E23K POLYMORPHISM IN IRANIAN PATIENTS WITH CORONARY HEART DISEASE

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Abstract

BACKGROUND: It has been shown that ATP-sensitive potassium (K_{ATP}) channels play an important role in physiology of myocardial adaptation to ischemia. In cardiomyocytes, the pore-forming subunits of these channels are coded by KCNJ11 gene. It was reported that the common polymorphism E23K of this gene is associated with higher susceptibility to coronary heart disease (CHD) in Chinese patients, but no other reports are available from other ethnic groups.

METHODS: Iranian patients with confirmed CHD, aged over 50 years were compared with healthy controls for allelic and genotypic frequencies of this polymorphism. Patients who did not suffer from diabetes mellitus were entered into this study if they showed coronary stenosis of > 50% in at least one artery in the angiography performed after a coronary event. The subjects and controls were matched for age, gender, blood glucose, body mass index and smoking.

RESULTS: No association could be found between CHD and frequencies of G and A alleles, single genotype frequencies of AA, AG, GG, and combine genotypes frequencies of AG + AA versus GG, and AG + GG versus AA.

CONCLUSION: This study did not find any association between coronary heart disease and E23K polymorphism in Iranian patients. But, this finding is not conclusive due to limitation of sample size. A subsequent study with a larger sample size is recommended.

Keywords: Single Nucleotide Polymorphism, Kir6.2 channel, KATP Channels, Caucasian.

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Introduction

Ischemic heart disease is one of the leading causes of mortality and morbidity worldwide. Therefore, numerous studies have been performed to identify the factors, which can contribute to development, prevention or treatment of this condition. It has been shown that ATP-sensitive potassium (K_{ATP}) channels play an important role in physiology of myocardial adaptation to ischemia.[1] The balance between inhibitory ATP and stimulatory MgADP, determines the closed or open state of the channel. On the other words, in a metabolically active cell, the gate is closed. When metabolism decreases, the gate opens and allows influx of K^+ ions.[2] This influx leads to membrane hyperpolarization, shortening of action potential duration, and a decrease in influx of calcium ions, which follows by decreased muscular contraction and energy consumption.[3,4] As a result, K_{ATP} channels can increase the adaptability of cardiomyocytes to stress and hypoxic conditions. Also, it has been shown that these channels prevent apoptosis in cardiomyocytes.[5]

In K_{ATP} channel, the pore is formed by a protein subunit, which is Kir6.2 in cardiomyocytes. Kir6.2 is coded by the gene KCNJ11. One of the common polymorphisms of KCNJ11 is substitution of G for A at nucleotide + 67 (counted from ATG start codon) of the only exon of this gene leading to substitution of glutamate (E) for lysine (K) at position 23 (E23K). K increases spontaneous open probability of the channel and decreases its sensitivity to inhibitory ATP.[6,7]

K_{ATP} channels have been identified in other cells, of which, pancreatic beta cells are of particular interest. Similar to cardiomyocytes, Kir6.2 acts as the pore-forming subunit in pancreatic beta cells. It has been

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shown that E23K polymorphism leads to decreased insulin secretion in these cells and is one of the etiologic factors of type II diabetes.[8] In a recent study in China,[9] it was demonstrated that the frequency of GG genotype of KCNJ11 at nucleotide + 67 is significantly higher in patients with coronary heart disease (CHD) compared with other genotypes of AA and AG. This finding can potentially help in identification of people at higher risk for development of ischemic events.

In this study, we have investigated the association of single nucleotide polymorphism (SNP) of KCNJ11 at nucleotide + 67 with coronary artery disease to see whether the finding of Chinese study could be duplicated in a sample of Iranian patients.

Materials and Methods

Subjects

The cases and controls were selected by simple sampling. 73 patients and 55 healthy individuals with ages over 50 years were entered into this study. Patients were selected from Shahid Rajaee Heart Hospital (Tehran, Iran) if they showed coronary stenosis of > 50% in at least one artery in the angiography performed after a coronary event.

Healthy individuals were those without history of angina pectoris who had never been treated for coronary diseases. Individuals with diabetes, hyperlipidemia, kidney and liver diseases, and pregnant women were not entered into this study.

A questionnaire was completed for all patients and controls to collect data regarding their demography, medical status and history and family history. Patients with diabetes mellitus were not entered into this study. Diabetes was ruled out in both patients and healthy controls, using the criteria published in 2006 by American Diabetes Association[10]. The project was approved by ethical committee in Cellular and Molecular Research Center, Iran University of Medical Sciences. Written consent was obtained from all participants.

Biochemical tests

The lipoprotein parameters (TG, cholesterol, HDL-cholesterol, LDL-cholesterol, ApoA-I, ApoB, and Lp(a)) were measured enzymatically with Cobas Mira Autoanalyzer, using conventional enzymatic methods with reagents from Parsazmoon Company, Iran. LDL concentration was calculated by the Friedewald formula:

[Total cholesterol] − [Total HDL] − 20% of the triglyceride value = Estimated LDL.

Genotyping

10 ml venous blood was obtained from each individual after 12 hours fasting. Half of this sample was used for DNA extraction, and the other half for biochemical analysis.

DNA extraction was performed from nucleated cells by salting out method as described before[11]. A segment of KCNJ11 gene containing the target polymorphic site was PCR amplified with forward primer of 5'-GACTCTGCAGTGAGGCCCTA-3' and reverse primer of 5'-AGAAAAGGAAGGCAGACGA GAAG-3'. In the 50-µL PCR reaction mix, the following were at the indicated final concentrations: DNA template 200 ng, primers 20 pmole each, $MgCl_2$ 3 mM, 400 µM of each dNTP, and Taq DNA polymerase 2.0 U.

DNA amplification was performed in Mastercycler 5330 (Eppendorf, Hamburg, Germany). PCR was performed by following thermal profile: DNA denaturation at 95°C for 10 min, and 35 cycles of template denaturation at 94°C (60 seconds), primer annealing at 60°C (30 seconds) and extension at 72°C (60 seconds). A final extension at 72°C was performed for 6 minutes.

The amplicon contained three *BanII* restriction enzyme recognition sites with G at position +67 and two *BanII* recognition sites with A at this position. 5 µL of PCR products were digested with *BanII* restriction enzyme for 16 hours at 37°C. The digestion products were visualized by ethidium bromide staining in 12% polyacrylamide gel.

Comparison with other reports

To make sure that the allelic frequency in our study is comparable with the frequencies reported in other Caucasian populations, our data were compared with other published reports.[12-14]

Statistical analysis

Allele frequencies were determined by counting alleles and calculating the proportions. Hardy-Weinberg equilibrium was confirmed using the $\chi 2$ test. Differences in lipid and lipoprotein values of the various genotypes were evaluated by unpaired Student's t test. Differences of genotypic and allelic distribution between patients and controls were analyzed with $\chi 2$ test.

Results

73 patients with confirmed CHD and 55 healthy controls were entered into this study. Tables 1 and 2 show the demographic data and results of serum biochemical tests. Both groups were matched for age, gender, fasting blood glucose, body mass index (BMI) and smoking. But, the mean blood pressure level, and total cholesterol and triglyceride were higher in CHD group. No significant difference was found between two groups in biochemical parameters.

Table 1. Baseline characteristics of CHD and control groups

	CHD patients (n = 73)	Controls (n = 55)
Age (mean ± SD)	61.2 ± 8.2	60.0 ± 7.9
Gender (male/female) (n)	54/19	39/16
Smoking	18 (24.6%)	6 (10.9%)
BMI (kg/m^2)	25.8 ± 1.8	25.0 ± 1.8
Blood pressure (mmHg)		
Systolic	130.5 ± 3.2	126.3± 2.7*
Diastolic	84.1 ± 4.1	81.8 ± 4.4*

* Significant difference (P < 0.05, t-test)
Abbreviations: CHD: coronary heart disease, BMI: body mass index

Table 2. Serum biochemical analysis in CHD and control groups

	CHD patients (n = 73)	Controls (n = 55)
FBS (mg/dl)	94.1 ± 14	96.4 ± 14
TG (mg/dl)	198.6 ± 108.8	147.3 ± 91.9*
Cholesterol		
TC (mg/dl)	207.9 ±53.5	180.8 ± 46.4*
HDL-C (mg/dl)	39.3 ± 8.5	49.5 ± 12.1
LDL-C (mg/dl)	124.7 ± 45.5	113.6 ± 44
Apolipoproteins		
ApoA-I (mg/dl)	116.6 ± 21.0	117.7 ± 25.0
ApoB (mg/dl)	105.2 ± 25.6	97.8 ± 26.6
Lp(a) (mg/dl)	4.9 ± 3.7	5.8 ± 5.6

* Significant difference (P < 0.05, t-test)
Abbreviations: CHD: coronary heart disease, FBS: fasting blood sugar, TG: triglyceride, TC: total cholesterol, HDL-C: high-density-lipoprotein cholesterol, LDL-C: low-density-lipoprotein cholesterol, ApoA-I: apolipoprotein A-I, ApoB :apolipoprotein B, Lp(a): lipoprotein (a)

Genotyping

The PCR-amplified product was a 449 bp amplicon. After digestion with *BanII* restriction enzyme, homozygote AA genotype was identified by presence of 227, 178, 44 and 28 bp bands, homozygote GG by 227 and 150 and 44 bp bands, and heterozygote AG by presence of five bands of 227, 178, 150, 44 and 28 bp (figure 1, the small bands of 44 and 28 bp are not visible in this figure).

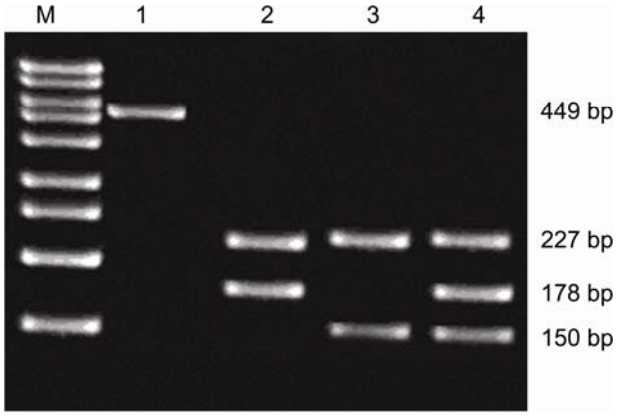

Fig 1. Representative ethidium bromide visualization of PCR products digested by BanII restriction enzyme in 10% polyacrylamide gel; M molecular marker, lane 1 undigested amplicon, lane 2, homozygote AA, lane 3 homozygote GG, and lane 4 heterozygote GA. Small 44 and 28 bp bands are not visible in this figure.

The association between the E23K SNP and CHD risk was examined as presented in table 3. The genotype frequencies for E23K variants were in Hardy–Weinberg equilibrium. There was no association between genotype frequencies, and also frequency of G and A alleles and CHD.

The association between the combined genotype AG+AA versus GG genotype and the combined AG+GG versus AA frequencies and baseline and biochemical parameters was assessed in CHD and control groups. But, no significant association was found (data not shown).

Comparison with other reports

Table 4 shows frequencies of alleles A and G in other studies on Caucasian populations. There was no difference between our study and other reported series.

Table 3. Genotype and allelic frequencies of Kir6.2-E23K SNP in CHD patients and controls

		Genotype n (%)					Allele n (%)	
	Number	GG	AG	AA	AG+AA	GG+AG	G	A
CHD	73	31(42.5)	33(45.2)	9(12.3)	42(57.5)	64(87.7)	95(65.1)	51(34.9)
Control	55	31(56.4)	19(34.5)	5(9.1)	24(43.6)	50(91)	81(73.6)	29(26.4)
P value*		> 0.05			> 0.05†	> 0.05‡	> 0.05	

* χ^2 test
† AG+AA genotypes in comparison with GG genotype
‡ GG+AG genotypes in comparison with AA genotype

Table 4. Comparison of alleles frequencies in different reports*

Reference study	Frequency of allele G	Frequency of allele A
Present study	0.848	0.152
Sakura et al, 1996†	0.645	o.355
Inoue et al, 1997‡	0.655	0.345
Hani et al, 1996§	0.63	0.37

* No significant difference (χ^2 test)
† Diabetologia 1996, 39:1233-1236
‡ Diabetes 1997, 46:502-507
§ Diabetologia 1998, 41:1511-1515

Discussion

In this study, we have shown that the E23K SNP of KCNJ11 gene is not associated with coronary heart disease in our sample of Iranian patients. But, this finding is not conclusive due to limitation of sample size. So far, several polymorphisms in KCNJ11 were reported and studied in regard to association with different conditions including diabetes, obesity, and cardiovascular diseases. The common E23K single nucleotide polymorphism, has attracted researchers' particular interest as its association with type 2 diabetes has been reported in different populations[15-17] and it was also linked to permanent neonatal diabetes.[18] Association of this SNP with mechanisms of tissue adaptation to stress and ischemia[19] and cellular apoptosis in cardiac tissue has been reported as well.[5] The key role of this SNP is gating alteration in K_{ATP} channels, which affects the membrane potential.[6,7]

According to the above pathophysiological background, relationship between E23K polymorphism and a number of cardiovascular conditions has been studied by different research groups. For example, in Japanese[20,21] and Korean patients,[22] it has been shown that this SNP is associated with high blood pressure levels. In American hypertensive patients, it was shown that E23K was associated with greater left ventricular size and hypertrophy.[23] In Japan, a study was performed to identify possible mutations for a number of candidate genes in patients with long QT syndrome and reported E23K as one of the polymorphisms found.[24]

It has been known for a long time that acquisition of most physiological phenotypes to exercise training is variable between individuals. In an interesting study, the association between E23K SNP and exercise training-induced cardiovascular physiological adaptations was investigated, but, no association could be identified.[25] Sudden cardiac death (SCD) after acute myocardial infarction (AMI) is another condition that attracted a polymorphism study. The association between this SNP with SCD in patients with AMI was investigated in Germans[26] and in a Chinese family with SCD pedigree,[27] but no association could be detected. The other area of interest is the level of expression of KCNJ11. It has been shown that the level decreases in patients with persistent atrial fibrillation.[28]

According to the existing evidence and function of K_{ATP}, it was possible to identify a relationship between E23K polymorphism and ischemic heart disease (CHD). This possibility was evaluated in Chinese patients and it was reported that E23K leads to higher susceptibility to CHD.[9] But, we did not find the same association in our sample from Iran. One of the possible explanations of this different observation could be population dependence of this genetic association. The other possibility is the different exclusion criteria set for our experiment. As a strong positive link has been reported between this polymorphism and risk of development of diabetes in numerous previous studies, we have excluded diabetic patients from both CHD and control groups and matched our groups for fasting blood glucose levels. But Xiong et al[9] have excluded diabetics only from their control group and reported that 4.2% of their CHD patients suffered from diabetes. This may have affected the results as a confounding factor. Furthermore, when we compared the frequencies of alleles A and G with other reports from Caucasian populations (Table 4), we found that our reported frequencies are in the range expected for Caucasians.

In conclusion, we have studied the association of E23K single nucleotide polymorphism of KCNJ11 gene with risk of development of coronary heart disease and found that they are not significantly associated. But, this finding is not conclusive due to limitation of sample size. A further study with higher sample size is recommended to establish whether lack of association is due to ethnical dependence of this observation or presence of confounder(s) in the previous study that reported a positive relationship.

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