Original Article

KCNJ11 E23K Polymorphism and Diabetes Mellitus with Adult Onset in Czech Patients

(diabetes mellitus / Kir6.2 / insulin / C-peptide)

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Abstract. In this work, we studied the association of the E23K polymorphism of the Kir6.2 ATP-sensitive potassium channels in 212 Czech patients with diabetes mellitus who were diagnosed after the age of 35. Patients were classified into T1DM, LADA and T2DM groups based on C-peptide and GADA levels. Carriers of the predisposing Kir6.2 E23K K allele showed no increased risk of either type of diabetes mellitus development. On the other hand, we found a correlation between E23K SNP of the *KCNJ11* gene and C-peptide levels, which may be considered a measure of pancreatic β-cell activity, although this correlation was not statistically significant. In conclusion, we failed to confirm the Kir6.2 E23K as a genetic marker for T1DM, LADA and T2DM in the Central Bohemian population of the Czech Republic.

Introduction

Diabetes mellitus (DM) with manifestation after the age of 35 is a very heterogeneous disease in which both genetic and environmental factors contribute to its development. The inwardly rectifying K^+channel (Kir) is one of two subunits in the pancreatic β-cell ATP-sensitive potassium channel complex. This complex plays a key role in glucose-stimulated insulin secretion, which makes it a potential candidate for a genetic defect contributing to the development of type 2 DM (T2DM). *KCNJ11*, a gene encoding Kir6.2 subunit, therefore belongs to well-established candidate genes of T2DM (Porte and Kahn, 2001; Kahn, 2003). Furthermore, functional studies show that E23K SNP is involved in increasing the "open probability" of the Kir6.2 channel, which should lead to diminished insulin secretion (Schwanstecher et al., 2002), and provide supporting evidence for *KCNJ11* being a diabetogene. After several initial reports with inconsistent data (Hani et al., 1998; Yamada et al., 2001) the association between the E23K polymorphism and the susceptibility to T2DM was recently confirmed (Gloyn et al., 2003).

The aim of this study was to investigate the Kir6.2 E23K gene polymorphism with regard to its possible role in the development and progression of DM in patients with disease onset occuring after 35 years of age, who were collected from the Central Bohemian population of the Czech Republic.

Material and Methods

Subjects

Two hundred and twelve diabetic patients (117 women and 95 men) from Central Bohemia were recruited, who were at least 35 years old at the time of DM diagnosis. The diagnosis of diabetes was established according to the current WHO criteria including the pres-

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Abbreviations: CP – C-peptide, DM – diabetes mellitus, GADA – glutamic acid decarboxylase antibodies, LADA – latent autoimmune diabetes in adults, PCR – polymerase chain reaction, RFLP – restriction fragment length polymorphism, SNP – single nucleotide polymorphism, T1DM – type 1 diabetes mellitus, T2DM – type 2 diabetes mellitus.

Table 1. Clinical features of diabetic patients genotyped for Kir6.2 gene E23K polymorphism

	T1DM N = 16	LADA N = 24	T2DM N = 172
BMI [kg/m^2]	28 (22.4-36.7)	32.5 (26.6-45.5)	31.2 (18.4-50.1)
Age at onset [years]	43 (36-55)	54 (35-71)	54 (35-81)
Duration of diabetes [years]	16.7 (3-32)	14 (4-29)	15.1 (1-65)
Mean C-peptide at onset [pmol/L][1]	66.2 (4-197.0)	677 (277.8-1752.3)	756.2 (202-3082)
Mean GADA [ng/mL][2]	223.8 (2.76-3000)	375.5 (50.6-2800)	7.69 (0.29-49.9)

All data shown as mean with min and max value; [1]C-peptide cut-off level: 200 pmol/l; [2]GADA cut-off level: 50 ng/ml

ence of antibodies against glutamic acid decarboxylase GAD65 (GADA) and serum C-peptide levels (CP) (Report, 2003). Patients were divided into three groups: 1) late onset T1DM which required insulin therapy within 6 months after diagnosis: CP-, GADA-/+; 2) latent autoimmune diabetes in adults (LADA): CP+, GADA+ and defined as having a minimum 6-month long period between diagnosis and initiation of insulin therapy; 3) T2DM: CP+, GADA- (Table 1). The control group consisted of 113 healthy individuals randomly selected from volunteers at the Blood Donors Center. Genomic DNA from peripheral blood samples was extracted by QiaAmp DNA Mini Kit spin columns (QIAGEN GmbH, Hilden, Germany).

PCR-RFLP

Genotyping was performed using the PCR-based restriction fragment length polymorphism (RFLP) method. Originally designed oligonucleotides ATPB and ATPC amplified product of the size of 169 bp under the following PCR conditions: initial denaturation at 94°C for 3 min, then 40 times denaturation at 94°C for 15 s, annealing at 56°C for 15 s and extension at 72°C for 15 s. To determine the presence of the E (glutamic acid) or K (lysine) allele, a *Hinf*I digestion was performed at 37°C for 1 h followed by heat inactivation. Within the reverse primer ATPC, an artificial *Hinf*I site was introduced and the resulting fragments were: undigested 169 bp (K/K homozygote), digested 145 + 24 bp (E/E homozygote). The digested and undigested products were visualized using ethidium bromide staining after electrophoresis on 4% agarose gel. The sequences of the original primers used in the Kir6.2 assays are available from the authors on request.

Statistical evaluation

Epi Info software (Version 3.3, October 2004, Atlanta, Georgia) was used for statistical analysis. Statistical differences in allele or genotype distributions were analysed by either the χ^2 or Fisher's exact probability test. Significance was defined using a Bonferroni corrected P value lower than 0.05. The strength of the observed associations was estimated by calculating the odds ratios (OR).

Results

We did not detect any statistically significant difference (P = 0.05) between T2DM patients and the control group. Similar results were obtained for both T1DM and LADA patient groups (Table 2a): the frequency of the K allele was 46.9% (P_{corr} = NS) in T1DM, 29.2% (P_{corr} = NS) in LADA and 36.9% (P_{corr} = NS) in T2DM patients as compared to 36.7% in the control group. Likewise, the genotype distribution did not differ between the three groups and was consistent with Hardy-Weinberg equilibrium.

Table 2b presents comparison of allele and genotype frequencies of the *KCNJ11* E23K polymorphism in all diabetic patients classified based on C-peptide levels (cut-off level 200 pmol/l). The K allele was increased in CP- compared to CP+ diabetic patients (46.9% vs. 36%, respectively), and similarly, the KK homozygous genotype was found more often in CP- (25%) than CP+

Table 2a. Frequency of alleles and genotypes of Kir6.2 E23K polymorphism in control subjects and patients with T1DM, LADA and T2DM diagnosed after the of age 35

Genes		Alleles and genotypes Control subjects	T1DM	P	LADA	P	T2DM	P
Kir6.2 E23K (E/K)	E	143 (63.3%)	17 (53.1%)	NS	34 (70.8%)	NS	217 (63.1%)	NS
	K	83 (36.7%)	15 (46.9%)	NS	14 (29.2%)	NS	127 (36.9%)	NS
	EE	48 (42.5%)	5 (31.2%)	NS	12 (50%)	NS	66 (38.4%)	NS
	EK	47 (41.6%)	7 (43.8%)	NS	10 (41.7%)	NS	85 (49.4%)	NS
	KK	18 (15.9%)	4 (25%)	NS	2 (8.3%)	NS	21 (12.2%)	NS

Table 2b. Frequency of alleles and genotypes of Kir6.2 E23K polymorphism between C-peptide-positive and C-peptide-negative diabetic patients

Locus		Alleles and genotypes CP- (N = 16)	CP+ (N = 196)	P
Kir6.2 E23K (E/K)	E	17 (53.1%)	251 (64%)	NS
	K	15 (46.9%)	141 (36%)	NS
	EE	5 (31.2%)	78 (39.8%)	NS
	EK	7 (43.8%)	95 (48.5%)	NS
	KK	4 (25%)	23 (11.7%)	NS

(11.7%) patients. Nevertheless, these differences were not statistically significant.

Discussion

Despite reports published elsewhere (Willer et al., 2007), with regard to the allele and genotype frequencies of the Kir6.2 E23K polymorphism, we cannot confirm recently published data (Love-Gregory et al., 2003; Florez et al., 2004; Willer et al., 2007) and the association between the genomic variations of Kir6.2 E23K SNP and the development of common T2DM. Although the E23K variant in *KCNJ11* showed no association with diabetes in Czech (for the K allele, OR 1.01 [95% CI 0.71–1.43], P = NS), 95% CI around the OR overlaps in meta-analysis of European populations (Gloyn et al., 2003; van Dam et al., 2005), suggesting that our results are not inconsistent with the previous studies.

Considering the physiological function of the ATP-dependent K channel of pancreatic β cells, we tested to see whether there was a correlation between C-peptide levels and genotypes/alleles of the *KCNJ11* E23K polymorphism. As shown in Table 2b, the K allele and KK genotype were observed more frequently in patients with low C-peptide levels. Although this finding did not reach statistical significance, it suggests an association of the K allele of E23K Kir6.2 polymorphism with decreased pancreatic β-cell secretory activity.

In conclusion, the presented study did not confirm that the Kir6.2 E23K SNP is a genetic marker of diabetes mellitus in Czech diabetic patients and rather proposes its direct impact on the secretory activity of pancreatic β cells.

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