Variants in *MEF2A* gene in relation with coronary artery disease in Saudi population

Seema Zargar¹ · Abdulaziz A. Aljafari¹ · Tanveer A. Wani²

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Abstract

This study investigated the association of variants in myocyte enhancer factor 2A (*MEF2A*) gene with coronary artery disease (CAD) via case control study on Saudi population. Several studies have indicated a high expression of *MEF2A* in the human coronary endothelium. The entire (exon 11 putative susceptibility exon) of *MEF2A* gene was sequenced using direct DNA sequencing method in 120 sporadic patients and 100 controls. Total number of variants were identified and crude odds ratio (OR) with 95% confidence interval (CI) was calculated. In total, three variants were identified, namely, CAG repeats, AGC deletion, and SNP rs: 325400. No significant link was observed between the common (CAG)_n polymorphism, AGC deletion, and CAD risk as reported in other populations, but interestingly, rs325400 (G1323T) in Saudis was found to be associated with the CAD with odds ratio 2.0102 (CI = 1.3405–3.0146) and significance of $p = 0.00048$. None of Saudi subjects (normal as well as diseased) showed 21-bp deletion as reported previously for other populations. In addition, genotype TT of rs325400 is associated with significantly higher levels of LDL-C and lower level of HDL-C. Among the quantitative parameters, lower HDL-C and higher LDL-C was found to be associated with disease. We report that *MEF2A* gene based on SNP rs325400 (G1323T) can be considered as a susceptibility factor for CAD and presence of T allele makes Saudis at more risk to CAD, while other variants detected in this gene do not have any association in Saudi population.

Keywords Coronary artery disease · *MEF2A* gene · Saudi population · rs: 325400 · (CAG)_n polymorphism

Introduction

Coronary artery disease (CAD) is one of the complex diseases, the incidence of which is attributable to lifestyle, environment, and genetics. In spite of great efforts to determine the genetic and molecular contributing factors that could account for variations in CAD, the etiology and the complex multigenic basis of atherosclerosis and MI is still not completely identified. *MEF2A* codes for one of the members of transcription factors of myocyte enhancer factor-2 (MEF2) family and plays an important role in certain biological functions (Wang et al. 2018; Scheuner 2003). There is high expression of *MEF2A* in dysfunctional endothelium that in

turn contributes to atherogenesis. MEF2 family also has been closely linked with important signaling pathways that include Ca²⁺, MAP kinase, Wnt and PI3K/Akt signaling, etc (Chen et al. 2017). *MEF2A* is reported as a strong candidate gene among other 93 genes of MEF2 family, because it is mRNA has been detected in blood vessels of mouse during early embryogenesis (Yamada et al. 2008). Furthermore, early *MEF2A* protein expression was seen on day 8.5 after reproduction in the embryonic cells and hence, behaving as an early marker in these cells. In addition, the *MEF2A* (reference sequence NM_005920.2) expression pattern was reported to be similar to vascular endothelial growth factor receptor 2 (*VEGFR2*), an endothelial cell marker, in endothelial cell precursors (Bhagavatula et al. 2004). This suggests *MEF2A* gene's possible role as early vasculogenesis marker and hence can be a potential candidate to evaluate its association in controlling CAD. The genomic sequence of *MEF2A* gene is very variable and, therefore, is interesting to explore as the sequence variations have functional importance to affect the gene expression. *MEF2A* gene consists of 11 exons and 10 introns. The exon 11 is claimed

✉ Seema Zargar
szargar@ksu.edu.sa

¹ Department of Biochemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

² Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

as the most polymorphic locus harboring various substitution and insertion/deletion (indel) polymorphisms such as a common variant (CAG)_n polymorphism (González et al. 2006; Han et al. 2007). A 21 bp coding sequence deletion leading to 7-aminoacid deletion in exon 11 of this gene in Scandinavian family was reported to be a causative mutation in single large CAD positive family (Liu et al. 2012; Wang et al. 2005). Furthermore, in vitro experiments have shown a disruption in the nuclear localization and decreased *MEF2A*-induced transcriptional activation post 21-bp deletion of the mature protein. Thus this genetic defect could produce defect in vascular endothelium that may originate atherosclerotic plaque or thrombosis and influence the whole process of atherogenesis (Wang and Anderson 2015). Since 21-bp deletion was recognized specifically in CAD family having an autosomal dominance and no other familial studies or genetic linkage analysis was done in this specific context so far, the molecular case-control association studies of samples belonging to same region without having relation to each other has become the alternative strategy for such studies. Many case control studies have claimed exon 11 of *MEF2A* gene as the most variant locus occupying various insertion/deletion (indel), substitution and (CAG)_n polymorphisms, the results, however, are in consistent (González et al. 2006; Han et al. 2007; Elhawari et al. 2010; Hsu et al. 2010; Dai et al. 2010; Wang et al. 2005; Guella et al. 2009). Therefore, whether *MEF2A* is a CAD causing gene or simply the findings are incidental remains controversial (Scacchi et al. 2011; Kajimoto et al. 2005). Therefore, this study evaluated all the possible variants of exon 11, the highly variable and susceptible region of *MEF2A* gene by direct sequencing, in 120 Saudi CAD patients against 100 normal control subjects of same region. Direct sequencing of exon 11 of *MEF2A* gene, was done to assess the possible variations in study population that could lead to individual's susceptibility to CAD.

Materials and methods

Study was hospital based case control study. Subjects were obtained from King Saud University hospital after informed written consent. The study was conducted after the approval of the Ethics Committee of King Saud University (Riyadh, Saudi Arabia) and. Informed written consent was obtained from all participant subjects prior to start of study.

Selection criteria and data collection

This study included 120 clinically confirmed cases of CAD patients (68 males and 52 females) and 100 healthy controls. The patients and controls were interviewed using a questionnaire and the data collected included the epidemiological/

demographic details, any disease history, addiction history (smoking or alcohol) and familial history of MI or CAD. The ECG and coronary angiographic studies of the subjects were collected and studied. The other relevant clinical laboratory parameters were also evaluated. The genetic analysis was carried on 120 CAD patients who gave a written consent to participate and provided blood samples.

Determination of risk factors

Following definitions were used for coronary artery disease risk factors: as per the WHO guidelines subjects with a blood pressure of > 140/90 mmHg or using antihypertensive treatment and subjects with blood sugar > 130 mg/dl or using any anti diabetic or hyperglycemia drugs were categorised as diabetic. Subjects with high levels of total cholesterol, high LDL and low HDL were categorised as dyslipidemic. A positive family history of CAD was considered, in case of presence of a first degree relative who suffered coronary artery disease at the age of <55 years for men and <60 years for women. The subjects on any lipid-lowering drugs were excluded for the study. The subjects with triglyceride (TG) levels > 400 mg/dl were also not recruited in the study.

Sample collection and variant detection

After informed consent was signed by all the subjects, venous blood samples were collected in EDTA tubes from 120 CAD patients. 100 blood samples were collected from healthy Saudi Arabians who were selected as controls.

DNA extraction

Genomic DNA was isolated using Genomic DNA extraction kit (Qiagen) according to the manufacturer's instructions and samples were stored at −20 °C for PCR amplification. The software Primer Premier 5.0 was used to design the Primers to amplify the whole exons and the exon-intron boundaries of the *MEF2A* gene. PCR products were purified with PCR purification kit (Invitrogen).

Direct sequencing

PCR products after purification were directly sequenced using an ABI Prism Big Dye Terminator v3.1 Cycle Sequencing Kit by the dideoxy chain-termination method. Samples were processed using an ABI3730XL capillary sequencer from Applied Biosystems, CA, USA. SeqMan 6.1 module of the Lasergene (DNA Star Inc. WI, USA) software package was used for Sequence analysis and compared with reference Gene Bank Sequence.

Lipid measurements

Total cholesterol and HDL-C were measured in plasma using BM/Hitachi 717 by an enzymatic method (Sera-Pak Bayer). TG levels were assayed by a Boehringer-Mannheim enzymatic method. LDL-cholesterol was calculated using Friedewald formula (Guella et al. 2009).

Statistical analysis

The Hardy–Weinberg equilibrium calculations were performed. Deviation from HWE for genotype distribution was tested with significant relation to CAD to study the disturbing influences of genotype frequency. Two-tailed $p < 0.05$ was accepted as statistically significant. The gene-counting method was used for calculating allelic frequencies. The association between occurrence of CAD, SNPs, allelic frequencies and variants detected were assessed by the odds ratio (OR) and confidence interval (CI). The significance was calculated by the Chi-square method. A p values ≤ 0.05 was considered statistically significant.

Results

The characteristics of the subjects are summarized in Table 1. The mean age of patients and normal controls was 39.8 ± 15.2 and 38.8 ± 16.2 years, respectively. To test genotype–phenotype associations, plasma lipid profile determinations were also carried out in the control ($n = 100$) and patient groups ($n = 120$). The plasma lipid parameters and genotypic data when compared revealed significant difference in the control and patient group showing significant differences. The lipid pattern of healthy controls and patients are shown in Table 1. All the patients showed significantly higher triglyceride (TG) and total cholesterol (TC) levels and lower high density lipoprotein (HDL-C) levels than controls ($p < 0.01$). Three variations observed by direct complete sequencing of exon 11 in 120 ethnically unrelated Saudis free from any fat lowering diet and smoking are presented in Table 2. There was no deviation detected from Hardy–Weinberg equilibrium for these polymorphisms in all subjects. Interestingly, no differences in Chi-square

Table 1 Clinical and biochemical characteristics of CAD patients and control subjects

Parameter	Characteristics	Controls ($n = 100$)	CAD group ($n = 120$)	p value
Age	Years	39.8 ± 15.2	38.8 ± 16.2	$< 0.001^*$
Gender	Male, %	60 (60%)	68 (56.66)	$< 0.001^*$
	Female, %	40 (40%)	52 (43.33)	
TG (mmol L ⁻¹)	Mean $\pm$ SD	1.06 ± 0.28	1.56 ± 0.7	$< 0.0001^*$
	Range	(0.53–1.72)	(0.57–3.77)	
TC (mmol L ⁻¹)	Mean $\pm$ SD	3.8 ± 0.48	4.24 ± 1.09	0.0002*
	Range	(3.01–5.11)	(0.77–7.5)	
HDL-C (mmol L ⁻¹)	Mean $\pm$ SD	1.70 ± 0.39	1.11 ± 0.88	$< 0.0001^*$
	Range	(0.76–2.11)	(0.53–5.1)	
LDL-C (mmol L ⁻¹)	Mean $\pm$ SD	1.65 ± 0.33	2.53 ± 0.91	$< 0.0001^*$
	Range	(1.0–2.5)	(1.01–4.89)	

Mean $\pm$ SD for all traits are presented. Student's t test was used to compare the values of each group. Lipid levels are expressed as mmol L⁻¹

* p value significant

Table 2 Nature of genetic variations in *MEF2A* exon 11 by sequencing in Saudi population

Categories	Variant	AA code	Minor allele frequency (%)		p value or (95% CI)
			CAD ($n = 120$)	Control ($n = 100$)	
STR	(CAG) _n (1235–1249)	(Q) _n ($n = 2$ –5)	5 (4.1)	0 (0)	0.0648 0.1045
Deletion	AGC (1244–1249)	Q deletion	6 (5.3)	0 (0)	$< 0.0001^*$ 0.03151
rs: 325400	G1323T	G443G	68 (56)	31 (31)	$< 0.0001^*$ 2.0102

Q glutamine, STR Short Tandem repeat polymorphism, AA amino acid, OR odds ratio, 95% CI 95% confidence interval

* p value significant

values between observed and expected genotypes were seen. Among all genotypes, the variants detected were ≥ 3.84 , suggesting that they are all in HWE. The rs325400 variants resulted in silent mutation at the 1323 codon (G1323T) and formed p.G443G of *MEF2A* gene. The allelic distributions and genotypes assessed for polymorphisms in the rs325400 are reported in Table 3. The observed genotypes include homozygous wild-type, heterozygous, and homozygous variants. The heterozygous (GT) and homozygous (TT) variants of rs325400 were in more frequency in the CAD patients than controls, indicating that these variants are at higher risk to develop the disease. The genotype analysis comparing heterozygous to wild-type homozygous showed significant association of rs325400 ($p=0.00048$) with CAD. Furthermore, we did not find the 21-bp deletion in any Saudi CAD patient and the control samples. This suggests that Saudi population is entirely different from other populations and 21 bp deletion has no association with CAD in Saudis. The lipid pattern of CAD patients, as illustrated in Table 1, was

used to investigate the effect of rs325400 polymorphism on plasma lipid levels (Table 4). ANOVA analysis showed the significant effect of this polymorphism on HDL-C levels in patients varying in three genotypes with TT genotype showing the highest frequency when the GT and TT genotypes were grouped together ($p=0.03$) (Table 5). Sequencing analysis showed Short Tandem Repeats (STR) (CAG)_n variants at position 1235–1249 (Fig. 1a) leading to change of amino acid glutamine [(Q)_n]. Most of the subjects had 2–5 repeats. Statistical significance was not observed ($p=0.0648$) for association of this polymorphism in spite of this no such variant was detected in control samples. Deletion variants at 1244–1249 with Q deletion were also found in 6 patients, while no such variant was found in control healthy subjects (Fig. 1b). Other sequence changes analyzed included synonymous SNP: rs325400 at position 1323 with G to T transition (Fig. 1c). Furthermore, no other sequence changes were observed in exon 11 of Saudi population.

Table 3 Correlation of rs325400 T > G MEF2 gene polymorphism among cases and controls

	<i>N</i>	TT	GG	GT	χ^2	<i>df</i>	<i>p</i> value ($\chi^2 > 20.978$)	OR (95% CI)	RR (95% CI)
Cases	120	68	6	46	20.978	2	0.00001	2.0102 (1.3405–3.0146)	1.4171 (1.1396–1.7623)
		51.27	7.64	61.09					
		–5.46	–0.35	–3.73					
Controls	100	26	8	66					
		42.73	6.36	50.91					
		–6.55	–0.42	–4.47					

OR odds ratio, (95% CI) 95% confidence interval, RR risk ratio

**p* value significant

Table 4 Allele frequencies of rs325400 T > G MEF2 gene in CAD

Group affected	Common Hz (TT)	Heterozygotes (GT)	Rare Hz (GG)	<i>p</i> allele freq	<i>q</i> allele freq
Common Hz	88.17	46	6	0.79	0.21
Heterozygotes	68	40.4	6	0.77	0.23
Rare Hz	68	46	7.78	0.75	0.25

p allele freq = 0.76; *q* allele freq = 0.24

Table 5 Significance of association of rs: 325400 polymorphism with lipoprotein levels

rs: 325400	CAD cases				Healthy controls			
	TT (<i>n</i> = 68)	GG (<i>n</i> = 6)	GT (<i>n</i> = 46)	<i>p</i> value	TT (<i>n</i> = 31)	GG (<i>n</i> = 8)	GT (<i>n</i> = 66)	<i>p</i> value
TC	2.38 ± 0.18	1.82 ± 0.21	2.13 ± 0.13	0.001	1.81 ± 0.22	1.62 ± 0.40	1.79 ± 0.42	0.48
HDL-C	0.36 ± 0.14	0.48 ± 0.16	0.42 ± 0.16	0.03	0.48 ± 0.14	0.41 ± 0.13	0.45 ± 0.12	0.32
LDL-C	1.18 ± 0.33	1.14 ± 0.16	1.20 ± 0.11	0.08	1.05 ± 0.18	1.19 ± 0.48	1.09 ± 0.31	0.44
TG	1.38 ± 0.64	1.20 ± 0.71	1.11 ± 0.55	0.069	1.38 ± 0.44	1.35 ± 0.62	1.25 ± 0.46	0.39

All data are represented as mean ± SD

TC total cholesterol, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, TG triglyceride

Fig. 1 Type of variants detected from exon 11 of *MEF2A* gene by direct sequencing

Discussion

Familial CAD/MI, is genetically heterogeneous disease. Myocyte enhancer factor 2A gene (*MEF2A*) has always been attraction with respect to CAD but the results are controversial. In this study, three variants in exon 11 of *MEF2A* in Saudi population were verified. The most remarkable variant in this population was the heterogeneous (CAG)_n polymorphism, the other two polymorphisms being within 100 bp downstream. This hypervariability in exon 11 of *MEF2A* gene made us to explore the association of exon11 *MEF2A* polymorphisms with CAD in Saudi population. Previous

studies on this exon in Chinese population reported a positive association of an allele (CAG)₉ with maximum severity and risk to CAD (Scacchi et al. 2011; Yuan et al. 2006). Another study in Chinese cohort from north ($n = 1139$) showed a borderline significance of $p = 0.052$ (Dai et al. 2010). However, in this study, we could not find any association of CAG repeats with CAD due to wide divergence in the number of these repeats in Saudi population carrying this disease.

We did not identify the 21-bp deletion in any of the affected individuals and controls. In 2003, a deletion in 21-bp (bp) coding sequence in exon 11 of gene *MEF2A* was indicated as

an associated mutation in a large single Scandinavian CAD/MI family (Guella et al. 2009). Since then, *MEF2A* gene has been associated as a candidate gene in causing CAD/MI and hence has been studied in different populations. Recently 6-bp deletion (CAGCCG) in exon 11 of *MEF2A* gene was reported in Chinese population (Xu et al. 2016). The incidence of the 21-bp deletion varied considerably (0.09–1.92%) in sporadic patients (0.16% in Whites and 0.65% in Asian) (Yan et al. 2012). Two other studies, that included Whites and Japanese confirmed the 21-bp deletion in controls (0.12 and 0.51%, respectively) with the drawback that the subjects did not underwent angiography (Guella et al. 2009; Scacchi et al. 2011; Kajimoto et al. 2005; Gulec et al. 2008). The overall frequency of the 21-bp deletion was approximately 0.2% in the combined populations published to date. Our data indicate that the common (CAG)_n variant might have a more compelling effect on CAD than rare 21-bp deletion, and *MEF2A* gene variants may, therefore, be a specific but rare causative agent of CAD/MI. In addition, we report that the mutation in rs:325400 of exon 11 is strongly associated with the development of CAD in Saudi population. Our results have been consistent with the results from previous studies (Guella et al. 2009; Scacchi et al. 2011; Kajimoto et al. 2005; Gulec et al. 2008) but contradicted by observations in few others (Liebet al. 2008; Horan et al. 2006; Maiolino et al. 2011; Li et al. 2005). The present data provides evidence of an association between rs:325400 polymorphisms in exon 11 of *MEF2A* gene with lipid levels of Saudis. Though this polymorphism is synonymous and is not involved directly in the altered production of mature protein, but may be in linkage disequilibrium with many other polymorphisms of *MEF2A* gene or many other nearby genes that may play an important role in onset of this disease. In addition, there can be differential activity of this gene with difference in SNP which could associate the allele of this SNP to CAD.

Among the quantitative parameters, only HDL-C was associated with genetic variations in CAD patients. In the *MEF2A* genotypes having T allele, the mean of HDL-C levels was found significantly higher than the genotypes not carrying it. A population which is at high risk (TT) was having low HDL cholesterol levels and high prevalence of CAD. The results are in the ranges of statistical significance ($p=0.03$). However, the true association of *MEF2A* variants with the CAD/MI in particular population may be confirmed by future studies with large number of samples and expression studies.

Conclusion

In conclusion, we found rs325400 polymorphism in *MEF2A* gene is significantly associated to CAD risk in Saudi population, but other variants did not show any association. In

addition, it was found that this polymorphism was significantly associated with lipid levels. Further studies with larger sample size are required to confirm this association. We did not find 21 or 6-bp deletion in any of our subjects also we found lot of heterogeneity in CAG repeats in Saudi population. SNP rs325400 is synonymous and does not appear to be directly implicated in the CAD, it is although possible that SNP rs325400 may be in linkage disequilibrium with other polymorphisms in *MEF2A* gene or in other nearby genes that could play an effective role in disease onset in Saudi population.

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Author contributions SZ proposed the theoretical frame and designed the experiments, AAJ contributed reagents, patient samples and ethical approval, TAW wrote manuscript and analyzed statistics.

Compliance with ethical standards

Conflict of interest Authors declare that there was no conflict of interest.

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