Sulfonylurea Receptor 1 Gene Variants Are Associated With Gestational Diabetes and Type 2 Diabetes but Not With Altered Secretion of Insulin

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OBJECTIVE — To investigate the possible association of the variants in the nucleotide binding fold regions of the sulfonylurea receptor 1 (SUR1) gene with gestational diabetes mellitus (GDM), type 2 diabetes, and altered insulin secretion in Finnish subjects.

RESEARCH DESIGN AND METHODS — The nucleotide binding fold regions of the SUR1 gene were amplified with polymerase chain reaction and screened by the single-strand conformational polymorphism analysis in 42 subjects with GDM and 40 subjects with type 2 diabetes. Detected variants were further investigated in 377 normoglycemic subjects by restriction fragment-length polymorphism analysis. The effect of the variants of the SUR1 gene on first-phase insulin secretion was studied in 295 normoglycemic subjects.

RESULTS — In subjects with GDM or type 2 diabetes, one amino acid change (S1369A), four silent substitutions (R1273R, L829L, T759T, and K649K), and three intron variants were identified in the nucleotide binding fold regions of the SUR1 gene. A tagGCC allele of exon 16 splice acceptor site was more frequent in subjects with GDM (0.55 allele frequency, $n = 42$) and type 2 diabetes (0.60, $n = 40$) than in normoglycemic subjects (0.43, $n = 377$) ($P_1 = 0.024$ and $P_2 = 0.009$, respectively). Similarly, an AGG allele of the R1273R polymorphism was more common in subjects with GDM (0.87) and type 2 diabetes (0.87) than in normoglycemic subjects (0.74) ($P_1 = 0.009$ and $P_2 = 0.001$, respectively). However, the S1369A, R1273R, and cagGCC→tagGCC variants of the SUR1 gene were not associated with altered first-phase insulin secretion in 295 normoglycemic subjects.

CONCLUSIONS — These results suggest that a functional variant that contributes to the risk of GDM and type 2 diabetes may locate close to the SUR1 gene.

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Approximately 1 to 3% of pregnant women develop gestational diabetes mellitus (GDM). GDM is characterized by metabolic disturbances similar to those of common type 2 diabetes, such as β-cell dysfunction and insulin resistance (1,2). In previous studies, defects in the glucokinase gene (3) have been shown to account for decreased insulin secretion in a minority of patients with GDM, but sulfonylurea receptor 1 (SUR1), a key regulator of insulin secretion as a part of the pancreatic ATP-sensitive K^+ channel (4), has not been previously studied in patients with GDM. Interestingly, several mutations of the SUR1 gene have been associated with neonatal hyperinsulism (5). Moreover, the ACT allele in codon 759 and the cagGCC allele or tagGCC allele of exon 16 splice acceptor site of the SUR1 gene have been shown to be associated with type 2 diabetes in Caucasian subjects (6–9). In accord with these results, a marker close to the SUR1 gene has been reported to be linked with 2-h glucose concentrations (10), and the AGA allele of a silent R1273R variant of the SUR1 gene has been shown to be associated with increased insulin levels in Mexican-Americans (11). Therefore, we screened the functionally important nucleotide binding fold regions of the SUR1 gene in Finnish patients with GDM or type 2 diabetes and investigated the association of the variants of the SUR1 gene with the first-phase insulin secretion in normoglycemic subjects.

RESEARCH DESIGN AND METHODS

Subjects

A total of 42 unrelated women with previous GDM (age 38 ± 1 years, BMI 29.1 ± 1.2 kg/m^2) were selected from patients who had a positive family history of diabetes and who had been treated for GDM at the Kuopio University Hospital between 1987 and 1991. Diagnostic plasma glucose values for GDM in an oral glucose tolerance test (OGTT) were as follows: fasting glucose, ≥4.8 mmol/l; 1-h glucose, ≥10.0 mmol/l; and 2-h glucose, ≥8.7 mmol/l. An individual with at least two pathological values in an OGTT was regarded to have GDM. In addition, 40 unrelated patients with type 2 diabetes (17 men and 23 women, age 66 ± 1 years, BMI 28.6 ± 0.7 kg/m^2) were selected randomly from our previous population-based study (12). These patients ful-

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Abbreviations: AUC, area under the insulin response curve; GDM, gestational diabetes mellitus; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; SSCP, single-strand conformation polymorphism; SUR1, sulfonylurea receptor 1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Allele frequencies of variants in the SUR1 gene in normoglycemic subjects, in subjects with GDM, and in subjects with type 2 diabetes*

Variant	Normoglycemia	GDM	Type 2 diabetes	P_1	P_2
n	377	42	40	—	—
Exon 14					
K649K (AAG→AAA)	ND	0.23 ± 0.13 (19)	0.21 ± 0.13 (17)	ND	ND
Intron 15*					
cagGCC→tagGCC	0.43 ± 0.05 (322)	0.55 ± 0.15 (46)	0.60 ± 0.15 (48)	0.024	0.009
Exon 18					
T759T (ACC→ACT)	0.03 ± 0.02 (26)	0.04 ± 0.06 (3)	0.05 ± 0.07 (4)	NS	NS
Intron 18					
ggtgct→tgtgct	0.01 ± 0.01 (9)	0.02 ± 0.05 (2)	—	NS	NS
Exon 21†					
L829L (CTG→TTG)	0.13 ± 0.07 (22)	0.15 ± 0.11 (13)	0.18 ± 0.12 (14)	NS	NS
Exon 31*					
R1273R (AGA→AGG)	0.74 ± 0.04 (559)	0.87 ± 0.10 (73)	0.87 ± 0.10 (70)	0.009	0.001
Exon 33*					
S1369A (TCC→GCC)	0.42 ± 0.05 (315)	0.50 ± 0.15 (42)	0.46 ± 0.15 (37)	NS	NS
Intron 33					
tggccg→cggccg	ND	0.14 ± 0.11 (12)	0.15 ± 0.11 (12)	ND	ND

Data are means ± SEM (number of alleles). *Variants of intron 15 and exons 31 and 33 correspond to the variants of intron 24 and exons 9 and 7, respectively, according to Inoue et al. (6); †the allele frequency of the L829L variant is available for 82 normoglycemic subjects. ND, not determined; P_1, statistical significance between the GDM and the normoglycemic groups; P_2, statistical significance between the type 2 diabetic and the normoglycemic groups.

filled the World Health Organization criteria for type 2 diabetes (13).

Normoglycemic subjects consisted of two groups. Group 1 included a random sample of 82 healthy men (age 54 ± 1 years, BMI 26.3 ± 1.4 kg/m^2), who had participated in our previous study (14) and in whom the euglycemic-hyperinsulinemic clamp had been performed to evaluate the degree of insulin sensitivity. They did not have any chronic diseases, abnormalities in an OGTT, or episodes of hypertension (use of antihypertensive drugs or systolic/diastolic blood pressure >160/95 mmHg); they were not obese (BMI >27 kg/m^2); and they did not receive any drug treatment that could influence glucose metabolism. Normoglycemic group 2, in whom an intravenous glucose tolerance test (IVGTT) had been performed, included a random sample of 295 subjects (150 men and 145 women, age 44 ± 1 years, BMI 25.6 ± 0.2 kg/m^2) from our previous population-based study (15). Impaired glucose tolerance and diabetes were excluded by an OGTT in these subjects, but other specific exclusion criteria were not applied. All subjects participating in this study were living in eastern Finland. Informed consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The protocol was approved by the Ethics Committee of the University of Kuopio.

OGTTs and IVGTTs

All study subjects underwent an OGTT (75 g glucose) after a 12-h overnight fast. The first-phase insulin secretion in 295 normoglycemic subjects was evaluated by an IVGTT after a 12-h overnight fast. Two successive samples of blood glucose (5 min apart) to measure fasting blood and plasma insulin levels were taken. An intravenous glucose bolus (0.3 g glucose/kg body wt as a 50% solution administered over 90 s) was then injected via the cannula in the nonsampled arm. Additional samples for measurements of blood glucose and plasma insulin were taken at 4, 6, 8, 10, 19, 22, 29, 37, 67, 90, and 180 min. The first-phase insulin secretion was evaluated by calculating the area under the insulin response curve (AUC) during the first 10 min of the IVGTT.

Identification of variants in the SUR1 gene

For the initial screening of the SUR1 gene, two highly conserved nucleotide binding fold regions that encompass exons 13–22 and 31–39 (exons are numbered consecutively, starting from the 5′ end of the SUR1 gene, in contrast to a previous study [6]) were amplified with the polymerase chain reaction (6) and screened by single-strand conformation polymorphism (SSCP) analysis, as previously described (16), in 42 patients with GDM and 40 patients with type 2 diabetes. Each sample was run in a 5–6% nondenaturing polyacrylamide gel containing 10% of glycerol at two different gel temperatures: 38°C for ~4 h and 29°C for ~5 h. The variants found in the SSCP analysis were identified by direct sequencing and digestion with an appropriate restriction enzyme. Prevalence of the variants in normoglycemic subjects was determined by restriction fragment-length polymorphism analysis.

RESULTS

Association study of the variants of the SUR1 gene

We found eight different variants in patients with GDM and seven different variants in patients with type 2 diabetes in the initial screening of the SUR1 gene (Table 1). The previously reported S1369A polymorphism of exon 33 was the only amino acid substitution observed (6); however, it displayed a similar allele frequency in subjects with GDM (n = 42) or type 2 diabetes (n = 40) compared with normoglycemic subjects (n = 377). In contrast, the tagGCC allele of exon 16 splice acceptor site was more common in patients with GDM (0.55) or type 2 diabetes (0.60) than in normoglycemic subjects (0.43) (P_1 = 0.024 and P_2 = 0.009, respectively). Similarly, the AGG allele of the R1273R polymorphism was more common in patients

with GDM (0.87) or type 2 diabetes (0.87) than in normoglycemic subjects (0.74) (P_1 = 0.009 and P_2 = 0.001, respectively). The R1273R and cagGCC→tagGCC were in linkage disequilibrium in patients with GDM ($P < 0.001$) and tended to be in patients with type 2 diabetes ($P = 0.130$) but were not in normoglycemic subjects ($P = 0.488$).

Effect of the variants of the SUR1 gene on insulin secretion

The influence of the cagGCC→tagGCC, T759T, ggtgct→tgtgct, R1273R, and S1369A variants of the SUR1 gene on insulin secretion were studied in 295 normoglycemic subjects. Fasting, 1-h, and 2-h insulin levels were similar during an OGTT in subjects with different genotypes of the variants (NS). Furthermore, the peak insulin secretion at 4 min and the AUC during the first 10 min of the IVGTT did not differ between different genotypes of the variants (AUC for S1369A polymorphism: SS genotype, 2,448 ± 141 pmol · l^{-1} · min^{-1}; SA, 2,762 ± 145 pmol · l^{-1} · min^{-1}; or AA, 2,379 ± 239 pmol · l^{-1} · min^{-1}) (NS). In addition, subjects with the combined at-risk genotypes (AGG/AGG—tagGCC/tagGCC, AGG/AGG—tagGCC/cagGCC, and/or AGG/AGG—cagGCC/cagGCC) (n = 184) had similar β-cell responses during the IVGTT as subjects with the nonrisk genotypes (n = 111) (NS). Furthermore, we could not show any influence of these variants of the SUR1 gene on fasting plasma glucose, BMI, waist-to-hip ratio, or the rates of whole-body glucose uptake during the euglycemic clamp in 82 normoglycemic men (data not shown).

CONCLUSIONS

— We investigated the highly conserved nucleotide binding fold regions of the SUR1 gene in Finnish subjects with GDM and type 2 diabetes. We report for the first time that the AGG allele of the R1273R variant and the tagGCC allele of exon 16 splice acceptor site of the SUR1 gene were associated with both GDM and type 2 diabetes, which supports the view that GDM and type 2 diabetes share a similar genetic predisposition.

Our results are in accordance with previous studies that have shown associations of the cagGCC→tagGCC and T759T variants of the SUR1 gene with type 2 diabetes in Caucasians (6–9,11) and the R1273R variant with elevated fasting and 2-h insulin levels in Mexican Americans (10). Because opposite alleles of the biallele variants of the SUR1 gene are associated with type 2 diabetes in different populations and because none of these variants are predicted to alter the amino acid sequence of the SUR1 gene, the associations are likely to be caused by indirect mechanisms. Indeed, we demonstrated a linkage disequilibrium between the AGG allele of the R1273R polymorphism and the tagGCC allele of exon 16 splice acceptor site of the SUR1 gene in patients with GDM, which supports the view that the cagGCC→tagGCC and R1273R variants are in linkage disequilibrium with the identical functional variant in the chromosomal region close to the SUR1 gene. This finding is not surprising, considering that our study population comes from quite a small area in eastern Finland.

In a recent study, 10 carriers of the combined exon 18/16 at-risk genotype (exon 18 C/T or T/T and exon 16 [−3] c/t or t/t) of the SUR1 gene had a decreased C-peptide and insulin response upon tolbutamide injection but a normal β-cell response upon glucose injection compared with 370 subjects with nonrisk genotypes (8). Furthermore, in Mexican-Americans, the homozygous subjects for the AGA allele of the R1273R variant had significantly higher fasting and 2-h insulin levels than subjects with the other genotypes (11). In the present study, neither the silent R1273R substitution and cagGCC→tagGCC variant of exon 16 splice acceptor site nor the combined at-risk genotypes of these variants was associated with altered insulin secretion in 295 normoglycemic subjects in an OGTT or IVGTT. Moreover, these variants were not associated with fasting plasma glucose, BMI, waist-to-hip ratio, or the rates of whole-body glucose uptake in 82 normoglycemic subjects. Therefore, how the disease locus contributes to the risk of GDM and type 2 diabetes remains unexplained, even though β-cell dysfunction that is expressed in a different manner than measured in our study may still exist. However, the disease locus is unlikely to play a major role in the pathogenesis of these diseases, because the microsatellite markers that flank the SUR1 locus have not shown any evidence of linkage with type 2 diabetes in Japanese sib pairs (17) or Caucasian families with type 2 diabetes (7,18). Furthermore, the screening of the whole coding region of the SUR1 gene (8,19) and the Kir 6.2 gene (20), which is located only 4.5 kb downstream from the SUR1 gene, has not revealed any mutations associated with GDM or type 2 diabetes. Our findings do not exclude, however, the possibility of a functional variant in the nonexamined coding region or in the promoter region of the SUR1 gene.

In conclusion, amino acid substitutions in the nucleotide binding fold regions of the SUR1 gene were uncommon in Finnish patients with GDM or type 2 diabetes. Therefore, variants in the nucleotide binding fold regions of the SUR1 gene are unlikely to contribute largely to an inherited risk of GDM or type 2 diabetes in Finnish subjects. However, the association of the R1273R and cagGCC→tagGCC variants with GDM and type 2 diabetes may be because of an unidentified variant in the unscreened part of the SUR1 gene. Alternatively, a functional variant of another gene that contributes to the risk of GDM and type 2 diabetes may locate close to the SUR1 gene.

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