E23K polymorphism of the *KCNJ11* gene in Korean children with type 1 diabetes

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Background: **This study was undertaken to evaluate the association of the E23K polymorphism of *KCNJ11* and type 1 diabetes in a Korean population.**

Methods: **Clinical variables from 70 Korean children with type 1 diabetes were analyzed. Patients' DNA was screened for the E23 locus in the *KCNJ11* gene. Each genotype frequency and clinical characteristics according to the genotypes were compared between the patient and control groups.**

Results: **The genotype frequencies of the *KCNJ11* E23 polymorphic locus in the patient group were 30.0% for EE, 44.3% for EK, and 25.7% for KK. We detected no differences in genotype frequencies between the patient and control groups. Additionally, in the patient group, no difference was detected in the clinical phenotypes among the three genotypes.**

Conclusion: **Although a rather small sample size constituted a limitation of this study, the association of the E23K polymorphism with type 1 diabetes was not statistically significant in the Korean population evaluated.**

World J Pediatr 2012;8(2):169-172

Key words: ***KCNJ11*****;**
type 1 diabetes;
polymorphism;
Kir6.2

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doi: 10.1007/s12519-012-0355-3

Brief report

Introduction

Autoimmune-mediated selective destruction of pancreatic β-cells constitutes the principal pathophysiology in type 1 diabetes mellitus (T1DM).[1] On the other hand, type 2 diabetes mellitus (T2DM) is generally regarded as a polygenic disorder,[2] and studies have shown some genetic variants with an increased risk of T2DM.[3,4] The most convincing candidate variants are p.Pro12Ala (P12A) of the *PPAR-γ2* gene, p.Gly972Arg (G972R) of the *IRS-1* gene, p.Ala98Val (A98V) of the *HNF-1α* gene, and p.Glu23Lys (E23K) of the *KCNJ11* gene.

Among them, the E23K polymorphism in *KCNJ11* has been shown, in *in vitro* studies, to reduce the ATP sensitivity of the ATP-sensitive potassium channel and to affect insulin secretion, reducing the ability of the channel complex to close and thereby inducing overactivity of the channel.[5] The impact of this variant on T2DM has been well established, as several studies have reported an association between the K23 allele and T2DM, with an odds ratio between 1.12 and 1.49 in various populations including Koreans.[6-8]

There are some indications of common etiological factors in T1DM and T2DM. The latent autoimmune diabetes of adults initially presents in a fashion similar to that of T2DM; however, it also involves a progressive β-cell failure, like in T1DM. This finding suggests an etiological overlap between T1DM and T2DM, and genes involved in insulin secretion and insulin signaling may also potentially be risk modifiers of T1DM. A few studies have been carried out to confirm the effects of E23K on the risk of T1DM, but no meaningful association has yet been observed in Caucasians.[9,10]

In this study, we evaluated the genotypic frequency of the *KCNJ11* E23 locus in a cohort of 70 Korean children with T1DM, and attempted to clarify the effect of this variation, which differed between the T1DM children and the controls.

Methods

Subjects

Seventy Korean children with T1DM were recruited from three hospitals (the Hallym University, the

Bundang Jeseang General Hospital, and the Ajou University Hospital) in Korea from 2003 to 2007. The study protocols were reviewed and approved by the Institutional Review Board of all three participating hospitals, and written informed consent was obtained from all subjects or from their parents.

All patients had no family history of T1DM and had been suffering from the disease for more than 2 years. The diagnoses were established in accordance with the most recent American Diabetes Association criteria.[11] As the non-diabetic control group, we employed data from a previous study in the Korean population.[7] The control group was composed of 630 non-diabetic healthy adults.

We reviewed retrospectively the patients' medical records. Clinical characteristics were recorded, including age at diagnosis, family history of T2DM, history of diabetic ketoacidosis, and body mass index (BMI) at diagnosis expressed as standard deviation score (SDS) according to age- and sex-matched control values (BMI-SDS). Biochemical data at diagnosis, including fasting serum C-peptide, hemoglobin A1C (Hb A1C), fasting serum lipid profiles, and the presence of autoantibodies (islet cell antibody, insulin antibody, glutamic acid decarboxylase antibody) were also assessed.

E23 locus genotyping in the *KCNJ11* gene

Genomic DNA was isolated from the peripheral blood leukocytes of the study subjects using a DNA isolation kit (QIAGEN, GmbH, Helden, Germany). Exon 1 of the *KCNJ11* gene was PCR-amplified using one pair of specific primers (forward: 5'-AGGTGGAGGTAAGGAAGAGTCTGG-3'; reverse: 5'-GTCACCCACACGTAGCATGAAG-3'). After amplification, the PCR mixtures were separated on 1.5% agarose gels with ethidium bromide to confirm the size and purity of the PCR products.

Subsequently, DNA sequencing reactions were carried out, and the sequencing reaction mixtures were electrophoresed and analyzed with an ABI3700xl Genetic Analyzer (Applied Biosystems, Foster city, CA, USA) and Sequencing Analysis v.5.2 software.

Statistical analysis

The allele frequencies of the E23K polymorphism were compared between the patient and control groups. Especially in the patient group, the clinical characteristics and results of the hormonal study were additionally compared between patients harboring the E23K polymorphism (homozygote or heterozygote) and the patients lacking this polymorphism.

Using the SPSS 11.2 software (SPSS Inc., Chicago, IL), the Chi-square test, Student's *t* test, and ANOVA test were carried out for data analysis, and two-tailed *P* values less than 0.05 were regarded as statistically significant.

Results

Clinical characteristics in patients with T1DM

In the patient group, 31 were male and 39 female, and the age at enrollment was 14.3 ± 4.1 years. The mean age at diagnosis was 8.9 ± 7.6 years, and the mean duration of disease was 5.5 ± 2.7 years. Twenty-four (34.3%) of 70 patients had one or more first-degree relatives with T2DM, and 27 (38.6%) patients experienced more than one episode of diabetic ketoacidosis. At diagnosis, the mean BMI of patients was -0.32 ± 1.17 SDS, the mean fasting serum C-peptide level was 0.85 ± 0.79 ng/mL, and the mean value of Hb A1C was 11.84 ± 2.43%. Their fasting serum lipid profiles were 139.5 ± 146.4 mg/dL for triglyceride, 52.19 ± 14.20 mg/dL for high-density lipoprotein, and 103.75 ± 43.49 mg/dL for low-density lipoprotein. Three autoantibodies were checked, and positive results were observed in 1 (1.4%) for the islet cell antibody, 11 (15.7%) for the insulin antibody, and 34 (48.6%) patients for the glutamic acid decarboxylase antibody.

We adopted the control group data from a previous study conducted with a Korean population composed of 630 non-diabetic healthy adults.[7]

Allele frequencies and genotype frequencies for E23 locus in the *KCNJ11* gene

We genotyped the E23K (c.67G>A) polymorphism in a cohort of 70 patients with T1DM, and the frequency of the A-allele (K23) was calculated as 0.48. The frequencies of each genotype were 0.30 for E/E, 0.44 for E/K, and 0.26 for K/K, and their distribution was consistent with Hardy-Weinberg equilibrium. Comparing the allele and genotype distributions between the patients and the controls, we were unable to detect any significant differences, and no meaningful association was detected between the presence of E23K and the occurrence of T1DM in

Table 1. Genotype frequencies of the *KCNJ11* E23K polymorphism in the Korean patients with type 1 diabetes and the non-diabetic controls

Genotypes	Patients *n* (%)	Controls[7] *n* (%)	*P* values		
			EE *vs.* EK *vs.* KK	EE+EK *vs.* KK	EE *vs.* EK+KK
E/E	21 (30.0)	255 (40.5)	0.078	0.066	0.116
E/K	31 (44.3)	273 (43.3)			
K/K	18 (25.7)	102 (16.2)			

all three inheritance models (additive, dominant, and recessive models) (Table 1). The allele frequencies of the minor A-allele at the E23 locus in the Korean patients and controls were also comparable with those previously reported in Chinese (0.39) and Caucasians (0.37).[12,13]

Clinical influence of E23K in patients with T1DM

In the patient group, we compared the clinical characteristics and biochemical values at diagnosis between the genotype subgroups in order to assess genotype-phenotype correlations. The genotype subgroups were classified as three inheritance models (additive, dominant, and recessive models).

However, clinical variables and biochemical data at diagnosis were not different between the three genotype subgroups of the T1DM patients (Table 2).

Discussion

The E23K (c.69G>A, rs5219) variant of *KCNJ11* results from a G-to-A transition in codon 23. Functional data have demonstrated that the E23K variant might alter T2DM susceptibility by increasing the threshold of ATP concentration necessary for insulin release, thus inducing the spontaneous overactivity of pancreatic β-cells.[5] Several analyses of the E23K variant in Caucasian populations have demonstrated that K/K homozygosity was more strongly associated with type 2 diabetes relative to E/K heterozygosity or E/E wild-type homozygosity.[14] Additionally, a significant reduction in estimates of serum insulin levels during an oral glucose tolerance test was also previously reported in a study of healthy individuals with E23K.[15]

On the other hand, no previous reports have confirmed Kir 6.2 E23K as a genetic risk factor for T1DM in Caucasian populations.[9,10] However, a few reports have already been carried out with other ethnic groups, including Asians. In this study, we investigated a consistently reported T2DM-associated polymorphism, E23K, with regard to its association with T1DM and its interaction with sex, age at diagnosis, biochemical profiles at diagnosis, and autoantibody status. However, the allele frequencies determined neither differed from those previously reported in other ethnic groups, nor was any evidence found suggesting an association between the development of T1DM and the presence of the E23K polymorphism in the *KCNJ11* gene.

Because we investigated just a single SNP in the known T2DM-associated loci for its association with T1DM in this study, it remains possible that some loci containing T1DM-associated variants do not affect T2DM. As known T2DM-associated SNPs explain less than 10% of genetic variation in terms of T2DM risk,[16] there may be some undiscovered T2DM-associated loci that are associated with T1DM. Moreover, considering that our T1DM patients were pediatric cases, we are currently unable to rule out the possibility that this T2DM-associated SNP has no effect on the development of adult-onset T1DM whatsoever.

Although our study has some limitations, most notably we studied only a single SNP locus and conducted the study with a rather small cohort, our results do appear to indicate that T2DM-susceptible genetic loci did not contribute to the risk of childhood-

Table 2. Clinical and biochemical characteristics of the type 1 diabetic patients classified according to genotypes of the *KCNJ11* gene

Variables	E/E (*n*=21)	E/K (*n*=31)	K/K (*n*=18)	*P* values		
				EE *vs.* EK *vs.* KK	EE+EK *vs.* KK	EE *vs.* EK+KK
Sex (male, %)	8 (38.10)	14 (45.16)	8 (44.44)	0.83	0.73	0.55
Age at diagnosis (y)	8.97 ± 3.50	8.48 ± 3.42	9.26 ± 4.06	0.75	0.56	0.82
Family history of T2DM (%)	7 (33.33)	11 (35.48)	6 (33.33)	0.99	1.00	1.00
History of DKA (%)	9 (42.86)	11 (35.48)	7 (38.89)	0.86	1.00	0.59
BMI-SDS	-0.71 ± 1.10	-0.12 ± 1.28	-0.19 ± 1.06	0.22	0.63	0.08
Fasting C-peptide (ng/mL)	0.67 ± 0.73	0.80 ± 0.87	1.06 ± 0.74	0.32	0.16	0.29
Hb A1C (%)	11.53 ± 2.30	11.10 ± 2.80	12.21 ± 2.40	0.37	0.20	0.98
Total cholesterol (mg/dL)	177.82 ± 49.20	182.32 ± 54.23	179.25 ± 48.68	0.96	0.93	0.82
Triglyceride (mg/dL)	126.73 ± 106.48	134.73 ± 109.46	160.33 ± 231.19	0.81	0.53	0.70
HDL-cholesterol (mg/dL)	53.27 ± 14.19	52.27 ± 16.11	50.67 ± 10.20	0.88	0.65	0.71
LDL-cholesterol (mg/dL)	97.29 ± 32.75	108.41 ± 57.28	100.47 ± 28.3	0.74	0.79	0.57
Islet cell Ab (%)	0 (0.00)	0 (0.00)	1 (5.56)	0.34	0.32	1.00
Insulin Ab (%)	3 (14.29)	6 (19.35)	2 (11.11)	0.47	0.47	1.00
GAD Ab (%)	13 (61.90)	10 (32.26)	11 (61.11)	0.12	0.75	0.20

T2DM: type 2 diabetes mellitus; DKA: diabetic ketoacidosis; BMI-SDS: body mass index - standard deviation score; Hb A1C: hemoglobin A1C; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GAD: glutamic acid decarboxylase; Ab: antibody.

onset T1DM. Therefore, consistent with the results of recent studies of T2DM loci in T1DM patients,[9,10,17] our results suggest that the association of the E23K polymorphism with Korean T1DM is not significant. Further large-scale studies will be necessary in the Korean population.

Funding: None.
Ethical approval: The Institutional Review Board of all three participating hospitals approved the study protocol, and written informed consent was obtained from all subjects or from their parents.
Competing interest: Nothing to declare.
Contributors: Ko JM wrote the first draft of this paper. Hwang IT and Hwang JS proposed the study and contributed equally to this study. All authors contributed to the intellectual content and approved the final version.

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Received May 9, 2011
Accepted after revision September 9, 2011