Full Length Research Paper

Association of E23K polymorphism of Kir6.2 gene with coronary artery disease

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The ATP-sensitive potassium (K\(_{ATP}\)) channels are generally cardioprotective under conditions of metabolic impairment, consisting of pore-forming Kir 6.X (Kir 6.1 and Kir 6.2) subunits in combination with regulatory sulfonylurea receptor (SUR1, SUR2A and SUR2B) subunits. E23K is a missense single nucleotide polymorphism (SNP) located in the cytosolic proximal N-terminal tail of the Kir6.2 subunit. We investigated the E23K polymorphism of Kir6.2 gene in coronary artery disease (CAD) patients to assess its role in susceptibility to CAD. The CAD group included 340 patients (257 male and 83 female; mean age, 60.5±9.1 years) who underwent coronary angiography after recent myocardial infarction or angina. The control group consisted of 91 non cardiac individuals (45 male and 46 female; mean age, 55.6±9.4 years) with normal coronary vessels. The E23K polymorphism of Kir6.2 gene was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in CAD and control groups. The frequency of the G allele was found to be significantly higher in patients than in control group (58.2% vs. 47.8, \(p = 0.01\)). There were also significant differences in GG and combined (GA+AA) genotypes frequencies (35.9 vs. 23.1% and 64.1 vs. 76.9%, \(p = 0.02\)). The E23K polymorphism of Kir6.2 gene may be associated with the development of CAD.

Key words: E23K polymorphism, Kir6.2 gene, coronary artery disease.

INTRODUCTION

Coronary artery disease (CAD) is a multifactorial disease influenced by environmental and genetic factors. The genes conferring susceptibility to CAD are largely unknown.

Potassium channels play important roles in vital cellular signalling processes in both excitable and non-excitable cells (Shieh et al., 2000). To date, four distinct types of K+ channels have been identified in vascular smooth muscle: Voltage-dependent K+ (Kv) channels, Ca\(^{2+}\)-activated K+ (BKCa) channels, ATP-sensitive K+ (K\(_{ATP}\)) channels, and inward rectifier K+ (Kir) channels (Nelson and Quayle, 1995; Noma, 1983).

K\(_{ATP}\) channels have been first identified in cardiac muscle and then, they have been found in various cells including endothelium and vascular smooth muscle (Noma, 1983). The K\(_{ATP}\) channel couples membrane excitability to cellular metabolism and is a critical mediator in the process of glucose stimulated insulin secretion from pancreatic \(\beta\)-cells (Seino and Miki, 2003). In heart, K\(_{ATP}\) channels are active under resting conditions and contribute to maintenance of basal coronary vascular tone (Samaha et al., 1992). They are generally cardioprotective under conditions of metabolic impairment (Gutterman et al., 2005). The inhibition of K\(_{ATP}\) channels leads to impaired coronary and cerebral autoregulation (Narishige et al., 1993; Hong et al., 1994). K\(_{ATP}\) channel activation is closely associated with several pathophysiological responses including systemic arterial dilation during hypoxia, reactive hyperemia in coronary and cerebral circulation and acidosis and endotoxic shock-induced vasodilation (Daut et al., 1990; Brayden,
The CAD group included 340 unrelated patients (257 male and 83 female; mean age, 60.5±9.1 years) who underwent coronary angiography after recent myocardial infarction or angina in Sani Konukoglu Medical Center. In all cases, CAD was established if >50% of 1 or more coronary artery had stenosis. The control group consisted of 91 unrelated non cardiac patients (45 male and 46 female; mean age, 56.0±9.4 years) who had a cardiacological check-up because of their medical history and their complaints, however subsequent angiography indicated normal coronary vessels. The classical risk factors such as presence of hypertension, smoking habit, body mass index and sex were noted in both patient and control groups. Body mass index (BMI) was calculated by dividing weight in kilogram by square of height in meters. The informed consents were obtained from all subjects. The population, approved by Ethics Committee of University of Gaziantep, met the declaration of Helsinki.

Genotyping

10 ml venous blood samples, drawn in EDTA as anticoagulant, were obtained from each subject. Genomic DNA was extracted from leukocytes manually by the method of Miller and Dykes and samples were stored at +4°C until further analysis (Miller et al., 1988). The mutation sites were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The target region in the Kir6.2 gene was amplified by PCR using forward primer 5'-GACTCTTGAGTGGCCTA-3'; reverse primer, 5'-AGAAAAAGGAAGCAGCAGAG-3'; 25 μL PCR mixture containing 5 ng genomic DNA, 1X Taq reaction buffer, 0.5 pmol of each primer, 0.05 mM dNTP mix, 1.5 mM MgCl2, and 0.5 U Taq DNA polymerase. After the DNA was denatured at 95°C for 5 min, the reaction mixture was subject to 35 cycles, each cycle comprising denaturation at 94°C for 30 s, annealing at 60°C for 1 min, and extension at 72°C for 30 s, with a final extension step at 72°C for 10 min. The PCR products were digested with 1 U BanII (Fermentas) for 4 h at 37°C, and the digested products were separated by electrophoresis on a 4.5% agarose gel at constant voltage of 80 V for 1.5 h. Then the gel was visualized under UV transilluminator with a 100 base pair ladder. The PCR products were completely digested into 3 fragments: 227-, 178-, and 150-bp products, the homozygous genotype GG present 227- and 150-bp products, the heterozygous present 227-, 178-, and 150-bp PCR products.

Statistical analysis

All data were analyzed using SPSS 13.0. Allele frequencies were calculated by allele counting and the chi-square test was used to apply for Hardy-Weinberg equilibrium and to compare allele and genotype frequencies between the CAD and control group. Standard descriptive and comparative statistics (χ² test, t-test) were used to compare clinical parameters in different groups (control, cases). Data were expressed as mean±standard deviation (SD). Results were presented as 95% confidence interval (95% CI). A value of P <0.05 was considered statistically significant.

RESULTS

Clinical characteristics of CAD patients and control subjects are shown in Table 1. BMI and prevalence of hypertension, DM and smoking habit were similar between CAD and control groups. However, there were significant differences in mean ages of female subjects and sex of subjects between CAD and control groups (Table 1). The E23K allele and genotype frequencies for the 340 CAD patients and for the 91 control subjects
Table 1. Characteristics of CAD patients and control subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CAD patient (n = 340)</th>
<th>Control subject (n = 91)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (W/M) (Mean±SD)</td>
<td>61.6±9.3/6.1±9.1</td>
<td>54.2±7.8/58.8±10.5</td>
<td>&lt;0.0001/0.4</td>
</tr>
<tr>
<td>Sex (W/M) (%)</td>
<td>24.4/75.6</td>
<td>*&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²) (Mean±SD)</td>
<td>29.3±3.8</td>
<td>28.9±4.4</td>
<td>0.3</td>
</tr>
<tr>
<td>HT (±) (%)</td>
<td>49.4±50.6</td>
<td>*0.09</td>
<td></td>
</tr>
<tr>
<td>Smoker (±) (%)</td>
<td>72.4±27.6</td>
<td>*0.6</td>
<td></td>
</tr>
<tr>
<td>DM (±) (%)</td>
<td>73.8±26.2</td>
<td>*0.9</td>
<td></td>
</tr>
</tbody>
</table>

N: Number of subjects; BMI: Body mass index; DM: Diabetes mellitus; CAD: Coronary artery disease, HT: Hypertension; W: Women; M: Men. * by χ² test.

Table 2. Observed and expected genotype distributions of the E23K polymorphism of Kir6.2 gene in CAD patients and controls.

<table>
<thead>
<tr>
<th>E23K genotype</th>
<th>Observed (n)</th>
<th>%</th>
<th>95%CI</th>
<th>Expected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>122</td>
<td>35.9</td>
<td>30.8-41.2</td>
<td>33.9</td>
</tr>
<tr>
<td>GA</td>
<td>152</td>
<td>44.7</td>
<td>39.3-50.2</td>
<td>48.6</td>
</tr>
<tr>
<td>AA</td>
<td>66</td>
<td>19.4</td>
<td>15.3-24.0</td>
<td>17.4</td>
</tr>
<tr>
<td>Total</td>
<td>340</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>21</td>
<td>23.1</td>
<td>14.9-33.1</td>
<td>22.8</td>
</tr>
<tr>
<td>GA</td>
<td>45</td>
<td>49.5</td>
<td>38.8-60.1</td>
<td>49.9</td>
</tr>
<tr>
<td>AA</td>
<td>25</td>
<td>27.5</td>
<td>18.6-37.8</td>
<td>27.2</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N: Number of subjects; CAD: Coronary artery disease.

Table 3. Allele and genotype frequencies of the E23K polymorphism of Kir6.2 gene in CAD patients and control subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>Allele (%)</th>
<th>Genotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>CAD (n = 340)</td>
<td>41.8</td>
<td>58.2</td>
</tr>
<tr>
<td>Controls (n = 91)</td>
<td>52.2</td>
<td>47.8</td>
</tr>
<tr>
<td>p</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>OR (%95CI)</td>
<td>1.52 (1.10-2.11)</td>
<td></td>
</tr>
</tbody>
</table>

N: Number of subjects; OR: Odds ratio; CI: Confidence interval; CAD: Coronary artery disease.

were in Hardy-Weinberg equilibrium (Table 2). The results were analyzed as allelic and genotypic frequencies of the E23K polymorphism (Table 3). The frequency of the G allele was found to be significantly higher in patients than in control group (58.2 vs. 47.8%, p=0.01). There were also significant differences in GG and combined (GA+AA) genotypes frequencies (35.9 vs. 23.1% and 64.1 vs. 76.9%, p = 0.02).

**DISCUSSION**

CAD is a leading cause of death among both men and women in most industrialized countries. Coronary artery atherosclerosis is the principal cause of CAD. It refers to the presence of atherosclerotic changes within the walls of the coronary arteries, which causes impairment or obstruction of normal blood flow with resultant myocardial ischemia. According to the response-to-vascular injury theory, injury to the endothelium by local disturbances of blood flow, along with systemic risk factors (eg, hyperglycemia, dyslipidemia, cigarette smoking, possibly infection) perpetuates a series of events that culminate in the development of atherosclerotic plaque (Celermajer et al., 1992). Disruption of normal endothelial function leads to loss of vasomotor control, reduced production of nitric
oxide (NO), formation of a procoagulant surface, and promotion of inflammation. As endothelial injury and inflammation progress, fibroatheromas grow and form the plaque (Celermajer, 1997).

Endothelial dysfunction is the initial step that allows diffusion of lipids and inflammatory cells. The most atherogenic type of lipid is the low-density lipoprotein (LDL) component of total serum cholesterol. The endothelium’s ability to modify lipoproteins is particularly important in atherosclerosis. LDLs appear to be modified by a process of oxidation in endothelial cells. Extensively oxidized LDL (oxLDL) is exceedingly atherogenic (Chen et al., 2010). Recently, ATP-sensitive potassium channel openers have been shown to enhance NO release, reduce levels of mRNA for endothelin-1, exert anti-apoptotic effects, and inhibit the overexpression of adhesion molecules in aortic endothelial cells under metabolic disturbances induced by oxidized low-density lipoprotein (Maurey et al., 2006). Furthermore, it has been demonstrated that the opening of K_{ATP} channels attenuates vascular and cardiac remodeling due to chronic inhibition of NO synthesis and induction of endothelin-1 synthesis (Gao et al., 2009). These experimental studies suggest that K_{ATP} channels play an important role in protecting endothelial function and in preventing cardiovascular remodeling.

The E23K variant is a missense SNP located within the N-terminus of the Kir6.2 subunit conferring a subsequent negative to positive change in residue charge. This change could cause decreased ATP sensitivity and enhance the open probability of the channel (Schwanstecher et al., 2002). In another aspect, the overactivity of K_{ATP} channel could facilitate the cellular membrane polarization and then cellular excitability reduction (Xiong et al., 2006). In this study, we analyzed the E23K polymorphism (G→A) of Kir6.2 gene in CAD patients and control group. The frequencies of combined (GA + AA) genotypes were significantly lower in CAD group than in controls. The frequency of the G allele was found to be significantly higher in patients than in control group, which implicated that the A allele may be a protective factor for CAD. Further analysis in different ethnic groups will provide evidence whether E23K polymorphism of Kir6.2 gene is a general risk factor or not.

**Conclusion**

Overall, E23K polymorphism of Kir6.2 gene may be associated with the development of CAD. According to our results, the presence of A allele may be a protective factor for CAD. Further analysis in different ethnic groups will provide evidence whether E23K polymorphism of Kir6.2 gene is a general risk factor or not.

**ACKNOWLEDGEMENT**

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**REFERENCES**


