International Scholars Journals

International Journal of Anatomy and Physiology ISSN: 2326-7275 Vol. 8 (2), pp. 001-005, February, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

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

Necla Benlier[1]*, Mustafa Erdogan[1,4], Mehtap Özkur [1], Oguzhan Yildiz[3], Muradiye Nacak[1], Birol Yamak[2] and A. Sükrü Aynacioglu[1]

[1]Department of Pharmacology, School of Medicine, University of Gaziantep, Gaziantep, Turkey.
[2]Sani Konukoglu Medical Center, Gaziantep, Turkey.
[3]Department of Pharmacology, Gulhane School of Medicine, Ankara, Turkey.
[4]Park Medical Hospital, Gaziantep, Turkey.

Accepted 21 November, 2018

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 ß -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,

*Corresponding author. E-mail: benlier@gantep.edu.tr. Tel: +90-342-3606060 (77730). Fax: +90-342-3601617.

2002; Kanatsuka et al., 1992; Landry and Oliver, 1992). Moreover, changes in K^+ channel function have been found to be associated with cardiac hypertrophy, failure and apoptosis (Shieh et al., 2000).

Coronary artery disease is characterized by the presence of atherosclerosis in the epicardial coronary arteries. Independent risk factors include a family history of coronary artery disease, cigarette smoking, diabetes mellitus, hypertension, hyperlipidemia and obesity. These risk factors accelerate or modify the chronic inflammatory process (Celermajer et al., 1992).

It is demonstrated that hypercholesterolemia impaires K_{ATP} channel function. Recent studies have provided evidence of the involvement of G protein dysfunction in altered function of the K_{ATP} channel induced by hypercholesterolemia (Shimokawa et al., 1991; Komaru et al., 1997; Tanikawa et al., 2000). A recent study by Pongo et al. (2001) demonstrated dysfunction in protein kinase C - K_{ATP} channel coupling by hypercholesterolemia in rabbit coronary arteries. Interestingly, impairment of K_{ATP} channel function in atherosclerotic vessels may not be reversible by dietary treatment (Sobey, 2001). K_{ATP} channels are formed by pore-forming Kir 6.X (Kir 6.1 and Kir 6.2) subunits in combination with regulatory sulfonylurea receptor (SUR1, SUR2A and SUR2B) subunits (Xiong et al., 2006). The molecular diversity of the K_{ATP} channel across species and tissue types is further expanded by the presence of multiple isoforms of the SUR (Noma, 1983). The Kir6.2 and SUR2A are located in heart (Narishige et al., 1993).

Intensive research has been focused on three particular single nucleotide polymorphisms (E23K, L270V, and I337V) found in several Caucasian populations (Hani et al., 1998; Hansen et al., 1997). E23K is a missense SNP located in the cytosolic proximal N-terminal tail of the Kir6.2 subunit and results in the substitution of a highly conserved glutamate (E) residue with lysine (K) and a subsequent negative-to-positive shift in residue charge (Cukras et al., 2002). Initial studies examining the effects of E23K on K_{ATP} channel function performed by Schwanstecher et al. (2002a) revealed an approximate 1.6-fold increase in open probability (Schwanstecher et al., 2002). This increase in open probability in E23K polymorphic K_{ATP} channels results from a slight reduction in ATP sensitivity (Schwanstecher et al., 2002). Thus, any alteration in K_{ATP} channel function due to a polymorphism might be one of the factors contributing to development of CAD. Therefore, we aimed to analyse the E23K polymorphism of Kir6.2 gene in CAD patients to assess its role in susceptibility to CAD.

MATERIALS AND METHODS

Subjects

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 cardiological 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'-GACTCTGCAGTGAGGCCCTA-3'; reverse primer, 5'-AGAAAAGGAAGGCAGACGAGAAG-3'. 25 L 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, 150. The homozygous genotype GG present 227- and 150-bp products, the homozygous genotype AA present 227- and 178-bp products, and those 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 (^{2} test, t- test) were used to compare clinical parameters in different groups (controls, cases). Data were expressed as mean±standard deviation (SD). Results were presented as 95% confidence internal (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.

Characteristic	CAD patient (n = 340)	Control subject (n = 91)	p
Age (W/M) (Mean±SD)	61.6±9.3/6.1±9.1	54.2±7.8/58.8±10.5	<0.0001/0.4
Sex (W/M) (%)	24.4/75.6	50.5/49.5	*<0.001
BMI (kg/m^{2}) (Mean±SD)	29.3±3.8	28.9±4.4	0.3
HT (±) (%)	49.4±50.6	59.3±40.7	*0.09
Smoker (±) (%)	72.4±27.6	74.7±25.3	*0.6
DM (±) (%)	73.8±26.2	73.6±26.4	*0.9

N: Number of subjects; BMI: Body mass index; DM: Diabetes mellitus; CAD: Coronary artery disease, HT: Hypertension; W: Women; M: Men. * by ^{2} test.

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

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

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.

Group	Allele (%)		Genotype (%)	
	A	G	GG	GA+AA
CAD (n = 340)	41.8	58.2	35.9	64.1
Controls (n = 91)	52.2	47.8	23.1	76.9
p	0.01		0.02	
OR (%95CI)	1.52 (1.10-2.11)		1.86 (1.09-3.19)	

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).

D SCUSS ON

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 atherogenesis. 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. In other words, A-to-G substitution at codon 23 of Kir6.2 gene may decrease the open probability of the channel Schwanstecher et al., 2002a, b). This decrease in channel function may cause impairment in maintenance of basal coronary vascular tone and disfunction in coronary endothelium, observed in coronary atherosclerosis (Hansen et al., 1997). Our results are in accordance with the results of Xiong et al., (2006). In their study, they found significant difference between the frequencies of combined (GA+AA) genotype and GG genotype but they could not find significant differences between the frequencies of alleles in controls and patients. That might be due to their small sample size.

It is known that presence of risk factors accelerates the rate of development of atherosclerosis. In our study, BMI and prevalence of hypertension, DM (Diabetes mellitus) and smoking habit were similar between the CAD and control groups. However, mean ages of female CAD patients were significantly higher when compared with the control female. It is known that premenopausal women have a lower incidence of CAD than men of similar age. For our female CAD patients, we do not know the menopause status but this result may be attributed to the increase in incidence of CAD by age (Kitler, 1994).

The present study was limited by its small control sample size, but it is the first study on this area with non cardiac patients who had a cardiological check-up because of their medical history with normal coronary vessels as control group.

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 analyzis in different ethnic groups will provide evidence whether E23K polymorphism of Kir6.2 gene is a general risk factor or not.

ACKNOWLEDGEMENT

This study was supported by Gaziantep University (BAP, TF.07.17, 2007).

REFERENCES

Brayden JE (2002). Functional roles of K_{ATP} channels in vascular smooth muscle. Clin. Exp. Pharmacol. Physiol., 29(4): 312-316.

Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE (1992). Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet., 340: 1111-1115.

Celermajer DS (1997). Endothelial dysfunction: Does it matter? Is it reversible? J. Am. Coll. Cardiol., 30(2): 325-333.

Chen TS, Liou SY, Wu HC, Tsai FJ, Tsai CH, Huang CY, Chang YL (2010). Low-density lipoprotein (LDL) apheresis reduces atherogenic and oxidative markers in uremic patients with hyperlipidemia. Int. Urol. Nephrol., 43(2): 471-474.

Sobey CG (2001). Potassium Channel Function in Vascular Disease. Arterioscler. Thromb. Vasc. Biol., 21(1): 28-38.

Cukras CA, Jeliazkova I, Nichols CG (2002). The role of NH2-terminal positive charges in the activity of inward rectifier K_{ATP} channels. J. Gen. Physiol., 120(3): 437-446.

Daut J, Maier RW, Von BN, Mehrke G, Gunther K, Goedel ML (1990). Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. Science, 247: 1341-1344.

Gao S, Long CL, Wang RH, Wang H (2009). K(ATP) activation prevents progression of cardiac hypertrophy to failure induced by pressure overload via protecting endothelial function. Cardiovasc. Res., 83(3): 444-456.

Gutterman DD, Miura H, Liu Y (2005). Redox modulation of vascular tone: focus of potassium channel mechanisms of dilation. Arterioscler. Thromb. Vasc. Biol., 25(4): 671-678.

Hani EH, Boutin P, Durand E, Inoue H, Permutt MA, Velho G, Froguel P (1998). Missense mutations in the pancreatic islet beta cell inwardly rectifying K^+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. Diabetologia, 41(12): 1511-1515.

Hansen L, Echwald SM, Hansen T, Urhammer SA, Clausen JO, Pedersen O (1997). Amino acid polymorphisms in the ATP-regulatable inward rectifier Kir6.2 and their relationships to glucose- and tolbutamide-induced insulin secretion, the insulin sensitivity index, and NIDDM. Diabetes, 46(3): 508-512.

Hong KW, Pyo KM, Lee WS, Yu SS, Rhim BY (1994). Pharmacological evidence that calcitonin gene-related peptide is implicated in cerebral autoregulation. Am. J. Physiol., 266(1): 11-16.

Kanatsuka H, Sekiguchi N, Sato K, Akai K, Wang Y, Komaru T, Ashikawa K, Takishima T (1992). Microvascular sites and mechanisms responsible for reactive hyperemia in the coronary circulation of the beating canine heart. Circ. Res., 71(4): 912-922.

Kitler ME (1994). Coronary disease: are the gender differences? Eur. Heart. J., 15(3): 409-417.

Komaru T, Tanikawa T, Sugimura A, Kumagai T, Sato K, Kanatsuka H, Shirato K (1997). Mechanisms of coronary microvascular dilation induced by the activation of pertussis toxin- sensitive G proteins are vessel-size dependent. Heterogeneous involvement of nitric oxide pathway and ATP sensitive K^+ channels. Circ. Res., 80(1): 1-10.

Landry DW, Oliver JA (1992). The ATP-sensitive K^+ channel mediates hypotension in endotoxemia and hypoxic lactic acidosis in dog. J. Clin. Invest., 89(6): 2071-2074.

Maurey C, Hislop AA, Advenier C, Vouhé PR, Israël BD, Lévy M (2006). Interaction of K_{ATP} channels and endothelin-1 in lambs with persistent pulmonary hypertension of the new born. Pediat. Res., 60(3): 252-257.

Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic. Acids. Res., 16: 1215-1218.

Narishige T, Egashira K, Akatsuka Y, Katsuda Y, Numaguchi K, Sakata M, Takeshita A (1993). Glibenclamide, a putative ATP-sensitive channel blocker, inhibits coronary autoregulation in anaesthetized dogs. Circ. Res., 73(4): 771-776.

Nelson MT, Quayle JM (1995). Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol., 268: 799-822.

Noma A (1983). ATP-regulated K+ channels in cardiac muscle. Nature, 305: 147-148.

Pongo E, Balla Z, Mubagwa K, Flameng W, Edes I, Szilvassy Z, Ferdinandy P (2001). Deterioration of the protein kinase C- K_{ATP} channel pathway in regulation of coronary flow in hypercholesterolemic rabbits. Eur. J. Pharmacol., 418(3): 217-223.

Samaha FF, Heineman FW, Ince C, Fleming J, Balaban RS (1992). ATP sensitive potassium channel is essential to maintain basal coronary vascular tone in vivo. Am. J. Physiol., 262: 1220-1227.

Schwanstecher C, Meyer U, Schwanstecher M (2002). K(IR)6.2 polymorphism predisposes to type2 diabetes by inducing overactivity of pancreatic beta-cell ATP-sensitive K(+) channels. Diabetes, 51(3): 875-879.

Seino S, Miki T (2003). Physiologic and pathophsiologic roles of ATP sensitive K^+channels. Prog. Biophys. Mol. Biol., 81'(2): 133-176.

Shieh CC, Coghlan M, Sullivan JP, Gopalakrishnan M (2000). Potassium channels: molecular defects, diseases, and therapeutic opportunities. Pharmacol. Rev., 52(4): 557-594.

Shimokawa H, Flavahan NA, Vanhoutte PM (1991). Loss of endothelial pertussis toxin-sensitive G protein function in atherosclerotic porcine coronary arteries. Circulation, 83: 652-660.

Tanikawa T, Kanatsuka H, Koshida R, Tanaka M, Sugimura A, Kumagai T, Miura M, Komaru T, Shirato K (2000). Role of pertussis toxin-sensitive G protein in metabolic vasodilation of coronary microcirculation. Am. J. Physiol. Heart. Circ. Physiol. 279(4): 1819-1829.

Xiong C, Zheng F, Wan J, Zhou X, Yin Z, Sun X (2006). The E23K polymorphism in Kir6.2 gene and coronary heart disease. Clin. Chim. Acta., 367: 93-97.