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Case-control study on the association between the *GATA2* gene and premature myocardial infarction in the Iranian population

Introduction

Coronary artery disease (CAD) and its severe complication, myocardial infarction (MI), are among the most common types of heart diseases in Western societies [1]. Heart diseases also account for almost half the total number of deaths in industrialized countries, whereas this mortality accounts for only 25% of all deaths in developing countries. Despite a large number of studies and subsequent development of new pharmacotherapies, an increase in the incidence of MI in young adults has recently been reported in both developed and developing countries [2]. The fact that MI is becoming increasingly prevalent in young adults especially emphasizes the elucidation of all contributory factors in the development of CAD and MI. CAD is a multifactorial complex disorder, and both environmental and genetic factors have been demonstrated to be involved in its etiology [3]. A gene demonstrated to be associated with early-onset CAD is *GATA2* [4]. *GATA2* is a member of the GATA zinc finger transcription factors family, joining (T/A)GATA(A/G) sequences on DNA and acting as a transcription activator [5, 6]. This gene is located on the long arm of chromosome 3 and plays a role in hematopoiesis and neovascularization of the aortic wall as well as in adipogenesis [7, 8]. It has also been demonstrated that *GATA2* regulates the expression of endothelial selective genes, such as Down syndrome critical region 1, endothelial nitric oxide synthase, platelet/endothelial cell adhesion molecule 1, von Willebrand factor, vascular endothelial cell adhesion molecule 1, kinase insert domain receptor (*KDR*), and *GATA2* itself [9–14]. Because of the strong potential of *GATA2* for modulating susceptibility to CAD, several studies to date have investigated the relationship between *GATA2* gene polymorphisms and atherosclerotic diseases. For instance, rs1573949, rs2713604, and rs3803 have been shown to be associated with MI [15] and early-onset CAD [4]. The results of such association studies can provide a basis for future functional studies and can also assist the identification of new therapeutic targets.

However, since such studies are too costly to conduct, a conclusive replication of results from the preliminary genotype–phenotype association studies should be previously performed. In addition, previous studies demonstrated that the results of association studies in populations of European descent may not be reproducible in the Iranian population [16, 17]. Therefore, the present study aimed to replicate the previously demonstrated association between rs2713604 and early-onset CAD in a case-control study, comparing the rs2713604 polymorphism frequency among those with and without premature MI in a sample of the Iranian population.

Methods

Participants

The Research Ethics Committee of Jahrom University of Medical Sciences (Jahrom, Iran) approved this study. Participant recruitment started in early 2014 and was completed at the end of April 2015 at Jahrom Hospital. The consent form approved by the Ethics Committee of Jahrom University of Medical Sciences was signed by all participants. Men below the age of 51 years and women below the age of 56 years with *de novo* acute MI were considered eligible as cases in this study. The incidence of MI was confirmed by two independent cardiologists according to the World Health Organization (WHO) criteria for MI,

Table 1 Baseline characteristics of participants

Characteristic		Premature MI cases *N* = 99	Controls *N* = 94
Male[a] (%)		67 (67.7)	64 (68.1)
Age[b,c] (years)		41.33 ± 4.8	41.82 ± 6.2
Hypertension[a] (%)		25 (25.3)	7 (7.4)
Hyperlipidemia[a] (%)		23 (23.2)	11 (11.7)
Type 2 diabetes mellitus[a] (%)		25 (25.3)	10 (10.6)
Smoking behavior[a] (%)		21 (21.2)	23 (25.5)
Family history of CAD[a] (%)		69 (69.7)	8 (8.5)
Lipid profile (mg/dl)	TG[b]	140.73 ± 69.9	140.74 ± 101.8
	Total C[b]	171.42 ± 46.5	159.19 ± 51
	HDL[b]	41.7 ± 10.41	38.28 ± 24.03
	LDL[b]	100.35 ± 39.95	91.75 ± 39.33

MI myocardial infarction, *CAD* coronary artery disease, *TG* triglyceride, *Total C* total cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein
[a]Reported as *N* (%)
[b]Reported as mean ± SD
[c]Age at diagnosis is considered for cases, and age at recruitment is considered for controls

Table 2 Association of rs2713604 with premature myocardial infarction

		MAF	AA *N* (%)	AG *N* (%)	GG *N* (%)	Odds ratio (95% CI)	*P*-value
GATA2	Rs2713604						
	Cases *N* = 99	0.3	16 (16.16)	27 (27.27)	56 (56.56)	0.72 (0.36–1.42)	0.34
	Controls *N* = 94	0.35	18 (19.15)	30 (31.91)	46 (48.94)	–	–

Adjusted for hyperlipidemia, hypertension, and type 2 diabetes mellitus
MAF minor allele frequency, *CI* confidence interval

which require typical symptoms plus either an elevation in cardiac enzyme levels or any diagnostic change on an electrocardiogram [18]. Controls were not diagnosed as having MI until the same age as that of their counterparts in the case group (51 years for men and 56 years for women). All of them underwent electrocardiography, and there was no evidence of a previous MI. In the control group, participants' ages ranged from 27 years to 55 years, with 31.9% identified as female and 88.8% reported married. Data related to medical history and demographic characteristics were collected from physicians' reports or by an interviewer on the basis of laboratory and clinical findings and/or current medications.

Variables of interest were sex; age; smoking behavior; history of hyperlipidemia (HLP); lipid profile, including serum concentrations of triglyceride (TG), total cholesterol (total C), high-density lipoprotein (HDL), and low-density lipoprotein (LDL); hypertension (HP); type 2 diabetes mellitus (T2DM); ischemic heart disease; and familial history of ischemic heart events. **Table 1** presents a comparison of these characteristics across cases and controls. Collectively, 193 participants were enrolled in this case-control study, including 99 cases and 94 controls. All participants were Iranian of Fars ethnicity.

DNA genotyping and *in silico* splicing analysis

A 3–5-ml blood sample was obtained from each participant. Genomic DNA was purified from peripheral blood lymphocytes using a commercially available DNA purification kit, DNPTM kit (CinnaGen Co., Tehran, Iran), according to the manufacturer's protocol. The target single-nucleotide polymorphism (SNP) was genotyped using the polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method. The RFLP procedure with the restriction enzyme AvaI (Fermentas, Thermo Fisher Scientific, Waltham, MA, USA) differentiates the A allele from the G allele. Two primer sequences for the target region of the *GATA2* gene containing rs2713604 were designed by the Primer3 web service. These primers were synthesized by Bioneer Corporation (Daejeon, South Korea). The following primer sequences were incorporated: forward (5′-AGAAGGGCTTCCCGTAAGAG-3′) and reverse (5′-ACCAGATGGCTACCATTCCA-3′). PCR amplification was conducted in a final volume of 20 µl. The reaction mixture contained 1X Taq DNA polymerase master mix with the final MgCl concentration of 1.5 mM (Bioneer, Daejeon, South Korea), 20 ng of template genomic DNA, and 1X from the forward and reverse primer. The thermal cycler (Applied Biosystems, Thermo Fisher Scientific, Paisley, UK) was set at 5 min of initial denaturation at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min. Final extension was performed at the temperature of 72 °C for 10 min. The PCR products were then subjected to RFLP via the AvaI restriction enzyme. The amplicons were digested with 4 U of the restriction enzyme in 1.5 µl of the 10X restriction buffer and incubated at 37 °C for 24 h. Afterward, the digested amplicons were analyzed by electrophoresis on a 2% agarose gel and photographed under an ultraviolet transilluminator. The NetGene2 web tool (http://www.cbs.dtu.dk/services/NetGene2) was also employed for splice-site analysis.

Statistical analysis

First, the polymorphism was tested for deviations from the Hardy–Weinberg equilibrium via the X2 test with two degrees of freedom in both case and control groups, and no deviation was found. The association between rs2713604 and premature MI was then assessed under an

additive genetic model using multivariable logistic regression modeling. This model was also adjusted for major CAD risk factors, which significantly differed across cases and controls. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.5 (IBM Corporation, Armonk, NY, USA) at the two-tailed significance level of <0.05.

Results

Table 1 represents the clinical characteristics of the participants. In terms of age, sex, and cigarette smoking, there were no significant differences between case and control groups. However, the number of individuals with HLP, HP, and T2DM was higher among those with premature MI compared to those without it ($P = 0.03$, $P = 0.001$, and $P = 0.008$, respectively).

These factors were considered as confounding factors for CAD and premature MI. Therefore, the association between premature MI and rs2713604 was adjusted for HLP, HP, and T2DM. PCR-RFLP differentiates between two alleles (A and G) and three genotypes (AA: 157 and 70 base pair [bp] bands; GG: 227 bp bands; and AG: 157, 70, and 227 bp bands). The results of the association between rs2713604 and premature MI are presented in **Table 2** after adjustment for HLP, HP, and T2DM. Also, information about clinical characteristics of recruited subjects grouped by genotypes are shown in **Table 3**. In the investigated population, the risk allele (A allele) of rs2713604 showed a slightly higher frequency in the control group compared to the case group. In addition, this variant had no correlation with the risk of premature MI. Moreover, the results provided by the NetGene2 web tool indicated that rs2713604 is not located within any splice acceptor/donor site.

Discussion

In this study, 193 cases and controls were genotyped for a common intron variant, rs2713604. The variant was not associated with premature MI in the investigated population. In a genome-wide

Abstract · Zusammenfassung

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Case-control study on the association between the *GATA2* gene and premature myocardial infarction in the Iranian population

Abstract

In recent decades, due to the high prevalence of coronary artery disease (CAD) and myocardial infarction (MI), numerous studies have attempted to elucidate genetic contributing factors in these complex disorders. A very interesting gene in this regard is *GATA*-binding protein 2 (*GATA2*), an important regulator of various gene expressions in vascular endothelial cells. Accordingly, the association of different *GATA2* polymorphisms with CAD and MI has already been evaluated. Rs2713604 is a genetic marker whose association with CAD has not been reproduced in previous studies. Considering the importance of replicating the initial association, the present case-control study aimed to examine the association of this intronic variant with premature MI in a sample of the Iranian population. In this study, 193 participants from Jahrom Hospital (Jahrom, Iran) were consecutively recruited during a 1.5-year period, and, following blood sampling, genomic DNA was extracted. We then proceeded to genotype rs2713604 using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and statistically analyzed the data. After adjustment for hyperlipidemia, hypertension, and type 2 diabetes mellitus, the results of the multivariate regression analysis showed no significant association between rs2713604 and premature MI. Interestingly, the risk allele (A-allele) of rs2713604 displayed a slightly higher frequency among controls compared to cases.

Keywords

Association · *GATA2* transcription factor · Coronary artery disease · Rs2713604 · Myocardial infarction

Fall-Kontroll-Studie zur Assoziation zwischen *GATA2*-Gen und frühzeitigem Myokardinfarkt in der iranischen Bevölkerung

Zusammenfassung

In den letzten Jahrzehnten haben aufgrund der hohen Prävalenz von koronarer Herzkrankheit (KHK) und Myokardinfarkten (MI) zahlreiche Studien versucht, die genetischen Einflussfaktoren auf diese komplexen Krankheitsbilder zu erklären. In dieser Hinsicht stellt das *GATA-bindende Protein 2 (GATA2)* als ein wichtiger Regulator verschiedener Genexpressionen bei vaskulären endothelialen Zellen ein sehr interessantes Gen dar. Folglich wurde die Assoziation von verschiedenen *GATA2*-Polymorphismen mit CAD und MI schon evaluiert. Der Zusammenhang des genetischen Markers rs2713604 und CAD konnte jedoch in früheren Studien nicht reproduziert werden. In Anbetracht dessen, wie wichtig die Replikation dieser initial festgestellten Assoziation ist, war es das Ziel der aktuellen Fall-Kontroll-Studie, den Zusammenhang dieser intronischen Variante mit frühzeitigem MI anhand einer Stichprobe der iranischen Bevölkerung zu untersuchen. In dieser Studie wurden 193 Teilnehmer des Jahrom Hospital (Jahrom, Iran) während eines Zeitraums von 1,5 Jahren konsekutiv rekrutiert. Nach einer Blutprobe wurde die genomische DNA extrahiert. Anschließend wurde rs2713604 mittels Polymerase-Kettenreaktion-Restriktionsfragmentlängenpolymorphismus (PCR-RFLP) genotypisiert, und die Daten wurden statistisch analysiert. Nach der Anpassung von Hyperlipidämie, Hypertension und Diabetes mellitus Typ 2 zeigte das Ergebnis der multivariaten Regressionsanalyse keine signifikante Assoziation zwischen rs2713604 und einem frühzeitigem MI. Interessanterweise wies das Risiko-Allel (A-Allel) von rs2713604 eine geringfügig höhere Häufigkeit in der Kontrollgruppe im Vergleich zu der Fallgruppe auf.

Schlüsselwörter

Assoziation · *GATA2*-Transcriptionsfaktor · Koronare Herzkrankheit · rs2713604 · Myokardinfarkt

Table 3 Clinical characteristics of subjects grouped by genotype

Group	Genotype	Smoking behavior[a]	*P*-value	T2DM[a]	*P*-value	HP[a]	*P*-value	HLP[a]	*P*-value
Case	GG	13 (23.2)	NS	15 (16.8)	0.008	14 (25)	0.03	16 (26.8)	0.004
	GA	4 (14.8)	NS	7 (25.5)	NS	8 (29.6)	0.02	6 (22.2)	NS
	AA	4 (25)	NS	3 (18.8)	NS	3 (18.8)	NS	1 (6.3)	NS
Control	GG	9 (19.6)	–	3 (6.5)	–	4 (8.7)	–	3 (6.5)	–
	GA	8 (26.7)		4 (13.3)		2 (6.7)		4 (13.3)	
	AA	6 (33.3)		3 (16.7)		1 (5.6)		4 (22.2)	

T2DM type 2 diabetes mellitus, *HP* hypertension, *HLP* hyperlipidemia, *NS* not significant
[a]Reported as *N* (%)

linkage study, Connelly et al. discovered the *GATA2* gene locus as a locus involved in the development of early-onset CAD [4]. A family-based association study was then conducted on a panel of 17 SNPs in the gene, reporting rs2713604 as a variant significantly associated with early-onset CAD [4]. It is evident that, in a family-based sample, cases run a higher risk of carrying common SNPs correlated with the susceptibility for the disease of interest than individuals belonging to an unrelated sample [19]. Thus, the authors then validated the observed association in an independent nonfamilial case-control sample (CATHGEN sample). The outcomes of that study contradict what we have observed in the present study. This apparent contradiction may have some potential sources. These sources can be categorized into two major groups: a difference in study populations, and a difference in study designs. Regarding the first, the two samples have ethnically different genetic backgrounds. While the CATHGEN sample was collected from a combination of American individuals of various ancestries, including European, Indian, African, and Asian, our study took advantage of an almost homogeneous sample composed exclusively of Iranians of Fars ethnicity. Furthermore, in the CATHGEN sample, the number of hypertensive participants was nearly three times more than that in our study.

In fact, 69% of their cases and 67% of their controls had hypertension. The authors had adjusted the analysis for hypertension, but hypertension has an established genetic background [20], and the association between a given phenotype and a genetic marker adjusted for a heritable confounding factor can be biased due to the genetic component of the covariate [21]. Thus, owing to the possibility of an overlap between genetic susceptibility to hypertension and CAD, the findings reported by Connelly et al. can be merely a false positive result, not necessarily exhibiting a CAD-specific genetic component.

Regarding the difference in study designs, another factor may be the difference between the investigated phenotypes. In the CATHGEN dataset, patients were selected based on the extent of CAD exhibited by coronary angiography, whereas our cases were patients showing typical symptoms of MI. However, it is very unlikely that the inconsistency between the outcomes should be due to the variation in the defined phenotype. This is because the majority of genetic markers associated with CAD are also associated with MI and vice versa, as long as the comparison between cases with either CAD or MI and controls without MI or CAD is performed [22].

There are also two independent studies [23, 24] that were unable to replicate the initial association meeting the $P < 0.05$ threshold, despite having a sufficient statistical power and being very similar to the study of Connelly et al. (CATHGEN dataset) in terms of design, especially with regard to the studied variables (smoking, HP, HLP, etc.) and case definition. Interestingly, in line with our findings, in one of these studies—the one by Dandona et al. [23]—risk-allele frequency was reported to be slightly higher in controls compared to cases. In our examined population, the frequency of the A allele was 0.3 and 0.35 among cases and controls, respectively. Therefore, it can be suggested that the variant may play a protective role in early-onset CAD and premature MI. Moreover, because of the weak effect of the variant (odds ratio <1.5), a large and well-powered study may be required in order to show the mild association between this polymorphism and CAD [25, 26]. In addition, Connelly et al. [4] did not use a multiplicity test in their analysis. Therefore, the initial finding may be a spurious association resulting from insufficiently straight criteria for the significance level. Taken together, with such conflicting evidence, it is impossible to generalize the correlation between rs2713604 and an increased early-onset CAD risk to diverse regions and ethnicities.

Variants located within the intron regions of genes can affect the function of either their host genes or distant genes in numerous ways. They can also regulate the susceptibility to many complex disorders. With respect to the splicing process, some intron polymorphisms occurring within the exon–intron splice junctions may cause aberrant splicing [27], whereas others can also be of great significance by acting as splicing-regulatory elements [28]. In the case of rs2713604, according to the results provided by the NetGene2 web tool, the polymorphism is not located within the splice acceptor/donor site. Still, further functional studies are required to reveal whether rs2713604 has any other effect on splicing [29]. In addition, the initially reported association may be merely reflective of another variant associated with CAD in perfect or very high-linkage disequilibrium with this variant. Accordingly, future evaluation of nearby SNPs can elucidate the real contributory variant in CAD in the vicinity of rs2713604.

Limitations of the study

As a case-control study on 193 subjects, our research has its own inherent limitations. It was designed to be a pilot study on the Iranian population, and, therefore, the sample size limited the statistical power. Furthermore, rs2713604 association may be affected by uncontrolled confounding factors. The results of our research may also be biased by misclassification, since the controls were not independently confirmed by a negative diagnosis of coronary lesions. The strength of this study lies in the fact that all participants were recruited from an Iranian population of Fars ethnicity. Thus, our study was free from population stratification to a considerable extent.

Conclusion

In summary, inconsistent with the initial reports, our study provides further evidence that rs2713604 has no association with premature MI and may even exert a protective role in these conditions. As a transcription factor being expressed in various types of cells in the aortic wall, it can regulate many downstream pathways important to CAD development [4, 30]. The *GATA2* gene can be considered a strong candidate for contribution in CAD. Accordingly, its polymorphisms can also be of potential benefit for utilization in clinical risk assessment. Nevertheless, prior to reaching such applications, the association of rs2713604 with premature MI or related conditions should be evaluated in studies on other populations with larger sample sizes.

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Compliance with ethical guidelines

Conflict of interest P. Izadpanah, E. Khabbzi, S. Erfanian, S. Jafaripour, and M. Shojaie declare that they have no competing interests.

All procedures performed in studies involving human participants or on human tissue were in accordance with the ethical standards of the institutional and/or national research committee and with the 1975 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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