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Association of KCNJ11 rs5219 E23K Polymorphism with Type 2 Diabetes

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Abstract

Diabetes mellitus (DM) is known as a major public health issue worldwide. Type 2 diabetes does not have a clear inheritance pattern, although many affected people have at least one close family member, such as a parent or sibling, who is affected with the disease. The KCNJ11 gene is a member of the potassium channel gene family. Polymorphisms in KCNJ11 (E23K, rs5219) result in neonatal diabetes and congenital hyperinsulinemia, which are associated with diabetes susceptibility where the K allele plays an important role in insulin secretion. This study evaluates the frequency of these polymorphisms in 85 Kurdish patients with type 2 diabetes. E23K polymorphism was genotyped by the PCR-RFLP method. Heterozygous carriers for AG are more in non-diabetic patients (P = 0.034)..

INTRODUCTION

Diabetes mellitus (DM) is a major public health issue affecting more than 400 million people worldwide [1]. This metabolic disorder progressively leads to chronic microvascular and life-threatening neuropathic complications. DM is caused either by a deficiency of insulin secretion, damage of pancreatic β cell or insulin resistance related to non-use of insulin [2]. Environmental factors (for example, obesity, an unhealthy diet, and physical inactivity) and genetic factors contribute to the multiple pathophysiological disturbances responsible for impaired glucose homeostasis in T2DM [3]. Insulin resistance and impaired insulin secretion remain the core defects in T2DM, but at least six other pathophysiological abnormalities contribute to glucose metabolism dysregulation [4]. The multiple pathogenetic disturbances present in T2DM dictate that multiple antidiabetic agents used in combination will be required to maintain normoglycemia [5]. In Iran, the prevalence of diabetes in adults aged 25–70 years was reported 11.9% (2011), indicating an increase of 35% compared to 2005. It is estimated that in the year 2030, nearly 9.2 million Iranians likely to have diabetes [6]. Many people with diabetes are unaware of their complications due to uncontrolled blood glucose levels. Type 2 diabetes does not have a clear pattern of inheritance. However, many affected people have at least one close family member, such as a parent or sibling, with the disease [7]. Estimates for the heritability of T2D range from 20%-80%. Evidence for heritability comes from a variety of population, family, and twin-based studies [8]. The lifetime risk of developing T2D is 40% for people who have a parent with T2D and 70% if both parents are affected. First-degree relatives of people with T2D are about three times more likely to develop the disease than those without a positive family history of the disease [9]. More than 100 genetic variants are currently thought to be associated with the risk of developing T2D. The majority of these genes affect insulin secretion [10]. One of such genes is the potassium inward rectifying channel subfamily J (KCNJ11). The KCNJ11 gene is a member of the potassium channel gene family located at 11p15, which encodes the islet ATP-sensitive potassium channel Kir6.2. The Kir6.2 protein, together with the high-affinity sulfonylurea receptor 1 (SUR1), forms the KATP channel that mediates insulin secretion [11]. Mutations in the KCNJ11 gene can promote diabetes by altering the functioning of the KATP channel. Among these genetic variants, common glutamate to lysine (E>K) change at position 23 (E23K) has consistently been shown to be associated with T2D, with an overall allelic odds ratio (OR) close to 1.15 when diabetic people were compared with non-diabetic control subjects [12]. More so, other studies have shown normoglycemic subjects with the lysine genotype to demonstrate a defect in secretion of insulin consistently. This has also been

confirmed in vitro, where the lysine risk allele seems to affect potassium channel properties. In this study, the frequency of these polymorphisms was evaluated in 85 Kurdish patients with type 2 diabetes.

METHOD AND MATERIALS

Eighty-five patients with type 2 diabetes and 85 normal people were selected as a test group and a control group, respectively. 5 ml of peripheral blood was taken from each person, and DNA was extracted using the salting-out method. The E23K polymorphism of the KCNJ11 gene was genotyped by the PCR-RFLP method. PCR was performed with forwarding primer 5'-GACTCTGCAGTGAGGCCCTA-3 and reversed primer 5'-ACGTTGCAGTTG CCTTTCTT-3'. After electrophoresis on 2% agarose gel, the PCR product was 209 bp and digested with 0.5 ul of BanII restriction enzyme and 8 ul of nuclease-free water, then separated on 3% agarose gels. The substitution of G with A eliminated the BanII site. The expected product sizes were 150 bp and 59 bp for the normal homozygote GG genotype, 209 bp only for the mutant homozygote AA genotype, and 209, 150, and 59 bp for the heterozygote GA genotype. Data was analyzed using Statistical Package for Social Science (SPSS) version 16.

RESULTS

The KCNJ11 E23K (G/A) gene fragment was successfully amplified for all study participants with a molecular size of 209 bp. After restriction enzyme digestion, the product sizes were 150 bp and 59 bp for the normal homozygous GG genotype and 209 bp only for the mutant homozygous AA genotype. Homozygous for AA genotype is susceptible to T2D, and in patients, the frequency of A allele was higher than control subjects. In addition, heterozygous carriers for AG are more in non-diabetic patients (P = 0.034). Genotype and allele frequency were summarized in Table 1.

Genotype Frequency	Case	Control	p. value
AA [KK]	31 (36.47%)	29 (34.11%)	0.034
AG [KE]	45 (52.94%)	53 (62.35%)	-
GG [EE]	9 (10.58%)	3 (3.52%)	-
Allele Frequency			-
G	63	59	-
A	107	111	0.047

DISCUSSION

Type 2 diabetes is a genetically heterogeneous disease. In twins, about 26% of this risk can be attributed to genetic factors, while in unrelated probands, this falls to 20% [13]. The inheritance pattern suggests that multiple genes and different combinations of genes are involved in the development of diabetes. Polymorphisms in KCNJ11 result in neonatal diabetes and congenital hyperinsulinemia, wherein the E23K (rs5219) polymorphism is associated with diabetes susceptibility where the K allele plays an important role in insulin secretion through reduction of ATP sensitivity of the KATP channel and suppression of insulin secretion [14, 15]. In the past decade, a number of case-control studies have been conducted to investigate the association between the KCNJ11 (E23K, rs5219) polymorphism and diabetes in different populations. However, the results have not been in full agreement. This study examined the association between this polymorphism and type 2 diabetes in the Kurdish population. Homozygous for AA genotype is susceptible to T2D, and in patients, the frequency of A allele was higher than control subjects. In addition, heterozygous carriers for AG are more in non-diabetic patients (P = 0.034). These results were consistent with the results of a study conducted by Rastegari et al. on the Iranian population [16]. Although obesity and lack of exercise are the leading causes of diabetes, these do not account for all diseases, and genetic factors are being increasingly recognized as having a role.

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