compared transcription factor ME F2A gene variations in individuals with coronary artery disease. The study included 225 CAD patients and 225 healthy controls. Coronary angiography in all CAD patients revealed at least one major coronary artery with higher than 50% stenosis. Iraqis without a history of cardiovascular disease were chosen as controls. Risk factors for CAD, such as hypertension, diabetes, hypercholesterolemia, and smoking, were evaluated based on medical history, current medications, or examination results obtained during hospitalization. Obesity was characterized as having a body mass index (BMI) of 27 kg/m2 or greater. Clinical features of those afflicted were noted. The practical portion of this research was carried out at Salahaddin University's College of Education, Department of Life Sciences, and Genetics Laboratory in Erbil, Iraq.

The following strategies were employed to reduce the likelihood of social-desirability bias:

- Researchers claim that in order to get more honest responses from respondents, the survey's positive responses were left intentionally imprecise.
- Confidentiality: After the questionnaires were completed, participants in our study were guaranteed the confidentiality of their replies.

1.2. Interview questionnaire

The researchers reviewed local and international literature to acquire a better understanding of the study and also produced the interview questionnaire form for (age, gender, and CAD in the family). Furthermore, information about (diet, smoking, and alcohol consumption) was provided from both the case and control groups.

1.3. Biochemical analysis

Fasting individuals' venous blood samples (2 mL) were collected, centrifuged, and tested for serum cholesterol, triglyceride, and fasting blood glucose levels.

1.4. PCR protocol

Each subject had three milliliters of peripheral blood collected from them, and their genomic DNA was extracted using conventional procedures [8]. Using a PCR-based RFLP approach, the genotypes of Mef2A 1250 C > T, Exon 11 452 G > T, and Exon 481 A > G were identified. PCR was carried out in 25 µl 0.2 ml tubes with 100 ng of genomic DNA. *Picomoles reverse and forward primers*, 200 µM of dNTPS and 1× PCR buffer. Primers used for amplification were referred to by previous reports [5, 9] (Table 1).

1.5. Statistical Analysis

SPSS version 20.0-statistic software was used for all statistical calculations. Prior to statistical calculations, the continuous variable, age, was evaluated for normality of distribution and presented as a mean and standard deviation. Categorical variables were displayed in both number and percentage form. An independent investigation compared the ages of the patients and controls using a random sample. The chi-square test was used to compare categorical variables. The odds ratio (OR) was developed to analyse the link between an exposure (the presence of alleles) and an outcome (the development of CAD). The 95% confidence interval (CI) was used to calculate the accuracy of OR [10]. The Hardy-Weinberg equilibrium (HWE) was evaluated using the goodness-of-fit X^2 test, which compared observed genotype frequencies to expected frequencies in controls to test the assumption that genotype frequencies in a population will remain constant from generation to generation [11]. The statistical significance of the variables was established at the level $P \leq 0.05$.

3. Results

The results showed that the average age of the individuals in the CAD patients and control groups was 47.5 ± 4.6 and 44.9 ± 5.8 years, respectively (Table 2). Males make up 68.9% of CAD patients and 62.7% of controls. Obesity affects 22.7% and 18.2% of CAD patients and controls, respectively. There were statistically significant differences in CAD family history between both groups ($P < 0.001$). However, gender and obesity were not detected as significantly different factors between the two groups.

All established risk factor measurements, such as food, smoking, and alcohol consumption, revealed statistically significant differences between CAD patients and controls ($P < 0.001$). Clinical data on classic CAD risk factors such as hypercholesterolemia, hypertriglyceridemia, hyperten-

Table 1. Primer sequences used for amplification and sequencing of Mef2A.

Primer name	Forward	Reverse
Mef11A	5'GCCAAGCACTGAAGGAAACGAC-3'	5'-CATGCACCCCTTTGCAACAGAC-3'
Mef11A2	5'-CGTGGGTGACCTAAGGCTTCC-3'	5'-CATACACACTCACACCCACATAC-3'
Mef11B	5'-ACTAGCTTGCAGAAACCTAGAC-3'	5'-GAAACCCCTTTATACAATCCAC-3'
Mef11C	5'-ATTTATCACCTTTGATTAAGTACC-3'	5'-CTTGTCACAAACAGCAGATGAC-3'
Mef11D	5'-CATGAGCAAAATTCAAAGTCCTG-3'	5'-GTTGGAAACTGTACTTTAACCAG-3'
Mef11E	5'-AGGGGACGACGCTAATGGTG-3'	5'-TGCAGGTGAAAAAGGTCTTCGTG-3'
Mef11F	5'-GGGATCTTTTTTCCTTGACC-3'	5'-TAGTCTTTCTTTCTATGCAGG-3'

sion, and diabetes were significantly higher in the CAD groups than in controls (P < 0.001, Table 3).

Figure 1 shows the pathological categorization of CAD patients. There were 134 (59.6%) patients with obstructive CAD, 62 (27.6%) patients with non-obstructive CAD and 29 (12.9%) patients with spontaneous CA dissection

The most common variants of the MEF2A gene were MEF2A 1250 C > T, MEF2A 452 G > T, and MEF2A 481 A > G (Table 4). The genetic analysis findings for MEF2A 1250, MEF2A 452, and MEF2A 481 were (X^2 = 2.985; P = 0.235), (X^2 = 4.371; P = 0.112), and (X^2 = 4.025; P = 0.134), respectively.

The genotype and allelic frequency distributions of Mef2A 1250 C > T in patients and controls have been shown in Table 5. In CAD patients, the CC, CT, and TT genotype distributions were 61.8%, 24.4%, and 13.8%, respectively, compared to 70.7%, 24.9%, and 4.4% in the control group. The genetic and allele prevalence was significantly different between the CAD patients and control groups (p = 0.002 and p = 0.001, respectively).

The genotype and allelic frequency distributions of Mef2a 452 G > T in patients and controls have been shown in Table 6. The percentage distribution of GG, GT, and TT genotypes in CAD patients was 62.2%, 23.1%, and 14.7%,

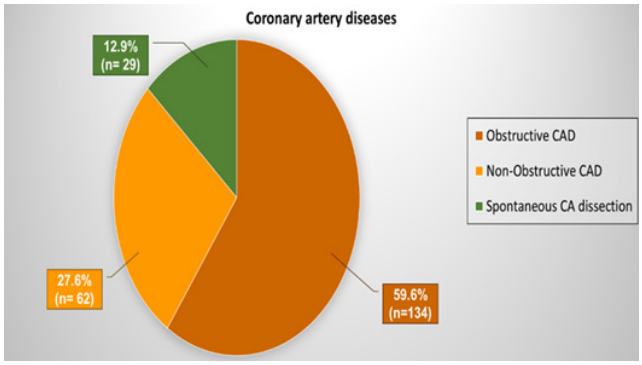

Fig. 1. The pathological classification of CAD.

Table 2. CAD patients' demographic features compared to controls.

Variables	CAD		Control		Chi-Square / Fisher's Text	
	N	%	N	%	X^2	P
Age years (mean ±SD)	47.5 ± 4.6		44.9 ± 5.8			< 0.001
Gender						
Female	70	31.1	84	37.3	1.935	0.164
Male	155	68.9	141	62.7		
Obesity						
No	174	77.3	184	81.8	1.366	0.242
Yes	51	22.7	41	18.2		
Family history						
Absent	175	77.8	213	94.7	27.012	<0.001
Present	50	22.2	12	5.3		

Table 3. The study groups' risk factors for coronary artery disease.

Variables	CAD		Control		Chi-Square / Fisher's Text	
	N	%	N	%	X^2	P
Diet						
Non- vegetarian	168	74.7	113	50.2	28.665	<0.001
Vegetarian	57	25.3	112	49.8		
Alcohol consumption						
No	164	72.9	198	88.0	16.330	<0.001
Yes	61	27.1	27	12.0		
Smoking						
No	155	68.9	185	82.2	10.829	0.001
Yes	70	31.1	40	17.8		
Hypercholesterolemia						
No	144	64.0	225	100.0	98.780	<0.001
Yes	81	36.0	0	0.0		
Hypertriglyceridemia						
No	139	61.8	225	100.0	106.319	<0.001
Yes	86	38.2	0	0.0		
Hypertension						
No	123	54.7	225	100.0	131.897	<0.001
Yes	102	45.3	0	0.0		
Diabetes Mellitus						
No	151	67.1	225	100.0	88.564	<0.001
Yes	74	32.9	0	0.0		

Table 4. The Hardy-Weinberg equilibrium of the Mef2a gene polymorphisms.

Polymorphisms	X^2	P
MEF2A 1250	2.985	0.235
MEF2A 452	4.371	0.112
MEF2A 481	4.025	0.134

Table 5. Comparison of Mef2a 1250 C > T mutations and sequencing distribution in CAD patients as well as controls.

	CAD patients		Controls		Chi-square test			
	N	%	N	%	X^2	P	OR	95% CI
Alleles								
C allele	333	74.0	374	83.1				
T allele	117	26.0	76	16.9	11.088	<0.001	0.58	0.42 – 0.8
Genotyping								
Co-dominant Model								
CC	139	61.8	159	70.7				
CT	55	24.4	56	24.9				
TT	31	13.8	10	4.4	12.107	0.002		
Dominant Model								
CC + CT	194	86.2	215	95.6				
TT	31	13.8	10	4.4	11.834	<0.001	0.29	0.14 – 0.61
Recessive Model								
CC	139	159	159	70.7				
CT + TT	86	66	66	29.3	3.974	0.046	0.67	0.45 – 0.99

Table 6. Mef2a 452 G > T allele and sequencing distribution in CAD patients and controls.

	CAD patients		Controls		Chi-square test			
	N	%	N	%	X^2	P	OR	95% CI
Alleles								
G allele	332	73.8	356	79.1				
T allele	118	26.2	94	20.9	3.554	0.059	0.74	0.55 – 1.01
Genotyping								
Co-dominant Model								
GG	140	62.2	146	64.9				
GT	52	23.1	64	28.4				
TT	33	14.7	15	6.7	8.117	0.017		
Dominant Model								
GG + GT	192	85.3	210	93.3				
TT	33	14.7	15	6.7	7.556	0.006	0.42	0.22 – 0.79
Recessive Model								
GG	140	62.2	146	64.9				
TT + GT	85	37.8	79	35.1	0.345	0.557	0.89	0.61 – 1.31

respectively, whereas it was 64.9%, 28.4%, and 6.7% in controls. The frequency of genotypic was significantly different between CAD patients and controls (X^2 = 8.117; P= 0.017).

The alleles and genotyping distribution of Mef2a 481 A > G between CAD patients and controls have been summarized in Table 7. The percentage distribution of AA, AG, and GG genotypes in CAD patients was 52.0%, 32.0%, and 16.0%, respectively, while it was 57.3%, 33.3%, and 9.3% in controls. There were no significant differences in genotypic and allele frequencies between CAD patients and controls (p=0.101 and p=0.407, respectively). Although the genotype distribution in CAD patients differed from the control group, no genotype was found to be significantly linked with the disease.

4. Discussion

CAD is one of the complicated illnesses whose prevalence is linked to lifestyle, environment, and genetics. Despite significant attempts to identify genetic and molecular features that may explain CAD differences[12]. MEF2A is a transcription factor of MEF2 that is involved in a range of biological activities. MEF2A expression is high in injured endothelial cells, which leads to CAD [13]. The purpose of this study was to investigate the link between three MEF2A polymorphisms and CAD in the Iraqi population.

There were statistically significant variations in every aspect of recognized CAD threat factors, including diet, smoking, and alcohol use, between CAD patients and controls in this research. Clinical data on traditional

Table 7. Comparison of alleles and genotyping distribution of Mef2a 481 A > G between CAD patients and controls.

	CAD patients		Controls		Chi-square test			
	N	%	N	%	X^2	P	OR	95% CI
Alleles								
A allele	306	68.0	333	74.0				
G allele	144	32.0	117	26.0	3.934	0.407	1.3394	1.00 - 1.79
Genotyping								
Co-dominant Model								
AA	117	52.0	129	57.3				
AG	72	32.0	75	33.3				
GG	36	16.0	21	9.3	4.594	0.101		
Dominant Model								
AA + AG	189	84.0	204	90.7				
GG	36	16.0	21	9.3	4.52	0.034	1.8503	1.04 - 3.28
Recessive Model								
AA	117	52.0	129	57.3				
GG + AG	108	48.0	96	42.7	1.291	0.256	1.2404	0.86 - 1.79

CAD risk factors such as hypertension, hypercholesterolemia, and diabetes are available. This is consistent with the findings of Maddhuri *et al*. (2018) reported that the risk of total cholesterol and LDL-C were considerably greater in patients than in healthy controls ($p<0.001$), but high-density level-C was higher in normal than in experimental groups [6]. Also, it was found that vegan diets may improve health and reduce the risk of CAD and heart failure [14]. In addition, smoking and excessive alcohol consumption are associated with a higher risk of acute myocardial infarction [15].

The results showed a much higher incidence of MEF2A in people with coronary artery disease. Therefore, MEF2A can be considered a crucial player in cardiovascular pathophysiology. Firstly, Wang et al. (2003) reported an association between mutations of MEF2A in an inherited disorder with features of CAD [16]. MEF2A is a transcription factor that activates many and stress-induced, growth factor-induced, and muscle-specific genes [17]. Due to its role in the activation of stress-induced genes, MEF2A may play a vital role in the incidence of CAD. However, some studies did not find any association between MEF2A and CAD [18].

The prevalence of Mef2A 1250 C > T genotypes and allelic frequencies in patients and controls was studied in this study. The percentage distribution of CC, CT, and TT genotypes in CAD patients was 61.8%, 24.4%, and 13.8%, respectively, vs 70.7%, 24.9%, and 4.4% in the control group. Patients and controls had substantially different genotypic and allele frequencies ($p=0.002$) and ($p=0.001$), respectively. This validates the findings of Foroughmand et al. (2014) who investigated the association of MEF2A gene polymorphisms with coronary artery disease [19]**.** Also, Maddhuri *et al.* (2018) found that the CT and TT genotypes were predominant in CAD with two and single-fold increased risk of CAD, respectively [6].

Also, the results of the investigation of the genotype and allelic frequency distributions of Mef2a 452 G > T in patients and controls showed that the frequency of genotypic was significantly different between CAD patients and controls. This showed a potential relation between this variant and CAD. The results of Maddhuri *et al.* (2018) study also found that 452 G > T was significantly associated with CAD (6).

The alleles and genotyping distribution of Mef2a 481 A > G between CAD patients and controls have been investigated. There were no significant differences in genotypic and allele frequencies between CAD patients and controls. The same result was achieved in the study of Maddhuri *et al.* (2018). They did not find any significant differences in genotypic and allele frequencies between the patients and controls (6).

In our study, the percentage distribution of GG, GT, and TT genotypes in patients was 62.2%, 23.1%, and 14.7%, respectively, and 64.9%, 28.4%, and 6.7% in controls. Patients and controls had significantly different genotypic and allele frequencies ($p=0.017$ and $p=0.059$, respectively). This difference indicates a potential association between the studied variant and the disease. The Mef2A 279 C > T SNP is a member of one of three clusters with significant synergy and independent effect. A redundant interaction exists between Mef2A rs325400 G > T and Mef2A rs34851361 A > G. This lends credence to the theory that CAD is inherited in a multifaceted manner, with major/minor gene(s) and environmental variables both having a role [20]**.** Zargar et al. (2018) investigated the relationship of the MEF2A gene with CAD in Saudi people. They concluded that the MEF2A gene based on SNP rs325400 can be considered a susceptibility factor for CAD, and the T allele presence makes people at more risk for this disease [21]. Also, the results of a study showed that there was an association between rs325400 SNP and CAD (19).

Notably, recent genetic studies on CAD have shown a distinct pathogenic route (or mechanism) underlying CAD development. The discovery of MEF2A alterations in CAD and MI patients with no obvious hypercholesterolemia, as well as high levels of MEF2A expression in coronary endothelium, suggests that endothelial dysfunction or abnormal development caused by altered transcriptional reprogramming of gene expression caused by genetic mutation may be an early result in for the progression of CAD [22]**.**

Genetic testing can identify many people who are

predisposed to having CAD. Because acute myocardial infarction (MI) and sudden death are frequently the first symptoms of CAD and MI, genetic testing is essential. Lifestyle changes and other treatment options may be employed in high-risk individuals to postpone the start of the illness or avert MI. CAD genes can also be exploited to create innovative medications to treat atherosclerosis [23].

5. Conclusion

In conclusion, the results showed a much higher incidence of MEF2A in people with coronary artery disease Therefore, MEF2A may play a crucial role in cardiovascular pathophysiology. Patients and controls had considerably different genetics and frequency of alleles. The results showed significant differences in MEF2A 1250 C > T and MEF2A 452 G > T variants of the MEF2A gene between the patients and controls. It showed that these variants can be considered as potential markers for diagnosis of CAD.

Recommendations

This will be an important area of future research, and human genetic investigations may uncover even more previously unknown roles for MEF2 transcription factors in development and disease.

It is recommended that MEF2A DNA testing for early detection and prevention of CAD in high-risk populations.

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Conclict of Interests

None declared.

Consent for publications

All authors read and approved the final manuscript for publication.

Ethics approval and consent to participate

All participants volunteered to take part after being told about the nature of the research and the purpose of the study. Everyone who took part in this study received a complimentary MEF2A screening test.

Informed Consent

This study was carried out in compliance with the Helsinki Declaration and with the consent of the ethics committee at the College of Education, University of Salahaddin-Erbil, Iraq.

Authors' contributions

Kazhal Muhammad Sulaiman did all the work alone.

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