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Association of *KCNJ11* with impaired glucose regulation in essential hypertension

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ABSTRACT. *KCNJ11* is one of the candidate genes for type 2 diabetes, confirmed by genome wide association study, but there are little data on the relationship between *KCNJ11* and impaired glucose regulation in essential hypertension patients. To identify the effect of E23K and I337V in the *KCNJ11* gene on susceptibility to impaired glucose regulation, we conducted a case control study in 1125 essential hypertension patients with or without impaired glucose regulation among a Han Chinese population. We also evaluated the impact of two SNPs on insulin sensitivity and glucose tolerance estimated through an oral glucose tolerance test. In our case control study, no association

of E23K and I337V with impaired glucose regulation was found using any genotypic models. However, lysine carriers of E23K showed a significant association with decreased insulin (30 min) and Cederholm index, and valine carriers of I337V showed association with a lower Cederholm index. All the quantitative tests were performed by linear regression, with adjustment for gender, age, body mass index, blood pressure, and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker treatment. These findings provided evidence that the *KCNJ11* gene plays a role in the pathogenesis of decreased insulin sensitivity in essential hypertension patients.

Key words: *KCNJ11*; Insulin sensitivity; Glucose tolerance; Essential hypertensive

INTRODUCTION

Epidemiologic studies suggest that the prevalence of type 2 diabetes mellitus (T2D) in patients with essential hypertension (EH) is much higher than in the common population (Hackam et al., 2010), and the risk for stroke and fetal myocardial infarction would nearly double in the case of EH accompanying T2D (De Marco et al., 2009). Moreover, the normotensive offspring of EH parents demonstrate elevated fasting insulin level and lower insulin sensitivity (Vlasakova et al., 2004), which suggests that certain specific factors may be involved in the pathogenesis of T2D in EH patients. Therefore, clarifying the mechanism underlying glucose metabolism disturbance in EH patients would contribute to detecting EH patients at high risk of T2D and delaying the process of target organ damage.

Several lines of evidence suggest that rectifying potassium channel subunits (*KCNJ11*) serves as a highlight candidate gene for T2D. First, *KCNJ11* knockout mice show severe defects in glucose-induced insulin secretion (Seino et al., 2000). Second, rare loss-of-function mutations in *KCNJ11* cause decreased function of the K_{ATP} channels, leading to persistent hyperinsulinemic hypoglycemia of infancy in humans (Lin et al., 2008); on the contrary, activating mutations in this gene lead to permanent neonatal diabetes due to overactive K_{ATP} channels (Gloyn et al., 2004). Third, a common glutamate (E) to lysine (K) change at position 23 (E23K) has been consistently associated with T2D, which leads to modest reductions in ATP sensitivity and insulin secretion (Saxena et al., 2007). Although many replications have been performed to determine the relationship between the *KCNJ11* gene and T2D, there are still few studies that explore the association of the *KCNJ11* gene with impaired glucose regulation (IGR) in EH patients.

Accordingly, we developed the following hypothesis: 1) the *KCNJ11* gene may increase the susceptibility of IGR in EH patients, and 2) it may influence glucose metabolism and insulin sensitivity. Therefore, we performed a case-control study to determine the susceptibility of two common nonsynonymous single nucleotide polymorphisms (SNPs) in the *KCNJ11* gene, E23K and I337V, to IGR in EH patients among a Han Chinese population. Moreover, we also determined the concrete effect of these two SNPs on insulin sensitivity and glucose tolerance.

MATERIAL AND METHODS

Participants

All the participants were inpatients registered in the Division of Hypertension of Ruijin Hospital affiliated with Shanghai Jiao Tong University (age = 54.2 ± 11.6, 59.8% men) from January 2000 to October 2004. The hypertensive status was determined according to blood pressure >140/90 mmHg or taking antihypertensive medication. Oral glucose tolerance test (OGTT) was applied to estimate the status of normal glucose tolerance (NGT), impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and T2D based on American Diabetes Association (ADA) criteria, whereas the patients with IGT and/or IFG were merged to form the IGR group. The subjects who reported taking either insulin or oral antidiabetic were also defined as T2D. Considering that the treatment effect may confuse the results of quantitative studies, all the patients suffering from T2D were excluded. In addition, patients that had taken β-receptor blockers or thiazine diuretic in previous two weeks, due to their influence on glucose metabolism, were also excluded. Furthermore, the patients who could not perform OGTT were also excluded, such as those suffering from cancer, hyper/hypothyroidism, eso-/gastric resection and so on. Thus, 667 NGT and 458 IGR were enrolled, and an association study was performed among these two groups. To estimate the effect of the *KCNJ11* gene on glucose metabolism, the EH patients with NGT and IGR were included for the quantitative study separately. All individuals were of Han Chinese origin residing in the Shanghai metropolitan area and provided a written informed consent for donating blood samples for genetic analysis and related assays. This study was approved by the Ethics Committee of Ruijin Hospital.

Study parameters

OGTT was performed after overnight fasting with a standard load of 75 g glucose, and blood samples were collected after 0, 30, 60, 120, and 180 min. Plasma glucose was measured by the glucose oxidase reaction and serum insulin concentrations were determined by radioimmunoassay.

Homeostatic model assessment index β (HOMA-β) was used to estimate β-cell function by the formula of 20 x Ins0 / (Glu0-3.5) (Matthews et al., 1985). Insulin sensitivity was estimated using HOMA-IR (insulin resistance) and plasma glucose increment in the first 30 min (ΔPI(30) / ΔPG(30)) as Glu0 x Ins0 / 22.5 and (PI(30) - PI(0)) / (PG(30) - PG(0)), respectively. Areas under the curve for glucose (AUCg) and insulin (AUCi) were calculated by the formulas (PG(0) + PG(180)) / 2 + PG(30) + PG(60) + PG(120) and (PI(0) + PI(180)) / 2 + PI(30) + PI(60) + PI(120) (Ryder et al., 2003). Cederholm and Wibell (1990) index was calculated by (75 - 0.19 x body weight) / (PG(0) + PG(30) + PG(60) + PG(120) + PG(180) / 5) / Lg(PI(0) + PI(30) + PI(60) + PI(120) + PI(180) / 5). Here PG(0), PG(30), PG(60), PG(120), PG(180), PI(0), PI(30), PI(60), PI(120), and PI(180) represented the plasma glucose and insulin concentrations at 0, 30, 60, 120, and 180 min separately.

The ambulatory blood pressure readings were obtained every 20 min during daytime and 30 min at night by oscillometric technique (SpaceLabs 90202 and 90207, USA). Daytime

was defined as the interval from 6:00 am to 10:00 pm, and nighttime from 10:00 pm to 6:00 am. The success ratio for all the participants was over 80%. At the same time, the baseline index for age, height and weight was recorded.

SNP genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the standard phenol/chloroform procedure for genetic analysis. The genotypes of E23K (rs5219) and I337V (rs5215) in *KCNJ11* were determined by using the MassARRAY SNP genotyping system (Sequenom, San Diego, CA, USA) based on matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). Primers for polymerase chain reaction (PCR) amplification and subsequent extension reactions were designed by using the SpectroDESIGNER assay design software (Sequenom). The primer sequences were as follows: E23K, forward: 5'-CGTTGGATGAGGAATACGTGCTGACACGC-3', reverse: 5'-ACGTTGGATGTTTCTTGGACACAAAGCGGG-3', extension: 5'-GGCACGGTACCTGGGCT-3'; I337V, forward: 5'-ACGTTGGATGTGGACTACTCCAAGTTTGGC-3', reverse: 5'-ACGTTGGATGAGGCTGTGGTCCTCATCAAG-3', extension: 5'-TGGTGTGGGCACTTTGA-3'. PCR conditions and extensive protocol design are described elsewhere. Genotypes were automatically called by the SpectroTyper software (Sequenom), and each genotype was manually inspected and verified. Fifty quality control individuals were placed randomly throughout the plates and checked for accuracy. Direct sequencing was performed in 59 samples of two SNPs to confirm the accuracy of genotyping, and one mistake was found among 118 reactions.

Statistical analysis

Hardy-Weinberg equilibrium for genotypic distributions was tested by the chi-square (χ^2) goodness-of-fit test. Multinomial regression analyses were performed to estimate the association of genotypes with IGR in codominant, dominant and recessive models, respectively. The codominant model compared three genotype groups per SNP separately. In the dominant model, the homozygotes and heterozygotes for the minor allele were compared as a group with homozygotes for the major allele; while in the recessive model, the homozygotes and heterozygotes for the major allele were considered as a group. Linear regression analyses were performed to study the associations of insulin sensitivity and glucose tolerance with genotypic groups in NGT. The logistic and linear regression analyses were both adjusted for gender, age, body mass index (BMI), mean systolic blood pressure (MSBP), mean diastolic blood pressure (MDBP) and whether receiving angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEI/ARB) therapy or not. The data were analyzed using the SPSS statistical software (version 13.0; SPSS Inc., Chicago, IL, USA). To estimate haplotype frequencies in each group and test for the association with IGR, glucose tolerance and insulin sensitivity, we employed haplo.score and haplo.cc functions implemented in the Haplo.stats program (Schaid et al., 2002) developed in the R language (www.r-project.org). The additive value, which gives the change in expected trait value due to the allele relative to the reference allele, was estimated for associations between the biochemical index and SNPs tested by the

UNPHASED program (version 3.0.13) (http://homepages.lshtm.ac.uk/frankdudbridge/software/unphased/) (Dudbridge, 2003). The significance level was set at a P value of 0.05, and the power of the present sample was 0.92 with effect size set at 0.1.

RESULTS

Comparison of characteristics in NGT and IGR

Table 1 summarizes the demographic and clinical data of EH patients. Compared to EH patients with NGT, the IGR group had higher age, BMI, PG(0), PG(60), PG(120), PG(180), PI(0), PI(30), PI(60), PI(120), PI(180), AUCg, AUCi, and HOMA-IR, and lower ΔPI(30) / ΔPG(30) and Cederholm and Wibell (1990) index. No significant difference was found for gender, MSBP, MDBP, PG(30), and HOMA-β between the two groups.

Table 1. Demographic and clinical data of subjects.

	NGT	IGR	P
Gender (male/female)	396/271	277/181	0.378
Age (years)	53.2 ± 11.8	55.7 ± 11.2	**<0.001**
BMI (kg/m^2)	25.0 ± 3.4	26.3 ± 5.7	**<0.001**
MSBP (mmHg)	130 ± 14	131 ± 15	0.104
MDBP (mmHg)	82.1 ± 10.4	81.5 ± 11.2	0.402
PG0 (mM)	5.2 ± 0.5	5.8 ± 0.6	**<0.001**
PG30 (mM)	8.9 ± 1.5	10.3 ± 1.6	0.097
PG60 (mM)	8.6 ± 2.0	11.3 ± 1.9	**<0.001**
PG120 (mM)	6.0 ± 1.0	8.9 ± 1.1	**<0.001**
PG180 (mM)	4.5 ± 0.9	5.6 ± 1.3	**<0.001**
PI0 (mU/L)	7.9 ± 6.1	10.5 ± 10.2	**<0.001**
PI30 (mU/L)	74.6 ± 50.6	69.5 ± 42.4	**<0.001**
PI60 (mU/L)	89.7 ± 61.0	105.1 ± 61.1	**<0.001**
PI120 (mU/L)	49.2 ± 41.3	100.5 ± 69.2	**<0.001**
PI180 (mU/L)	14.1 ± 15.5	32.9 ± 29.6	**<0.001**
ΔPI30/ΔPG30 (mmol/mU)	17.8 ± 35.1	9.4 ± 26.6	**<0.001**
AUCg (mM)	26.6 ± 6.9	34.0 ± 8.0	**<0.001**
AUCi (mU/L)	204 ± 144	275 ± 254	**<0.001**
HOMA-β (U/mmol)	99.0 ± 92.9	87.31 ± 169	0.158
HOMA-IR (mmol·mU^{-1}·L^{-2})	1.9 ± 1.5	2.8 ± 2.7	**<0.001**
Cederholm and Wibell (1990) index	15.0 ± 2.4	12.8 ± 2.3	**<0.001**

Means ± SD for continuous variables. NGT = normal glucose tolerance; IGR = impaired glucose regulation; BMI = body mass index; MSBP = 24 h mean systolic blood pressure; MDBP = 24 h mean diastolic blood pressure; PG0, PG30, PG60, PG120, PG180, PI0, PI30, PI60, PI120, and PI180 represent the plasma glucose and insulin concentrations at 0, 30, 60, 120, and 180 min separately. AUCg = areas under the curve for glucose; AUCi = AUC for insulin; HOMA-β = homeostatic model assessment index β; HOMA-IR = HOMA insulin resistance. P values less than 0.05 are shown in bold.

Association of genotypes and haplotype with IGR

The χ^2 goodness-of-fit test showed that the genotypic distributions of E23K and I337V did not deviate from the Hardy-Weinberg equilibrium in NGT and IGR patients. In the case control study, no association of E23K and I337V was found with IGR using any genotypic models (Table 2). The two SNPs in a nearly complete linkage disequilibrium region (D' = 0.97, r^2 = 0.85), and the haplotypes of E23K-I337V also did not distribute differently between the two groups (data not shown).

Table 2. Association of E23K and I337V with impaired glucose regulation (IGR).

Variant	Gene	NGT (%)	IGR (%)	Codominant model OR (95%CI)	Dominant model OR (95%CI)	Recessive model OR (95%CI)
E23K	E/E	39.8	41.6	0.96 (0.78-1.18) P_{codom} = 0.698	0.94 (0.70-1.27) P_{dom} = 0.672	0.96 (0.65-1.43) P_{rec} = 0.848
	E/K	42.4	42.4			
	K/K	17.9	16.0			
I337V	I/I	36.6	37.9	0.95 (0.79-1.13) P_{codom} = 0.544	0.93 (0.71-1.22) P_{dom} = 0.601	0.92 (0.66-1.29) P_{rec} = 0.632
	I/V	43.9	44.1			
	V/V	19.5	18.0			

Data are reported as number of subjects with each genotype (% of each group). P values compare genotype distributions between normal glucose tolerance (NGT) and IGR subjects applying a codominant (P_{codom}), dominant (P_{dom}) or recessive (P_{rec}) logistic regression model with adjustment for age, gender, BMI, MSBP, DSBP, and ACEI/ARB treatment. OR = odds ratio; CI = confidence interval.

Association of genotypes and haplotypes with glucose metabolism

Although the SNPs in the *KCNJ11* gene showed no significant association with IGR, it is probably because they influence the metabolism of glucose and sensitivity of insulin in some quantitative trait pattern. Therefore, we compared all the indexes of serum glucose and insulin among the different genotypes of each SNP in all the genetic models in the EH patients with NGT and IGR separately. No significant association was detected in either SNP with any phenotype (data not shown). However, in the EH patients with NGT, E23K showed a nominal association with PG(120) in both the codominant (P_{codom} = 0.048) and recessive (P_{rec} = 0.028) model, with PI(0) (P_{dom} = 0.031), PI(60) (P_{dom} = 0.023) and AUCi (P_{dom} = 0.033) in the dominant model, with PI(30) (P_{codom} = 0.008; P_{dom} = 0.001), ΔPI(30) / ΔPG(30) (P_{codom} = 0.035; P_{dom} = 0.033) and with the Cederholm and Wibell (1990) index (P_{codom} = 0.016; P_{dom} = 0.002) in both the codominant and dominant models. In the case of I337V, it displayed an association with PG(30) in the codominant model (P_{codom} = 0.041), with PI(30) (P_{codom} = 0.046; P_{dom} = 0.017), ΔPI (30) / ΔPG(30) (P_{codom} = 0.038; P_{dom} = 0.016), AUCg (P_{codom} = 0.006; P_{dom} = 0.007), and with the Cederholm and Wibell (1990) index (P_{codom} = 0.004; P_{dom} = 0.003) in both codominant and dominant models. All the comparisons were performed by linear regressions with the adjustment for age, gender, BMI, MSBP, MDBP and whether receiving ACEI/ARB therapy (see Table 3). Since two SNPs and three genetic models were considered with two kinds of index (plasma glucose and insulin), the threshold for significant P value should be less than 0.004 by Bonferroni's corrections. Therefore, E23K still associated significantly with PI(30) and Cederholm and Wibell (1990) index, whereas the lysine carriers suffered lower PI(30) and Cederholm and Wibell (1990) index. In addition, the association of I337V with Cederholm and Wibell (1990) index remains significant after correction, in which the valine carriers demonstrated lower Cederholm and Wibell (1990) index.

Then, we compared all measurements of serum glucose and insulin in different E23K-I337V haplotypes. We found that the level of PI(60) was nominally different among three haplotypes (global P = 0.038; Table 4). Such difference was still significant after the adjustment for age, gender, BMI, MSBP, MDBP, and ACEI/ARB treatment. However, when the P value was adjusted for two kinds of metabolic phenotype, the difference was not significant (adjusted P = 0.076). Such evidence may indicate that E23K and I337V influence the glucose metabolism and insulin sensitivity through the single pattern rather than the combined model.

Table 3. Association of E23K and I337V with glucose tolerance and insulin sensitivity in normal glucose tolerance subjects with essential hypertension (EH).

E23K (rs5219)	E/E	E/K	K/K	P_{codom}	P_{dom}	P_{rec}
PG0 (mM)	5.3 ± 0.4	5.3 ± 0.5	5.2 ± 0.5	NS	NS	NS
PG30 (mM)	8.8 ± 1.5	8.9 ± 1.5	9.0 ± 1.5	NS	NS	NS
PG60 (mM)	8.4 ± 1.9	8.6 ± 2.1	8.6 ± 2.0	NS	NS	NS
PG120 (mM)	6.0 ± 1.0	6.0 ± 1.0	6.2 ± 0.9	0.048	NS	0.028
PG180 (mM)	4.5 ± 0.8	4.5 ± 1.0	4.7 ± 1.1	NS	NS	NS
PI0 (mU/L)	8.9 ± 7.1	7.5 ± 5.3	7.9 ± 6.3	NS	0.031	NS
PI30 (mU/L)	86.7 ± 56.5	69.6 ± 47.1	71.0 ± 54.3	0.008	0.001	NS
PI60 (mU/L)	99.3 ± 64.4	80.4 ± 52.9	93. 9 ± 74.4	NS	0.023	NS
PI120 (mU/L)	53.4 ± 45.1	44.6 ± 35.0	54.2 ± 50.8	NS	NS	NS
PI180 (mU/L)	15.7 ± 15.7	13.2 ± 12.4	15.2 ± 23.7	NS	NS	NS
ΔPI30/ΔPG30 (mmol/mU)	23.2 ± 56.0	15.7 ± 19.5	14.7 ± 9.6	0.035	0.033	NS
AUCg (mM)	25.9 ± 7.2	26.5 ± 7.3	26.6 ± 7.4	NS	NS	NS
AUCi (mU/L)	224 ± 160	187 ± 125	201 ± 176	NS	0.048	NS
HOMA-IR (mmol·mU^{-1}·L^{-2})	2.1 ± 1.7	1.8 ± 1.2	1.9 ± 1.6	NS	0.033	NS
HOMA-β (U/mmol)	108 ± 96	92.9 ± 97.4	103 ± 110	NS	NS	NS
Cederholm and Wibell (1990) index	15.5 ± 2.6	14.7 ± 2.4	14.9 ± 2.6	0.016	0.002	NS
I337V (rs5215)	I/I	V/I	V/V	P_{codom}	P_{dom}	P_{rec}
PG0 (mM)	5.2 ± 0.4	5.2 ± 0.5	5.2 ± 0.5	NS	NS	NS
PG30 (mM)	8.8 ± 1.5	8.9 ± 1.5	9.1 ± 1.5	0.041	NS	NS
PG60 (mM)	8.4 ± 1.8	8.6 ± 2.1	8.8 ± 2.0	NS	NS	NS
PG120 (mM)	6.0 ± 1.0	6.0 ± 1.0	6.2 ± 0.9	NS	NS	NS
PG180 (mM)	4.4 ± 0.8	4.5 ± 1.0	4.6 ± 1.0	NS	NS	NS
PI0 (mU/L)	8.3 ± 6.8	7.6 ± 5.2	7.8 ± 6.7	NS	NS	NS
PI30 (mU/L)	81.4 ± 54.8	71.6 ± 47.4	69.8 ± 50.1	0.046	0.017	NS
PI60 (mU/L)	95.6 ± 65.2	83.9 ± 53.6	90.8 ± 67.2	NS	NS	NS
PI120 (mU/L)	52.3 ± 44.9	46.3 ± 36.4	49.4 ± 44.4	NS	NS	NS
PI180 (mU/L)	15.2 ± 15.6	13.1 ± 12.5	14.2 ± 20.8	NS	NS	NS
ΔPI30/ΔPG30 (mmol/mU)	22.9 ± 52.9	14.9 ± 18.0	15.7 ± 21.0	0.038	0.016	NS
AUCg (mM)	25.8 ± 7.1	27.1 ± 6.5	27.1 ± 7.0	0.006	0.007	NS
AUCi (mU/L)	215 ± 155	197 ± 129	199 ± 158	NS	NS	NS
HOMA-IR (mmol·mU^{-1}·L^{-2})	2.0 ± 1.6	1.8 ± 1.2	1.8 ± 1.7	NS	NS	NS
HOMA-β (U/mmol)	102 ± 90.5	96.3 ± 93.3	99.0 ± 99.3	NS	NS	NS
Cederholm and Wibell (1990) index	15.4 ± 2.4	14.7 ± 2.4	14.6 ± 2.5	0.004	0.003	NS

Data are reported as means ± SD. P values compare measurements among EH patients with different genotypes applying a codominant (P_{codom}), dominant (P_{dom}) or recessive (P_{rec}) linear regression model with adjustment for age, gender, BMI, MSBP, MDBP, and ACEI/ARB treatment. NS = nonsignificant. For abbreviations, see legend to Table 1.

Table 4. Association of haplotypes with plasma insulin concentration at 60 min (PI60).

Haplotype	Frequency	Addval	Hap-Score	Individual P	Global P
E-I	0.58	0.0	1.51	0.131	0.038
E-V	0.03	0.002	2.08	0.038	
K-V	0.38	-0.002	-2.10	0.036	

Data are reported as frequency of each haplotype (E23K and I337V). P values were adjusted for age, gender, BMI, MSBP, MDBP, and ACEI/ARB treatment. Haplotype E-I was chosen as the reference. Addval = the change in expected trait value due to this haplotype, relative to the reference value.

DISCUSSION

In the present study, we explored the relationship between *KCNJ11* and IGR in EH patients of Han Chinese origin for the first time. Although no meaningful association was found in the case control study, the quantitative trait analysis revealed a significant difference in glucose metabolism and insulin sensitivity among the diverse genotype carriers in both E23K

and I337V. Such evidence suggested that *KCNJ11* may contribute to the process of glucose metabolism disturbance in EH patients.

KCNJ11 E23K was one of the highlight candidate loci for T2D, which has been verified by a number of studies. In the genome wide association studies of T2D involving a variety of international consortia, Zeggini et al. (2007) and Scott et al. (2007) confirmed an association of E23K polymorphism with T2D susceptibility. In 2008, Vaxillaire et al. analyzed 19 common polymorphisms of 14 known candidate genes for their contribution to prevalence and incidence of glucose intolerance in the DESIR prospective study of 3877 middle-aged Caucasian subjects, and found that E23K may predict the incidence of T2D in the DESIR cohort. On the contrary, Florez et al. (2007) found that lysine carriers were less likely to develop diabetes than E/E homozygotes, in spite of an association with reduced insulin secretion at baseline. In the present study, we detected no significant association of *KCNJ11* E23K with IGR in EH, which was consistent with the results detected in a 2834-subject Japanese cohort (Yokoi et al., 2006). The divergence among different studies may be explained by the small sample size and different ethnic background. In addition, the distinct accompanying condition may also influence the incidence of IGR or T2D. Here, we focused on IGR in EH patients, and no significant difference was detected. It may be caused by the accelerating effect of EH on IGR progression toward T2D, which lessened the difference between NGT and IGR groups. Therefore, T2D patients should be discussed in a future study and the specific underlying mechanism needs further verification by prospective studies on EH patients.

Moreover, we found that normoglycemic E23K lysine carriers display a defect in insulin secretion, which was in accordance with the results from previous studies. Nielsen et al. (2003) compared 803 T2D patients and 862 NGT subjects in a Danish population, and associated E23K with significant reductions in the insulinogenic index and AUCi. In 2009, Villareal et al. assessed insulin secretion among nine subjects with the K/K genotype and nine matched subjects with the E/E genotype, and the results revealed that insulin secretory responses to oral and intravenous glucose were reduced by about 40% in glucose tolerant subjects with lysine homozygous for E23K. Here, we did not find a significant variation of AUCi according the different genotypes; however, the lysine carriers displayed significantly lower PI(30) and Cederholm and Wibell (1990) index after adjustment. This suggests that the K allele may affect insulin secretion in the early phase and insulin sensitivity rather than the total amount of insulin stimulated by OGTT in the EH patients.

I337V was another common missense variant in *KCNJ11*, which has received much less attention than E23K. We found that valine carriers of I337V showed lower Cederholm and Wibell (1990) index after adjustment. In addition, there was no metabolism index showing an association with E23K-I337V haplotypes, which indicated that E23K and I337V may influence the glucose metabolism and insulin sensitivity through the single pattern rather than the combined model.

CONCLUSION

We found significant association of E23K and I337V in the *KCNJ11* gene with poor insulin secretion and impairment of insulin sensitivity. Large-scale and prospective cohort studies on a variety of ethnic groups are suggested to elucidate the relationship between these SNPs and IGT in EH. In addition, whether I337V polymorphism is the functional variant responsible for abnormal insulin secretion still needs further verification.

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REFERENCES

Cederholm J and Wibell L (1990). Insulin release and peripheral sensitivity at the oral glucose tolerance test. *Diabetes Res. Clin. Pract.* 10: 167-175.

De Marco M, de Simone G, Roman MJ, Chinali M, et al. (2009). Cardiovascular and metabolic predictors of progression of prehypertension into hypertension: the strong heart study. *Hypertension* 54: 974-980.

Dudbridge F (2003). Pedigree disequilibrium tests for multilocus haplotypes. *Genet. Epidemiol.* 25: 115-121.

Florez JC, Jablonski KA, Kahn SE, Franks PW, et al. (2007). Type 2 diabetes-associated missense polymorphisms KCNJ11 E23K and ABCC8 A1369S influence progression to diabetes and response to interventions in the Diabetes Prevention Program. *Diabetes* 56: 531-536.

Gloyn AL, Pearson ER, Antcliff JF, Proks P, et al. (2004). Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N. Engl. J. Med.* 350: 1838-1849.

Hackam DG, Khan NA, Hemmelgarn BR, Rabkin SW, et al. (2010). The 2010 Canadian Hypertension Education Program recommendations for the management of hypertension: part 2 - therapy. *Can. J. Cardiol.* 26: 249-258.

Lin YW, Bushman JD, Yan FF, Haidar S, et al. (2008). Destabilization of ATP-sensitive potassium channel activity by novel KCNJ11 mutations identified in congenital hyperinsulinism. *J. Biol. Chem.* 283: 9146-9156.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, et al. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412-419.

Nielsen EM, Hansen L, Carstensen B, Echwald SM, et al. (2003). The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* 52: 573-577.

Ryder E, Gomez ME, Fernandez V, Campos G, et al. (2003). Presence of impaired insulin secretion and insulin resistance in normoglycemic male subjects with family history of type 2 diabetes. *Diabetes Res. Clin. Pract.* 60: 95-103.

Saxena R, Voight BF, Lyssenko V, Burtt NP, et al. (2007). Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316: 1331-1336.

Schaid DJ, Rowland CM, Tines DE, Jacobson RM, et al. (2002). Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am. J. Hum. Genet.* 70: 425-434.

Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, et al. (2007). A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316: 1341-1345.

Seino S, Iwanaga T, Nagashima K and Miki T (2000). Diverse roles of K(ATP) channels learned from Kir6.2 genetically engineered mice. *Diabetes* 49: 311-318.

Vaxillaire M, Veslot J, Dina C, Proenca C, et al. (2008). Impact of common type 2 diabetes risk polymorphisms in the DESIR prospective study. *Diabetes* 57: 244-254.

Villareal DT, Koster JC, Robertson H, Akrouh A, et al. (2009). Kir6.2 variant E23K increases ATP-sensitive K+ channel activity and is associated with impaired insulin release and enhanced insulin sensitivity in adults with normal glucose tolerance. *Diabetes* 58: 1869-1878.

Vlasakova Z, Pelikanova T, Karasova L and Skibova J (2004). Insulin secretion, sensitivity, and metabolic profile of young healthy offspring of hypertensive parents. *Metabolism* 53: 469-475.

Yokoi N, Kanamori M, Horikawa Y, Takeda J, et al. (2006). Association studies of variants in the genes involved in pancreatic beta-cell function in type 2 diabetes in Japanese subjects. *Diabetes* 55: 2379-2386.

Zeggini E, Weedon MN, Lindgren CM, Frayling TM, et al. (2007). Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316: 1336-1341.