

Genetic Study of Saudi Diabetes (GSSD): significant association of the *KCNJ11* E23K polymorphism with type 2 diabetes

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Abstract

Background The E23K variant of *KCNJ11* has been associated with type 2 diabetes (T2D) in several but not all populations studied. Thus far, despite a high incidence of T2D, the role of this variant in Arabs has not been established.

Methods We performed a case-control association study using 550 T2D Saudi patients (WHO criteria), and 335 controls (age ≥ 60 ; fasting plasma glucose < 7 mmol/L). E23K genotyping was performed by using molecular beacon-based real time PCR assays.

Results The difference in K or risk allele frequency of cases and controls was significant with an OR of 1.7 ($p = 0.0001$). The K allele is more common among T2D patients (21%) than in the age and sex matched controls (13.6%). This was consistent with a likely eventual conversion to T2D of younger normoglycemic individuals as they grow older.

Conclusions Our results report for the first time a positive association of the E23K variant with T2D in an Arab population. Confirmation by a larger study is indicated. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords E23k polymorphism; type 2 diabetes; association

Background

Insulin is secreted from pancreatic β -cells in response to nutrients, predominantly glucose but also fatty acids and some amino acids. Glucose metabolism is increased in response to rising cellular glucose levels and results in the production of ATP from ADP. Increased cytosolic ATP:ADP ratios trigger closure of K_{ATP} channels and membrane depolarization via reduced K^+ efflux and subsequent activation of voltage gated calcium channels giving rise to transient increases in intracellular calcium. This in turn induces the exocytosis of insulin-containing granules [1]. While voltage-gated and calcium-activated potassium channels are involved in membrane repolarization, K_{ATP} channels transduce glucose-mediated metabolic signals into electrical activity which modulates insulin secretion.

The K_{ATP} channel consists of two types of subunit: an inward-rectifier potassium channel subunit (Kir6.2) [2,3], and a sulfonylurea receptor (SUR) subunit [4]. The K_{ATP} channel is made of 4 Kir6.2 subunits coupled to four high-affinity SUR subunits [2,5–7]. The Kir6.2 subunit is encoded by *KCNJ11*, and the SUR subunit is encoded by *ABCC8*. Both genes reside adjacent to

one another on chromosome 11. The four Kir6.2 subunits form the pore of the channel through which K^+ passes and also contain the ATP-binding sites. The four SUR subunits modulate the activity of the channel and contain the binding site of sulfonylurea drugs [4]. Mutations in either *KCNJ11* or *ABCC8* can dramatically affect K_{ATP} channel activity, leading to either increased or decreased insulin release [8–12].

Common polymorphisms of *ABCC8* and *KCNJ11*, particularly the E23K variant, have been associated with type 2 diabetes (T2D) in several populations including non-European populations [13–23]. Current status of the E23K polymorphism and the implications for T2D is discussed by Riedel *et al.* [24]. Direct effects of polymorphisms in *ABCC8* (exon 16–3t/c, exon 18 C/T) have not been demonstrated, however, a functional role is proposed for the E23K variant of *KCNJ11*, which is reported to stimulate increased pancreatic β -cell activity, thus increasing the ATP threshold for insulin secretion [15]. The E23K variant of *KCNJ11* results from a G \rightarrow A transition in codon 23. Analysis of the E23K variant in several Caucasian populations showed that KK homozygosity had a stronger association with T2D relative to EK heterozygosity or EE wild-type homozygosity [16]. To date, incidence of the E23K polymorphism and its association with T2D has not been studied in any Arab population.

Research design and methods

Subjects

Non-familial random T2D cases of Saudi ancestry (550) were recruited through a program for the Genetic Study of Saudi Diabetes (GSSD). Diagnosis was based upon WHO 98 criteria (fasting plasma glucose >7.0 mmol/L, and/or 2 h OGTT ≥ 11.1). Their age ranged between 60 and 88 years. Anonymized control subjects (335), aged between 60 and 95 years, were selected on the basis of a fasting plasma glucose of <7.0 mmol/L. Patient's participation in this study was with full informed consent based on the principles of the Declaration of Helsinki and as required by the Institutional Review Board of King Faisal Specialist Hospital and Research Center.

Blood sample collection and DNA extraction

Peripheral blood was drawn from T2D subjects and controls and anticoagulated with EDTA. DNA was extracted from the blood using a PUREGENE DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) as recommended by the manufacturer.

PCR amplification and sequencing analysis

A DNA fragment (404 bp) incorporating the E23K SNP (rs5219) site was amplified from 50 individuals (100

chromosomes) and direct sequencing reactions were performed on both strands using an Amersham ET Dye Terminator sequencing kit (Amersham Biosciences, Code No. 81090) following the manufacturers instructions. Sequencing reactions were desalted and unincorporated nucleotides removed using ethanol precipitation, and then resuspended in a formamide EDTA solution for injection on a Megabace 1000 capillary electrophoresis system. Sequence analysis was performed using the SeqMan module of the Lasergene (DNA Star Inc. WI, USA) software package. Sequencing results were used to identify EE, EK, and KK controls for validation of the molecular beacon-based real-time PCR assay for genotyping of E23K.

Genotyping

Real-time PCR assays using allele specific molecular beacons were used to genotype the study samples. The forward (5'-GCTCCCGGATGTTCTTGTGG-3') and reverse primer (5'-TGTCCCGCAAGGGCATCATC-3') for PCR generated a 99 bp amplicon incorporating codon 23 of *KCNJ11*. Two molecular beacons were used simultaneously in the PCR mix, each specific for one E23K allele and labelled with a unique fluorescent tag at its 5' end. The beacons were: 5'-FAM-CGCGATCGTACCTGGGCTCGGCAGGGTCCTGATCGCG-DABCYL-3' and 5'-TET-CGCGATCGTACCTGGGCTTGGCA GGGTCCTGATCGCG-DABCYL-3'. Thermocycling conditions were 95 °C for 15 min followed by 40 cycles of 95 °C for 20 s; 55 °C for 30 s; 72 °C for 20 s. Upon completion of thermocycling using an ABI 7900HT instrument, data was analyzed using ABI SDS2.1 software (Applied Biosystems, Foster City, CA, USA). Fluorescence was normalized and threshold cycle numbers for both fluorescence channels (FAM and TET) were used to identify the resulting genotypes. Sequence-validated genotyping controls were added within every run and in quadruplicate to control assay performance and positional effects if any. A genotyping success rate of 97% was achieved with 100% concordance and accuracy of sequenced quadruplicate controls.

Statistical analysis

Genotype and allele frequencies among cases and controls were compared by chi-square test. Mean age of cases and controls was compared by t-test. To study the effect of allele on the risk of diabetes adjusting for age and sex we used multiple logistic regression. Statistical analyses were performed using the SPSS software version 14 (SPSS Inc., Chicago, USA). A two-tailed p -value $<.05$ was considered statistically significant. Power calculations were performed using Power and Sample Size Calculation software (PS version 2.1.31; <http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>) with $\alpha = 0.05$.

Results

We conducted a case-control association study comprising 550 T2D patients and 335 controls that both aged above 60 years or higher (Table 1). Both groups were of Saudi ancestry and represented the first such study of an Arab population. In this study, our hypothesis was that an individual who is ≥ 60 years old with a normal fasting plasma glucose level (≤ 7.0 mmol/L) is unlikely to become diabetic at a later age due to genetic predisposition. On the basis of this hypothesis, the controls were selected from individuals whose ages were ≥ 60 years. The T2D cases in this study were also selected to be ≥ 60 years. Cases and controls are comparable with respect to age and sex (Table 1) and therefore these two variables are unlikely to confound the association between the genotype or allele and the risk of becoming diabetic. Both groups utilized in this study (cases and controls) were each in Hardy-Weinberg equilibrium.

The K allele frequency of the cases (231; 21%) was significantly higher than that observed in the controls (91; 13.6%) (Table 1). The crude odds ratio (OR) was 1.7 with a 95% CI of 1.3–2.2 ($p = 0.0001$) with statistical power of 32% ($\alpha = 0.05$). The GA genotype frequency of the cases was also significantly higher than that of the controls when compared to the GG reference genotype. The OR was 1.84 with a 95% CI of 1.35–2.52 ($p = 0.0001$). Finally the AA genotype frequency of both the cases and controls was also compared to the GG reference genotype. The statistical analysis resulted in a p -value of 0.092, and an OR of 2.03 with a 95% CI of 0.89–4.64. This suggests no significant difference between the cases and controls, which was probably due to the modest number of AA genotypes observed in both groups; this was apparent from the wide CI range (0.89–4.64) obtained and statistical power, which was only 40% ($\alpha = 0.05$). Table 2 shows the results from multiple logistic regression. It shows the joint effect of sex, age, and allele/genotype on the risk of the disease. The results suggested that both allele and genotype are significantly associated with the disease, whereas sex and age were not.

Discussions

Variable prevalence rates of T2D among different Arabian countries have been reported and are most likely due to

Table 2. Results for multiple logistic regression

Variable	Odds ratio (95.0% C.I.)	p -Value
Model 1: for Allele controlling for age and sex		
Age	0.99 (0.99–1.0)	0.527
Sex (reference females)	1.01 (0.84–1.2)	0.869
ALLELE (reference G)	1.7 (1.3–2.2)	0.0001
Model 2: for genotype controlling for age and sex		
Age	0.99 (0.99–1.0)	0.571
Sex (reference females)	1.01 (0.8–1.3)	0.896
GG (Reference)	–	–
GA	1.85 (1.4–2.5)	0.0001
AA	2.04 (0.89–4.7)	0.091

We report the first association study of the *KCNJ11* E23K variant with T2D in an Arab population. Cases (550) and controls (335) were selected to be above the age of 60 in order to minimize the likelihood of including among controls individuals who may develop T2D later in life. A strong association of the K allele with T2D (OR 1.7; 95% CI of 1.35–2.52; $p = 0.0001$) was established. However, given statistical power (32%; $\alpha = 0.05$), confirmation of this finding is required in a larger study of this ethnic group.

genetic and environmental heterogeneity among Arabs. Economic development and urbanization have led to rising incidence of this disorder among Arabs. No previous genetic studies, but limited epidemiological reports appeared in the literature that discussed T2D in the Arab world [25]. Our study is the first to explore the effect of E23K in T2D in Arabs, and has been confirmed in the Saudi population, its association with T2D as seen in several other populations [13–23]. This is consistent with approximately 90% of common genetic variants being shared by all human populations [26]. In our study, controls were chosen to be > 60 years old with a fasting plasma glucose < 7.0 mmol/L to reduce the likelihood including individuals who may develop T2D later in life. Being age matched T2D individuals were also aged > 60 years and may under-represent the true frequency of the risk allele in the event that its effect is associated with significant mortality prior to age 60. While association with the K allele was evident, the sample size did not allow us to establish whether the KK genotype was more strongly associated with T2D. Other studies have indicated stronger association of T2D with KK homozygosity [19] indicating a dominant or perhaps a co-dominant model for increased risk, which is supported

Table 1. Frequency of allele, genotype, and distribution of age and sex among cases and controls

Variable	Outcome		OR (95% CI)	p -Value	
	Cases ($n = 550$)	Controls ($n = 335$)			
ALLELE	A variant	231 (21%)	91 (13.6%)	1.7 (1.3–2.0)	0.0001
	G wild type	869 (79%)	579 (86.4%)	Reference	
Genotype	GG	341 (62%)	252 (75.2)	Reference	
	GA	187 (34%)	75 (22.4%)	1.8 (1.3–2.5)	0.00001
	AA	22 (4%)	8 (2.4%)	2.0 (0.89–4.6)	0.092
Age	Mean age (SD)	67.1 (5.9)	68.8 (7.1)		0.595
Sex	%(Male)	57.8%	57.6%		0.952

by our data. This is perhaps not surprising given the octameric structure of the K_{ATP} channel, which includes a central quadramer of Kir6.2. E23K heterozygotes generate both wild-type and variant Kir6.2 and would be expected to result in heterogeneous quadramers of Kir6.2 in which function may be modulated in a transdominant fashion. A co-dominant model for E23K risk is supported by a meta-analysis [15] and functional studies describing a greater risk associated with KK homozygosity.

In our study, significant *p*-values were obtained for incidence of the A or risk allele, as well as the GA genotype, in cases and controls who were ≥ 60 years old. Our data is consistent with E23K being a risk allele of weak individual effect, but one that may have a substantial population attributable risk [15]. Also, the multiple logistic regression analysis suggested that the A allele and the GA genotype are significantly associated with the disease, whereas sex and age were not. A relatively strong OR of 1.7 was observed for the K or risk allele in this study. This is substantially higher than the OR of 1.23 reported in a meta-analysis of K allele association with T2D [12]. Given statistical power of our study (32%; $\alpha = 0.05$), confirmation of this result in a larger group including other Arabs is required to draw firm conclusions on the role of *KCNJ11* E23K as a diabetic risk factor in this ethnic group.

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Conflict of interest

None declared.

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