Variation in KCNQ1 is associated with therapeutic response to sulphonylureas

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Summary

Background: We aimed to analyse quantitative effects of treatment with sulphonylurea in addition to metformin on parameters of glycemic control in relation to KCNQ1 genotypes, and to identify factors predictive for the response to sulphonylurea treatment.

Material/Methods: Effect of 6-month sulphonylurea therapy in addition to metformin on glycemic control according to KCNQ1 genotypes was evaluated in 87 patients with type 2 diabetes who failed to achieve glycemic control on metformin monotherapy. KCNQ1 rs163184 (T>G) polymorphism was determined by real-time PCR with melting analysis of unlabeled probe.

Results: The reduction in fasting plasma glucose (ΔFPG) after 6-month sulphonylurea therapy significantly differed among 3 KCNQ1 genotype groups (ANOVA, p=0.017). In a recessive genetic model, carriers of the T-allele (TT+TG) achieved significantly lower FPG levels in comparison with patients with the GG genotype (6.95±0.13 vs. 7.50±0.21 mmol/L, p=0.033). Consequently, ΔFPG was significantly higher in the TT+TG group compared to the GG group (1.58±0.13 vs. 1.04±0.18 mmol/L, p=0.016). In multiple linear regression analysis KCNQ1 genotype (p=0.016) and baseline FPG (p<0.001) were the only significant independent predictors of ΔFPG (R²=0.48).

Conclusions: Our results suggest that the magnitude of FPG reduction after 6-month sulphonylurea treatment in addition to metformin in patients with type 2 diabetes is related to the variation in KCNQ1. The FPG response to sulphonylureas was significantly lower in carriers of the risk GG genotype.

key words: pharmacogenetics • sulphonylureas • KCNQ1 • glycemic control • type 2 diabetes

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BACKGROUND

Type 2 diabetes is a disease with significant genetic predisposition. In recent years almost 40 genes associated with type 2 diabetes have been identified by genome-wide association studies [1]. Among them, 2 independent genome-wide studies in East Asian populations reported that several polymorphisms of potassium voltage-gated channel KQT-like subfamily member 1 (KCNQ1) gene were consistently associated with type 2 diabetes [2,3]. This association was subsequently replicated in several population cohorts of Asian and European ancestry [4–7].

KCNQ1 gene encodes the pore-forming subunit of a voltage-gated K+ channel (KvLQT1) that plays a key role in the repolarization of the cardiac action potential, as well as water and salt transport in epithelial tissues [8,9]. Both human and animal studies suggest that mutations in KCNQ1 can result in the K+ channel dysfunction and cause the hereditary long QT syndrome and familial atrial fibrillation [10,11]. KCNQ1 is also expressed in pancreatic islets and insulin-secreting cell lines [2,3,12]. Several studies indicated that various KCNQ1 polymorphisms were related either to impaired insulin secretion [6,7,13–15] or to impaired incretin secretion [15].

The recommended initial therapeutic interventions in type 2 diabetes include lifestyle changes and pharmacotherapy with metformin [16]. In patients with metformin monotherapy failure, sulphonylureas are frequently used as a second-line treatment. Sulphonylureas act as insulin secretagogues through the stimulation of insulin secretion via the sulphonylurea receptor 1 in pancreatic β-cells [17]. Considerable interindividual variation in the hypoglycaemic response to sulphonylureas likely reflects variations in the β-cell secretory reserve, and may relate to variations in genes involved in regulating β-cell function [18–21].

Since genetic variation in KCNQ1 is associated with fasting glucose and β-cell function [13], we hypothesised that the magnitude of sulphonylurea treatment effect might be related to the KCNQ1 genotype. Therefore, the aim of the present pharmacogenetic pilot study was to analyse quantitative effects of treatment with sulphonylurea in addition to metformin on parameters of glycaemic control with respect to KCNQ1 genotypes in patients with type 2 diabetes.

MATERIAL AND METHODS

Patients

Patients with type 2 diabetes diagnosed according to the American Diabetes Association criteria [22] recruited from 3 outpatient clinics participated in the study, which was conducted in a university hospital setting. Patients were eligible for the study if they were on previous metformin monotherapy for at least 6 months, and failed to maintain HbA1c <7.0% on maximal tolerated doses of metformin at 2 consecutive visits within a 3-month period. Inclusion criteria were HbA1c of 7.0–11.0%, fasting glycaemia of 6–15 mmol/L, age 35–70 years, and BMI 20–35 kg/m². Patients with malignancies, hypothyroidism, chronic renal failure, severe liver disease, systemic inflammatory disease, and receiving corticosteroid treatment were excluded. The study was approved by the L. Pasteur University Hospital Review Board, and all subjects gave written consent to participate in the study.

Anthropometric data and diabetes duration were recorded at the baseline visit. Blood samples were taken for biochemical measurements of FPG, HbA1c, lipid levels and genotyping. Sulphonylurea treatment was started with gliclazide, glimepiride, glipizide or glibenclamide. Sulphonylurea dose could have been adjusted after 3 months based on blood glucose self-monitoring results. Measurements of body weight, FPG, HbA1c, and serum lipids were repeated after 6 months following initiation of sulphonylurea therapy.

Biochemical analyses

In all patients, peripheral venous blood samples were collected between 7–8 a.m. following an overnight 12-hour fast. Glucose was measured by glucose oxidase method, and cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were measured by routine enzymatic methods (Pliva-Lachema, Czech Republic) on a Beckman autoanalyzer. HbA1c was measured using an immunoturbidimetric method (Roche Diagnostica, France).

Genotyping of KCNQ1 rs163184 (T>G)

Genomic DNA was extracted using a Wizard Genomic DNA purification kit (Promega Corp., Wisconsin, USA). PCR was performed in 10 µL of reaction volume on a LightScanner 32 instrument (Idaho Technology Inc., Salt Lake City, USA) at asymmetric primer ratio (1:10). Master mix was composed of 1x LGreen Plus+ (Idaho Technology Inc.), 200 nM dNTPs (Jena Bioscience, Jena, Germany), 0.06 µM forward primer, 0.6 µM reverse primer, 1.2 µM unlabeled blocked probe, 3 mM MgCl₂, 250 µg/ml BSA (Fermentas, Burlington, Canada), 0.5M betaine (Sigma-Aldrich, Germany), 1 U BioThermAB polymerase with 1x corresponding buffer (GeneCraft, Munster, Germany), and approximately 10 ng DNA. The sequences of oligonucleotides (Sigma-Aldrich, Germany) were:

CTTTGCTCAGTAACGGACTGGAACCA (forward primer),
CTGTGTGGAAAAGGTTGCCTGC (reverse primer), and
TGGATTTTGAGTAAAGAGAGA-phos (probe).

PCR conditions were the following: initial denaturation at 95°C for 5 min, 60 cycles at 95°C for 10 s, 56°C for 15 s and 72°C for 15 s. Amplification was performed at the thermal transition rate of 10°C/s for all steps, and was immediately followed by melting analysis with a denaturation at 95°C for 30 s and renaturation at 45°C for 1 minute. Data were acquired over a 45–90°C range at the thermal transition rate of 0.1°C/s. Genotypes were identified by the melting temperatures indicated by peaks on the derive plots after normalization using LightScanner 32 software 1.0.0.23 (Idaho Technology Inc.). The probe was designed to exactly match the allele T. The T allele homozygotes had a derivative melting peak at 60°C and G allele homozygous samples had a melting peak at 53°C, whereas heterozygotes showed both peaks.

Statistical analyses

Statistical analyses were performed using SPSS 17.0 for Windows software (SPSS Inc., Chicago, IL, USA). Relative
In further analyses, a recessive genetic model was tested in which carriers of the T-allele were pooled (TT+TG) and compared with patients with the GG genotype (Table 3). After sulphonylurea therapy, patients in the TT+TG group achieved significantly lower FPG levels in comparison with patients with the GG genotype (6.95±0.13 vs. 7.50±0.21 mmol/L, p=0.033). Consequently, ΔFPG was significantly higher in the TT+TG group compared to the GG group (1.58±0.13 vs. 1.04±0.18 mmol/L, p=0.016) (Table 3).

In multiple linear regression analysis with ΔFPG as a dependent variable and KCNQ1 genotype, age, sex, BMI, and baseline FPG as independent variables, KCNQ1 genotype (p=0.016) and baseline FPG (p<0.001) were the only significant independent predictors of ΔFPG (R²=0.48).

**DISCUSSION**

In the present study we have shown that the homozygous carriers of the risk G-allele of the KCNQ1 rs163184 gene polymorphism responded to treatment with sulphonylurea by significantly lower reduction in fasting glycaemia. In multivariate analysis, KCNQ1 genotype and baseline FPG levels were the only independent significant predictors of FPG reduction after sulphonylurea treatment.

The reports on the potential relationships between the effect of sulphonylurea therapy and genes variants associated with type 2 diabetes are scarce. Sulphonylurea receptor 1 is encoded by ATP-binding cassette transporter sub-family C member 8 (ABCC8) gene. A recent study in 115 Chinese patients revealed that carriers of the risk Aa-allele in the non-synonymous ABCC8 Ser1369Ala polymorphism were more sensitive to glitazide treatment with respect to reduction of HbA₁c level in comparison with Ser/Ser homozygotes [18]. This finding was confirmed in a larger study of 1268 Chinese subjects with type 2 diabetes, in which 8-week treatment with glitazide showed higher therapeutic efficacy on fasting glycaemia, but not on HbA₁c levels, observed in patients with Aa/Ala genotype when compared with patients with Ser/Ser genotype [19].

The Genetics of Diabetes Audit and Research Tayside Study (GoDARTS) was another robust pharmacogenetic study which addressed the relationship between 2 transcription

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**Table 1. Baseline characteristics of the subjects and the effect of 6-month sulphonylurea treatment in addition to previous metformin monotherapy in the entire group of patients.**

|                      | Before treatment | After treatment | p   |
|----------------------|------------------|-----------------|-----|
| Weight (kg)          | 84.0±1.8         | 84.3±1.9        | 0.228 |
| BMI (kg/m²)          | 30.4±0.4         | 30.5±0.4        | 0.605 |
| FPG (mmol/L)         | 8.47±0.14        | 7.12±0.11       | <0.001 |
| HbA₁c (%)            | 7.98±0.10        | 6.97±0.06       | <0.001 |
| Cholesterol (mmol/L) | 5.03±0.15        | 4.90±0.15       | 0.472 |
| LDL cholesterol (mmol/L) | 2.74±0.13    | 2.64±0.10       | 0.542 |
| HDL cholesterol (mmol/L) | 1.20±0.05       | 1.22±0.05       | 0.588 |
| Triglycerides (mmol/L) | 2.07±0.12       | 1.86±0.13       | 0.036 |

Data are expressed as mean ±SE. p-values refer to Student’s tests. BMI — body mass index; FPG — fasting plasma glucose; LDL — low-density lipoprotein; HDL — high-density lipoprotein.
factor 7-like 2 (TCF7L2) polymorphisms and the response to sulphonylurea therapy in type 2 diabetic patients. In that study, 901 Scottish patients with type 2 diabetes treated with sulphonylurea were analysed. The probability for early sulphonylurea treatment failure was almost doubled in subjects with the risk TT genotypes of both rs12255372 and rs7901346 polymorphisms compared to those homozygous for the reference non-risk genotypes [20]. Furthermore, our recent study demonstrated that carriers of the risk T-allele of TCF7L2 rs7903146 polymorphism responded to treatment with sulphonylurea by significantly smaller reductions in HbA1c and FPG levels in comparison with patients with the CC genotype [21].

In the present study we observed a significant association between the KCNQ1 rs163184 gene polymorphism and the efficacy of sulphonylurea treatment in type 2 diabetic patients who failed to achieve adequate glycaemic control on monotherapy with metformin – patients carrying 2 risk G-alleles of KCNQ1 (GG genotype) had significantly smaller...
reductions in FPG levels after 6-month therapy than those with at least 1 non-risk T allele (TT+TG genotypes). The differences in mean FPG (0.54 mmol/l) observed between different genotypes of KCNQ1 rs163184 polymorphism after sulphonylurea treatment in the present report were similar to those observed for TCF7L2 polymorphism [21]. To our best knowledge the present findings are the first to provide evidence of differences in the FPG reductions between various KCNQ1 genotype groups. Nevertheless, similar to Feng et al who analysed the treatment effect with respect to ABCC8 genotypes [19], we did not observe significant effect of the KCNQ1 rs163184 gene polymorphism on ΔHbA1c following sulphonylurea treatment. Besides the KCNQ1 genotype, baseline FPG also independently predicted the FPG reduction after sulphonylurea therapy. Importantly, the effect of KCNQ1 gene polymorphism on the reduction in FPG was independent of the baseline FPG levels in the present study.

The mechanism underlying the effects of KCNQ1 polymorphism on the therapeutic effect of sulphonylurcas is poorly understood. In a physiological study in INS-1 cell cultures, the blockade of the KvLQT1 channel with KCNQ1 protein inhibitor 293B stimulated insulin secretion in the presence of sulphonylurea drug tolbutamide [12], suggesting that KvLQT1 channels might play a role in fine-tuning of insulin secretion during sulphonylurea treatment.

There are limitations in the current pilot study, such as the small size of the study group. However, the approximately 35% difference in ΔFPG after 6-month therapy between the carriers of risk GG genotype and the carriers of non-risk T allele suggests that the sample size of this study, although limited, is enough to gain understanding on the role of sulphonylurea drug tolbutamide [12], supporting that KvLQT1 channels might play a role in fine-tuning of insulin secretion during sulphonylurea treatment. Such observations might lead to the development of genotype-based personalized strategies for the treatment of type 2 diabetes in the future.

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