Kir6.2 E23K polymorphism is related to secondary failure of sulfonylureas in non-obese patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: The Kir6.2 E23K polymorphism was studied with a special reference to secondary sulfonylurea (SU) failure in non-obese patients with type 2 diabetes.

Materials and Methods: We recruited 278 non-obese (body mass index ≤30.0 kg/m²) Japanese patients with type 2 diabetes who had a history of SU treatment (for 11.2–6.3 years) and compared the frequency of the secondary SU failure among the genotypes of the polymorphism. Genotyping of the Kir6.2 E23K was carried out by polymerase chain reaction-restriction fragment length polymorphism.

Results: The genotype frequencies of the polymorphism were similar to those previously reported in Japanese patients with type 2 diabetes. The frequency with which patients deteriorated into secondary SU failure was significantly higher in those with the KK genotype than those with EE or EK genotypes. Among 214 patients who eventually received insulin therapy because of secondary SU failure, the period of SU treatment in those with the KK genotype was significantly shorter than those with the EE or EK genotype, although the period from diagnosis to the start of SU treatment was not significantly different.

Conclusions: These data suggest that the Kir6.2 E23K polymorphism is related to the acceleration of secondary SU failure in non-obese Japanese patients with type 2 diabetes. (J Diabetes Invest, doi: 10.1111/jdi.12070, 2013)

KEY WORDS: Kir6.2 E23K polymorphism, Secondary failure of sulfonylurea, Type 2 diabetes

INTRODUCTION

The adenosine triphosphate (ATP)-sensitive K⁺ channel (KATP channel) in pancreatic β-cells is a heterooctameric protein complex composed of sulfonylurea receptor (SUR1) subunits and inwardly rectifying K⁺ channel (Kir6.2) subunits. As the Kir6.2 mutation is reported to cause neonatal diabetes, the findings provide new insight into the structure and function of the Kir6.2 subunit of the KATP channel. A meta-analysis study in French Caucasians first suggested that the single nucleotide polymorphisms (SNPs) at codon 23 (E23K) in Kir6.2 (encoded by KCNJ11, rs5219) were associated with type 2 diabetes mellitus, and a recent large-scale meta-analysis confirmed that the E23K variant increased the risk of type 2 diabetes. There are also some reports regarding the association of this polymorphism with type 2 diabetes in the Japanese population.

Because of its unique composition, Kir6.2 is also thought to be associated with impairment of SU-stimulated insulin secretion in patients with type 2 diabetes. Glycemic control in patients treated with SU agents gradually becomes poor, known as secondary failure to SU, and patients’ treatment needs to be converted to insulin therapy. The decline of the β-cell function as a result of longtime overstimulation is thought to be one of the causative mechanisms for secondary SU failure; however, the genetic basis for this is unknown, and long-term clinical observation of the β-cell decline in combination with secondary SU failure is rare. Therefore, we studied the Kir6.2 E23K gene polymorphism with a special reference to SU treatment in non-obese Japanese patients with type 2 diabetes using a long-term clinical observation.

METHODS

Participants

We recruited 485 (287 men) unrelated Japanese patients with type 2 diabetes (type 2 diabetes group) from the diabetes outpatient clinic of Wakayama Medical University Hospital in Wakayama, Japan. In order to study the type 2 diabetic patients as uniformly as possible, patient ages were limited to between 26 and 59 years in order to eliminate early onset diabetes, such as, maturity onset-type diabetes of the young and those diagnosed at an advanced age. Patients with a body mass index (BMI) equal to or more than 30 kg/m² were also
excluded. Genomic DNA was subjected to the genotyping of the \( \text{Kir}6.2 \) E23K polymorphism. Among these, patients who had a history of SU treatment (for 11.2 ± 6.3 years) and whose treatments had been followed at the outpatient clinic for more than 10 years (22.9 ± 8.8; \( n = 278 \), SU group) were used to study the frequency of secondary failure of the SU. Among the SU group, patients who eventually received insulin treatment as a result of secondary failure of SU (SUF group, \( n = 214 \)) were selected to compare the time period of the treatments; the remaining 64 patients who did not deteriorate into insulin therapy were classified as the sulfonylurea contine (SUC) group. Genomic DNA of the SUF group was also subjected to the genotyping of \( c/g \) SNP of \( \text{CDKAL1} \) (rs7754840). Clinical characteristics of the type 2 diabetes, the SU, the SUF and the SUC groups at the final point during the observation period are shown in the upper portion of Table 1.

Treatment regimen was as follows. First, standard treatment to correct excessive consumption and lack of activity were initiated after diagnosis. Some patients were treated with metformin 500–750 mg/day. Next, they were additionally treated with SU to obtain a glycated hemoglobin (HbA1c) level <6.9%. Secondary SU failure was clinically determined when HbA1c was higher than 9.4% for more than 6 months or 8.4% for more than 12 months under treatment with glibenclamide 6 mg/day or a submaximal dose of glibenclamide (equal or more than 5.0 mg/day), at which time insulin therapy was initiated. Before starting the insulin therapy, anti-glutamic acid decarboxylase (GAD) antibodies were measured by the radioimmunoassay using GADAb Cosmic (Cosmic Corporation, Tokyo, Japan; cut off line <1.4 U/mL) in order to detect slowly progressive type 1 diabetes; patients carrying the antibodies were excluded. None of the patients were treated with thiazolidinediones, glinides, \( \alpha \)-glucosidase inhibitors, dipeptidyl peptidase IV inhibitors or glucagon-like peptide-1 receptor agonists. During the entire study period, all patients were instructed to follow a standard diet (25–35 kcal/kg per standard bodyweight) as recommended by the Japan Diabetes Society. The recommended energy intake was readjusted at every visit to the hospital to account for any weight lost during the preceding period. Patients received dietary counseling every 2 weeks for the first 3 months and at every visit thereafter. The patients visited our hospital at least every 2–3 months throughout the study.

**Typing for \( \text{Kir}6.2 \) E23K Polymorphism and \( c/g \) SNP of \( \text{CDKAL1} \)**

Genomic DNA was extracted from peripheral leukocytes of the participants using a QIAamp DNA Blood Kit (QIAGEN, Tokyo, Japan). Genotyping of the \( \text{Kir}6.2 \) E23K variant was carried out by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method with Ban II restriction enzyme (New England Biolabs, Beverly, MA, USA) as previously described. The genotyping was carried out with duplicated samples, and when the RFLP needed to be confirmed, direct sequencing was carried out using ABI prism 310 (PE Biosystems, Tokyo, Japan). Genotyping of the \( c/g \) SNP of \( \text{CDKAL1} \) was carried out by direct sequencing in the SUF group (\( n = 214 \)).

C-peptide was measured by immunoenzymometric assay (ST-E test Tosoh II C-peptide; Tosoh, Tokyo, Japan). The within-run and day-to-day precisions (coefficients of variation) were 1.3–2.2% and 3.1–3.8%, respectively. The assay sensitivity was 0.017 nmol/L. The value for HbA1c (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the following formula, HbA1c = 1.02 HbA1c (Japan Diabetes Society [JDS]%) + 0.25%, which expresses the relationship between HbA1c (JDS%) measured with the Japanese reference system, and HbA1c measured by with NGSP (NGSP%).

Informed consent was obtained from all participants. The study was approved by the Ethical Committee of Wakayama Medical University and was in accordance with the principle of the Declaration of Helsinki.

**Table 1 | Clinical characteristics at the final point and genotype frequencies of the \( \text{Kir}6.2 \) E/K polymorphism of the patients**

| Group  | Type 2 diabetes | SU  | SUF  | SUC  |
|--------|----------------|-----|------|------|
| N      | 485            | 278 | 214  | 64   |
| Male   | 287            | 146 | 118  | 28   |
| Age at diagnosis (years) | 440 ± 85 | 43.2 ± 8.6 | 46.5 ± 7.7 |
| Duration (years)         | 229 ± 8.8 | 23.2 ± 9.4 | 21.8 ± 6.2 |
| Body mass index (kg/m²)  | 23.6 ± 3.3 | 23.8 ± 3.5 | 23.0 ± 2.7 |
| Period for SU (years)    | 11.2 ± 6.3 | 10.6 ± 6.1 | 13.3 ± 6.7 |
| \( \text{Kir}6.2 \) polymorphism | | | |
| EE (%)  | 185 (38.1%)    | 101 (36.3%) | 80 (37.4%) | 21 (32.8%) |
| EK (%)  | 228 (47.0%)    | 135 (48.6%) | 100 (46.7%) | 35 (54.7%) |
| KK (%)  | 72 (14.8%)     | 42 (15.1%)  | 34 (15.9%)  | 8 (12.5%)  |

The sulfonylurea (SU) group: among the type 2 diabetes group, patients who had a history of SU treatment and were followed for more than 10 years. SUF group: among the SU group, patients who deteriorated into insulin treatment as a result of secondary failure of SU. SUC group: among the SU group, patients who did not deteriorate into insulin treatment.
Statistical Analysis
Data is shown as mean ± standard deviation. Categorical variables were compared by \(\chi^2\)-test. Differences in continuous variables between two groups were analyzed by Student’s or Welch’s \(t\)-test, and those among three groups by Scheffe’s multiple comparison test. The rate of change of patients to insulin therapy from SU treatment, and the rate of change of patients to SU treatment from standard treatment were compared by the Kaplan–Meier method. A \(P\)-value < 0.05 was considered to be statistically significant.

RESULTS
Genotype Frequencies of the Kir6.2 E23K Variant
Frequencies of the genotype of the Kir6.2 E23K polymorphism in the type 2 diabetes, the SU, the SUF and the SUC groups are summarized in the lower portion of Table 1. The genotype frequencies in these groups were similar to those in patients with type 2 diabetes previously reported in Caucasian populations\(^8,16\) and other Japanese populations\(^24,25\). The KK genotype frequency in the SUC group was slightly lower compared with that in the SUF group, although not significantly different. The genotyping was concomitant with Hardy–Weinberg equilibrium.

Therapeutic Changes Among the Kir6.2 E23K Variant
We analyzed the therapeutic changes among the Kir6.2 E23K variants evaluated with the Kaplan–Meier method (Figure 1 for standard treatment to SU and Figure 2 for SU to insulin). The KK carriers were started on SU therapy within the same timeframe as other genotype carriers (EE or EK) on the diagnosis of diabetes (Figure 1). However, the KK carriers started insulin treatment significantly earlier (log–rank test \(P = 0.0043\)) after the start of the SU treatment than the others (EE or EK carriers; Figure 2). The sex and BMI adjusted Cox hazard ratio was 1.712 (95% confidence interval 1.18–2.48, \(P = 0.0046\)).

Fasting serum C-peptide levels in the patients of the SU group were compared between KK carriers and the others (EE or EK). Fasting serum samples were obtained after starting the SU treatment. The patients with renal dysfunction (serum creatinine >97.2 \(\mu\)mol/L) or those carrying anti-insulin antibodies in the patients treated with insulin were excluded. Although the duration of diabetes and BMI were not different between the groups, the fasting C-peptide level was significantly lower in the KK carriers (Table 3).

In order to compare the time period of the treatment, the patients with type 2 diabetes who eventually received insulin treatment because of secondary failure of SU (SUF group, \(n = 214\)) were selected, and their periods of standard treatment and those of SU treatment were compared among the E23K genotypes, respectively (Table 2). The time period of SU treatment of the KK genotype was significantly shorter than that of the EE or EK genotype, although the periods of the standard treatment were not statistically different. Multiple regression analysis showed that the E23K polymorphism (scoring: 0 for

![Image](73x151 to 283x302)

**Figure 1** | Analysis of therapeutic changes (standard treatment to secondary sulfonylurea [SU]) in the Kir6.2 E23K variants in patients with type 2 diabetes evaluated by the Kaplan–Meier method. The KK and the other (EE or EK) genotype carriers are shown as a thick solid line and dotted line, respectively.

**Table 2** | Comparison of fasting C-peptide levels between Kir6.2 E23K variants in sulfonylurea group

| Kir6.2 E23K variant | EE/EK | KK | Significance |
|--------------------|-------|----|-------------|
| \(N\) | 226 | 38 | |
| Age (years) | 61.7 ± 10.4 | 61.5 ± 8.9 | NS |
| Duration (years) | 17.9 ± 10.6 | 17.0 ± 8.0 | NS |
| Body mass index (kg/m\(^2\)) | 23.8 ± 3.2 | 23.5 ± 2.5 | NS |
| HbA1c (%) | 8.1 ± 1.3 | 7.9 ± 1.1 | NS |
| Fasting C-peptide (nmol/L) | 0.65 ± 0.33 | 0.50 ± 0.20 | \(P < 0.001\) |

HbA1c, glycated hemoglobin; NS, not significant.
EE or EK genotype, 1 for KK genotype) significantly correlated with the period of SU treatment (standardized regression coefficient = -0.2047, P = 0.002) when adjusted by sex, age at diagnosis and BMI as the modulators. In contrast, neither the period of standard treatment nor that of SU treatment had a significant difference among the three genotypes of the CDKAL1 SNP (Table 3).

**Table 3 | Comparison of time period of each treatment among genotypes of Kir6.2 E/K polymorphism, CDKAL1 c/g single nucleotide polymorphism in the group of patients who deteriorated into insulin treatment as a result of secondary failure of sulfonylurea**

| Kir6.2 E/K polymorphism | EE | EK | KK | Significance |
|-------------------------|----|----|----|--------------|
| n (%)                   | 80 | 100| 34 | NS           |
| LS period (years)       | 4.8±5.2 | 4.6±5.5 | 4.6±5.2 | NS           |
| SU period (years)       | 11.1±6.1* | 11.2±6.3* | 7.7±4.6# | *P = 0.015 |

| CDKAL1 c/g SNP | cc | cg | gg | Significance |
|---------------|----|----|----|--------------|
| n (%)         | 56 | 90 | 68 | NS           |
| LS period (years) | 3.9±4.9 | 4.9±5.7 | 60±6.2 | NS           |
| SU period (years) | 11.1±6.2 | 10.5±6.6 | 10.6±5.8 | NS           |

Lifestyle (LS) period: time period in which the patient received only standard treatment for life style improvement before beginning the sulfonylureas treatment. Sulfonylurea (SU) period: time period of treatment with sulfonylureas before the beginning of insulin treatment.

**DISCUSSION**

Previous studies showed that analysis in a recessive model (KK vs EK/EE) of the Kir6.2 polymorphism showed a significant association of the KK genotype with type 2 diabetes, we thus carried out Kaplan–Meier analysis to examine the frequency of secondary failure of SU as a recessive model using type 2 diabetic patients treated with SU (SU group). As shown in Figure 2, the patients with the KK genotype deteriorated into insulin therapy significantly earlier compared with the other genotypes (EE or EK). In response to this finding, fasting serum C-peptide level was significantly lower in the patients with the KK genotype than those with others, although it did not really support the accelerating effect of the KK genotype. In order to compare the time period of SU treatment before beginning insulin therapy, patients who finally received insulin treatment as a result of secondary failure of the SU (SU group, n = 214) were selected from the SU group. As shown in Table 3, the time period of SU treatment of the KK genotype was significantly shorter than that of the EE or EK genotype, respectively, although the periods of standard treatment in these groups were not statistically different. The SNP of CDKAL1 has been reported to be a strong candidate for type 2 diabetes susceptibility, together with Kir6.2 (KCNJ11), in the Japanese population. We thus compared the time period of standard treatment and SU treatment among the genotypes of the CDKAL1 SNPs in the SUF group. As shown in Table 3, there was no significant difference in these time periods among the genotypes of the SNP, respectively. The SNP (rs7903146) of TCF7L2 has also been reported to be a candidate for type 2 diabetes susceptibility. We also carried out the genotyping of the TCF7L2 c/t SNP by a duplex real-time PCR for LightCycler instrument (Roche Diagnostics, Mannheim, Germany) based on the hybridization probe format in 129 patients of the SUF group (cc: 89.9%, ct: 10.1% and tt: 0%), and compared each treatment period between the cc and the ct genotype. As same as the results of CDKAL1, neither the period of standard treatment (cc: 4.9±5.6 years, ct: 5.2±4.2 years, not significant) nor that of SU treatment (cc: 10.6±6.2 years, ct: 11.2±6.0 years, not significant) had significant difference. These data strongly suggest that the unique association of the Kir6.2 E23K polymorphism with the period of SU treatment.

Secondary SU failure is a very serious issue in the treatment of patients with type 2 diabetes. However its definition is still obscure. The target level of HbA1c for reconsideration of the present treatment was recommended as 8.4% by the JDS. We, thus, tentatively defined secondary SU failure as continuation of HbA1c level of above 8.4% for 12 months or above 9.4% for 6 months under treatment with a submaximal dose of the SU. No significant difference of the SU period among the genotypes of two SNPs other than Kir6.2 also supports the position that our treatment policy is acceptable.

There is a possibility that KK carriers have smaller β-cell mass left at the start of the SU treatment. However, our findings, which show that the period of standard treatment was not significantly different among the E/K genotypes refute the aforementioned possibility.

A recent ex vivo insulin secretion study using human islets showed that the glibenclamide-stimulated insulin secretion was selectively impaired in the islets obtained from K allele donors without perturbation of the glucose-stimulated insulin secretion. These data support our notion that the E23K polymorphism might be associated with decreased insulin secretory capacity, and is related to the secondary failure to SU agents, although the precise mechanism is not known at present and further examination will be required.

Recent genome-wide association studies carried out among several populations, including Japanese, identified multiple susceptible variants associated with an increased risk of type 2 diabetes. However, it is still unclear whether these variants are causative for type 2 diabetes, and the role of these variants in the clinical setting of SU treatment remains to be elucidated.
diabetes. However, there have been no reports regarding a gene or a SNP that affects the clinical course of patients with type 2 diabetes after diagnosis, such as secondary failure of SU by a long-term observation study. Taking these into consideration, our results prove valid and the marker will provide options for choosing the treatment of patients with type 2 diabetes.

In conclusion, we have shown the E23K polymorphism in Kir6.2 is related to the acceleration of secondary SU failure in the course of treatment for non-obese Japanese patients with type 2 diabetes.

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The authors declare no conflicts of interest.

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