

Carriers of an Inactivating β -Cell ATP-Sensitive K^+ Channel Mutation Have Normal Glucose Tolerance and Insulin Sensitivity and Appropriate Insulin Secretion

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OBJECTIVE — Insulin release from the pancreatic β -cells is controlled by ATP-sensitive K^+ (K_{ATP}) channels, which consist of a hetero-octameric complex of four sulfonylurea receptor 1 (SUR1) and four Kir6.2 proteins. Mutations in the SUR1 gene are the major cause of congenital hyperinsulinemia (CHI). Despite the hereditary nature of CHI, studies of glucose homeostasis in heterozygous relatives of CHI patients are lacking. Theoretically, in the heterozygous state of the SUR1 gene mutation, only 1 of 16 K_{ATP} channels consists of entirely normal subunits. The aim of our study was to investigate in vivo the glucose homeostasis of heterozygous SUR1 mutation carriers.

RESEARCH DESIGN AND METHODS — We studied 8 parents of CHI patients, all 8 of whom were heterozygous for the inactivating SUR1 mutation V187D, and 10 matched control subjects. We evaluated glucose tolerance and insulin secretory capacity with oral and intravenous glucose tests, rates of whole-body glucose uptake with hyperinsulinemic-euglycemic clamps, C-peptide response to hypoglycemia during hyperinsulinemic-hypoglycemic clamp, and function of the K_{ATP} channels with intravenous tolbutamide test.

RESULTS — Carriers of the V187D substitution had normal glucose tolerance, normal tissue sensitivity to insulin, and no signs of inappropriate insulin secretion. The normal insulin response to tolbutamide indicated that heterozygosity for the V187D mutation did not impair K_{ATP} channel function.

CONCLUSIONS — We conclude that the heterozygous carriers of the SUR1 mutation had normal glucose metabolism and insulin secretion, indicating that carriers of recessive K_{ATP} channel mutations are unlikely to be at an increased risk of hypoglycemia or other disturbances in glucose metabolism.

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Abbreviations: CHI, congenital hyperinsulinemia; K_{ATP} , ATP-sensitive K^+ channel; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; RIA, radioimmunoassay; SUR1, sulfonylurea receptor 1.

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

Insulin secretion of the pancreatic β -cells is regulated by ATP-sensitive K^+ (K_{ATP}) channels, which consist of two protein subunits: sulfonylurea receptor 1 (SUR1) and the inward-rectifying K^+ channel Kir6.2 (1). In the resting β -cell, the K_{ATP} channels are open and the outward flow of K^+ ions maintains membrane hyperpolarization, thus preventing insulin release. An increase in the cytoplasmic ATP-to-ADP ratio results in the closure of K_{ATP} channels. This in turn depolarizes the cell membrane to a critical level, sufficient to open the voltage-gated Ca^{2+} channels, resulting in an influx of Ca^{2+} ions. The resulting rise in the cytoplasmic Ca^{2+} concentration is a powerful stimulus for the exocytosis of insulin (2).

Recessive autosomal mutations in the K_{ATP} genes are known to be the major cause of congenital hyperinsulinemia (CHI), which is a rare disorder characterized by persistent hypoglycemia caused by dysregulated insulin secretion (3). Even though the understanding of the genetic basis and pathophysiology of CHI has increased rapidly (3–5), very little is known about glucose homeostasis and the control of insulin secretion in heterozygous relatives of CHI patients. A high frequency of diabetes in CHI families has been previously reported (6,7), but no detailed studies are available concerning insulin secretion or glucose metabolism among these heterozygous kin (8).

The regulation of insulin secretion in heterozygous SUR1 mutation carriers is interesting both theoretically and clinically. In the heterozygous state, half of the SUR1 protein produced is normal, whereas the other half is totally inactive. This leads to the formation of different SUR1 tetramers with various degrees of defective protein. Theoretically, only 1 of 16 octameric K_{ATP} channels in the heterozygous relatives consists of entirely normal subunits. Therefore, it could be

assumed that heterozygotes would have abnormalities in insulin secretion.

We have identified a missense mutation, V187D, in the SUR1 gene that is responsible for the majority of severe CHI cases in Finland. This mutation leads to the inactivation of pancreatic β -cell K_{ATP} channels. The patients carrying this mutation in either homozygous or compound heterozygous form have a severe form of CHI (9). Many parents of these patients have reported symptoms that could be classified as hypoglycemic during fasting. To determine *in vivo* the effects of the heterozygous state of this mutation on the regulation of β -cell secretion, whole-body glucose metabolism, and glucose homeostasis, including the counterregulatory system against hypoglycemia, we studied eight parents of CHI patients, all eight of whom are carriers of the V187D substitution.

RESEARCH DESIGN AND METHODS

Subjects

Study subjects. The subjects for the present study were the parents of five patients with diffuse CHI caused by the homozygous SUR1 mutation V187D. All 10 parents were invited to take part in the study, but 2 of them were unwilling to participate. Genetic analysis confirmed that all eight parents were heterozygous carriers of the V187D mutation. One of them had been diagnosed with gestational glucose intolerance, but none of them had a history of diabetes.

Control subjects. The control group consisted of 10 age-, sex- and BMI-matched healthy control subjects who had to fulfill the following criteria: 1) age between 30 and 40 years, 2) no diabetes, 3) no first-degree relatives with a history of diabetes, 4) no drug treatment nor any disease that could potentially modify carbohydrate metabolism, and 5) no strenuous physical activity more than three times per week.

Study protocol

The subjects were admitted to the metabolic ward of the Department of Medicine, Kuopio University Hospital, for 2 days. The subjects were asked by questionnaire about the symptoms of hypoglycemia (tiredness, shaking, sweating, and headache) in normal daily life when fasting. On the first day, after 12-h fasting,

the basal clinical and the biochemical data were collected, and then a 4-h oral glucose tolerance test (OGTT) was performed. On the second day, an intravenous glucose tolerance test (IVGTT) was performed. Immediately after the IVGTT, hyperinsulinemic-euglycemic and hypoglycemic clamp studies were performed. The validation of measuring insulin sensitivity by the hyperinsulinemic-euglycemic clamp after an IVGTT has been reported elsewhere (10). Several weeks after the other tests, four carriers of the V187D mutation and six control subjects participated in the tolbutamide test. All study subjects who were able to participate were included. The study protocol was approved by the Ethics Committee of Kuopio University Hospital. Informed consent was given by all subjects studied.

OGTT. In the 4-h OGTT (75 g of glucose per person), samples for blood glucose, plasma insulin, and plasma C-peptide were drawn at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min to evaluate the degree of glucose tolerance and the β -cell response to the oral glucose load.

IVGTT. The IVGTT was performed to determine the first-phase insulin secretory capacity. An intravenous catheter was placed in the antecubital vein for the infusion of glucose. Another cannula for blood sampling was inserted into a wrist vein, which was surrounded by a heated box (40°C). After baseline blood collection, a bolus of glucose (300 mg/kg in a 50% solution) was administered into the antecubital vein within 30 s to acutely increase the blood glucose level. Samples for the measurement of blood glucose and plasma insulin were drawn at -5, 0, 2, 4, 6, 8, and 10 min.

Euglycemic-hypoglycemic clamp. The degree of insulin resistance was evaluated with the hyperinsulinemic-euglycemic clamp technique (11). After the IVGTT, a priming dose of insulin (Actrapid 100 IU/ml; Novo Nordisk, Gentofte, Denmark) was infused at a rate of $160 \text{ mU} \cdot \text{m}^{-2}$ body surface area $\cdot \text{min}^{-1}$ during the initial 10 min to acutely raise plasma insulin, followed by a continuous insulin infusion at a rate of $80 \text{ mU} \cdot \text{m}^{-2}$ body surface area $\cdot \text{min}^{-1}$. Blood glucose was clamped at 5.0 mmol/l for the next 120 min (euglycemic clamp 0–120 min) and then at 3.0 mmol/l for the next 120 min (hypoglycemic clamp 120–240 min) by infusing 20% glucose at various rates according to blood glucose measurements performed

at 5-min intervals (mean coefficient of variation of blood glucose was <3% during the euglycemic clamp and 3% during the hypoglycemic clamp in both groups).

During the euglycemic clamp, the blood glucose levels were averaged over each 20-min interval. The mean value for the period from 60 to 120 min was used to calculate the rates of whole-body glucose uptake (*M* value).

The hypoglycemic clamp was performed to investigate C-peptide levels and counterregulatory hormone responses during hypoglycemia. The measurements of plasma insulin, plasma C-peptide, serum glucagon, serum norepinephrine, serum cortisol, serum growth hormone, and serum epinephrine were drawn at 90 min during the hyperinsulinemic-euglycemic clamp (normoglycemic state) and again at 225 min during hypoglycemia and at 240 min during the hyperinsulinemic-hypoglycemic clamp.

The symptoms of hypoglycemia were recorded at 0, 30, 60, and 120 min from the beginning of the hypoglycemic clamp, using a questionnaire previously described (12–14). The study subjects were asked to evaluate the severity of autonomic symptoms (such as sweating, shaking, nervousness, and pounding of the heart) and the neuroglycopenic symptoms (such as blurred vision, weakness, hunger, tiredness, dizziness, difficulty in thinking, faintness, and tingling) on a visual scale from 0 (absent) to 10 (severe). The sums of these scales at each of the four time points constituted the hypoglycemia symptom score.

Tolbutamide test. The tolbutamide test was performed in four V187D heterozygotes and six control subjects to investigate the β -cell response to the K_{ATP} channel antagonist tolbutamide. It is known that the sulfonylurea drug tolbutamide induces insulin secretion through direct interaction with the SUR1 protein. It was thus hypothesized that the increment in insulin secretion would be lower in the V187D carriers if their K_{ATP} channels are defective. After a 12-h overnight fast, the baseline values of blood glucose, plasma insulin, and plasma C-peptide were measured in duplicate at 5-min intervals. A glucose bolus (300 mg/kg in a 50% solution) was injected into the antecubital vein within 30 s (15). At 20 min after the end of the glucose injection, a rapid bolus of tolbutamide (300 mg Ori-

Table 1—Clinical and biochemical characteristics of the study groups

	Control subjects	V187D carriers	<i>P</i>
<i>n</i>	10	8	
Age (years)	35.6 ± 1.1	35.3 ± 0.9	NS
Sex (M/F)	5/5	4/4	NS
BMI (kg/m ²)	23.2 ± 0.6	24.0 ± 1.2	NS
Fasting blood glucose (mmol/l)	4.3 ± 0.2	4.7 ± 0.2	NS
Fasting insulin (pmol/l)	49.2 ± 6.6	42.0 ± 5.4	NS
Fasting C-peptide (pmol/l)	530 ± 35	450 ± 51	NS
Systolic blood pressure (mmHg)	128 ± 4	129 ± 3	NS
Diastolic blood pressure (mmHg)	84 ± 2	76 ± 2	NS
HbA _{1c} (%)	5.4 ± 0.1	5.4 ± 0.1	NS
Fasting hepatic insulin extraction (pmol C-peptide/pmol P-insulin)	10.7 ± 0.3	10.7 ± 0.4	NS

Data are means ± SEM or *n*.

nase DiagnosticR; Pharmacia & Upjohn, Kalamazoo, MI) was injected. Venous blood was collected at 19, 22, and 29 min after the glucose injection for the measurement of blood glucose, plasma insulin, and plasma C-peptide. Plasma insulin and plasma C-peptide responses to tolbutamide injection (at -1 and 2 min) were calculated as the difference between the measurements obtained at the two time points. The tolbutamide-stimulated plasma insulin and plasma C-peptide responses during 1–9 min after the tolbutamide bolus were calculated by means of the trapezoidal method as the incremental area under the curve.

Assays and calculations. Blood glucose was measured with the glucose oxidase method (Glucose & Lactate Analyzer 2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH). Blood was collected into EDTA tubes for the determination of plasma insulin and C-peptide. After centrifugation, plasma for the determination of insulin and C-peptide was stored at -20°C until the analysis. Radioimmunoassay (RIA) kits were used to measure plasma insulin (Phadeseph Insulin RIA 100; Pharmacia Diagnostics, Uppsala, Sweden) and C-peptide (insulin and C-peptide 125J RIA kit; Incstar, Stillwater, MN). This insulin assay also detects proinsulin and proinsulin conversion products, with a cross-reactivity of 47%. The incremental insulin and C-peptide areas under the curve were calculated by the trapezoidal method. The counterregulatory hormone responses were analyzed by high-pressure liquid chromatography (epinephrine and nor-

epinephrine, in-house method), chemiluminometric enzyme immunoassay (Immulite 2000 cortisol/growth hormone hGH; Diagnostic Products, Los Angeles, CA), and RIA (glucagon double-antibody RIA; Diagnostic Products).

Statistical analysis. All calculations were performed with SPSS for Windows software (SPSS, Chicago, IL). Data are shown as the means ± SEM. Differences between the different variables in two groups were analyzed using nonparametric Mann-Whitney *U* tests. The repeated-measurements analysis of variance test was used when analyzing the results of the OGTT and IVGTT.

RESULTS

Clinical and biochemical characteristics of the study groups

Table 1 shows the clinical and biochemical characteristics of the study groups. The groups were comparable with respect to age, sex, and BMI. Furthermore, there were no significant differences in fasting glucose, insulin, C-peptide, HbA_{1c}, or blood pressure levels. Altogether, 7 of 8 V187D carriers and 4 of 10 control subjects had suffered from hypoglycemic symptoms.

OGTT

All individuals in both study groups had normal glucose tolerance according to World Health Organization criteria (16), as determined by OGTT (fasting blood glucose: V187D heterozygotes 4.2–5.5 mmol/l, control subjects 2.8–6.2 mmol/l; 2-h blood glucose: V187D heterozygotes

2.8–6.2 mmol/l, control subjects 3.4–6.4 mmol/l).

Figure 1A shows the glucose response during the OGTT. The blood glucose levels were similar in both groups (V187D heterozygotes and control subjects) at all time points measured after the oral glucose load. Figure 1B depicts the plasma insulin response during the OGTT. There were no significant differences in plasma insulin levels at any time point. Moreover, the plasma insulin response, expressed as the incremental insulin area under the curve, was similar in the two groups (V187D heterozygotes 519 ± 98 pmol/l · h, control subjects 553 ± 89 pmol/l · h). The plasma C-peptide response during the OGTT (Fig. 1C) was of the same magnitude in both study groups. Similarly, the incremental C-peptide area under the curve was comparable between the study groups (V187D heterozygotes 3,125 ±

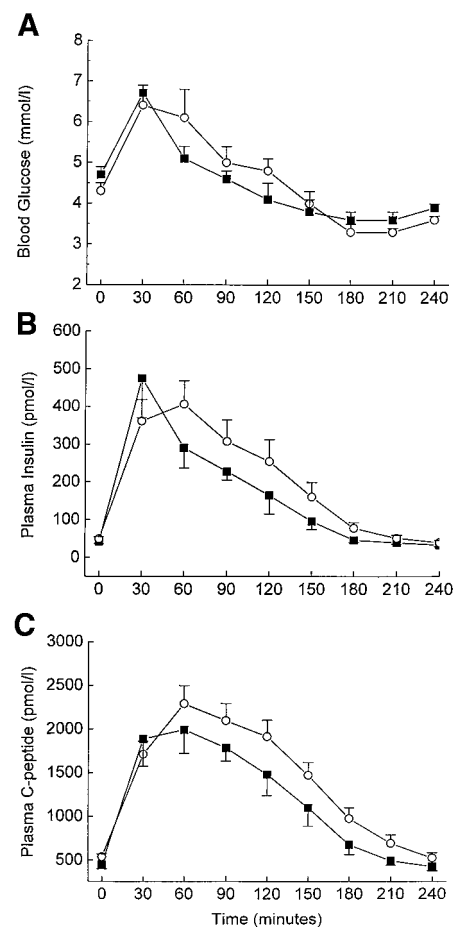


Figure 1—Blood glucose (A), plasma insulin (B), and plasma C-peptide (C) concentrations during the OGTT. ○, Control subjects; ■, SUR1 V187D heterozygotes.

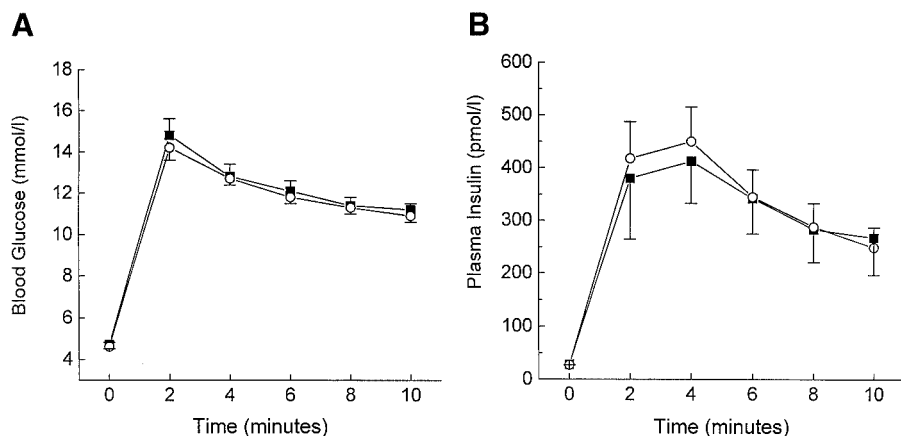


Figure 2—Blood glucose (A) and plasma insulin (B) concentrations during the IVGTT. ○, Control subjects; ■, SUR1 V187D heterozygotes.

441 pmol/l · h; control subjects 3,726 ± 426 pmol/l · h).

IVGTT

Figure 2 shows the acute blood glucose (Fig. 2A) and plasma insulin (Fig. 2B) response during the IVGTT. Fasting plasma insulin levels were similar in the study groups. There were no differences in plasma insulin levels nor in the incremental plasma insulin areas under the curve (2,683 ± 659 vs. 2,758 ± 448 pmol/l · min). Furthermore, the incremental glucose area under the curve was similar in both study groups (71.1 ± 3.9 and 70.2 ± 2.7 mmol/l · min for the V187D heterozygote group and control subjects, respectively).

Euglycemic-hypoglycemic clamp

The rates of whole-body glucose uptake did not differ significantly between the groups (61.1 ± 5.0 vs. 56.1 ± 4.2 μmol · kg⁻¹ · min⁻¹). Also, the steady state insulin levels during the euglycemic clamp were similar in both study groups (949 ± 62 and 1,058 ± 84 pmol/l in carriers and control subjects, respectively). Figure 3A shows that there were no differences in blood glucose levels during the hypoglycemic clamp or in plasma insulin and C-peptide levels (Fig. 3B) at the end of the study. Finally, neither the counterregulatory hormone responses in normoglycemia (serum glucagon 84.3 ± 5.5 vs. 81.0 ± 9.9 pmol/l, serum epinephrine 0.14 ± 0.03 vs. 0.24 ± 0.05 nmol/l, serum norepinephrine 1.5 ± 0.2 vs. 1.8 ± 0.3 nmol/l, serum cortisol 213.3 ± 37.4 vs. 253.8 ± 30.7 nmol/l, and serum growth hormone 0.88 ± 0.13 vs. 0.46 ±

0.15 μg/l in the V187D heterozygote group and in the control group, respectively) and in hypoglycemia (serum glucagon 95.1 ± 4.2 vs. 120.7 ± 18.9 pmol/l, serum epinephrine 1.3 ± 0.4 vs. 1.6 ± 0.3 nmol/l, serum norepinephrine 1.9 ± 0.3 vs. 2.1 ± 0.3 nmol/l, serum cortisol 355.9 ± 85.4 vs. 493.4 ± 59.3 nmol/l, and serum growth hormone 11.4 ± 2.9 vs. 14.7 ± 3.9 μg/l in the V187D heterozygote group and control subjects, respectively) nor the symptoms of hypoglycemia evaluated during the hypoglycemic clamp differed significantly between the groups.

Tolbutamide test

Figure 4 shows that plasma insulin and C-peptide responses to the tolbutamide injection were similar in V187D heterozygotes and control subjects when expressed as the difference between the hormone levels measured at 0 and 3 min after the tolbutamide bolus. The incremental areas under the curve (0–10 min) did not differ, either (P-insulin 1,744 ± 338 vs. 2,226 ± 491 pmol/l · min and C-peptide 5,950 ± 1,266 vs. 6,607 ± 870 pmol/l · min for V187D carriers and control subjects, respectively).

CONCLUSIONS — CHI is a rare inherited monogenic disease characterized by inappropriate insulin secretion during hypoglycemia. In the affected infant, the condition leads to severe, persistent hypoglycemia and, if not treated adequately, to neurological damage (17). Mutations in at least four genes expressed in the pancreatic β-cell are known to cause CHI (18). Understanding of the molecular ba-

sis of CHI has raised questions concerning possible disturbances in glucose metabolism of individuals carrying recessive CHI-associated gene variants. In this study, we investigated in detail the glucose metabolism of such individuals, who carried the previously described SUR1 loss of function mutation V187D (9). According to our results, the heterozygosity for this mutation does not lead to impaired glucose tolerance, tissue sensitivity to insulin, or defective insulin secretion.

It is not clear how the formation of the hetero-octamers in the K_{ATP} channels is regulated and what the impact is of one single SUR1 subunit on the function of the entire hetero-octamer forming the K_{ATP} channel. Theoretically, only 1 of 16 octameric K_{ATP} channels in the SUR1 heterozygotes consist of entirely normal subunits. Therefore, it could be assumed that they would have abnormalities in insulin secretion. However, it is not clear whether wild-type SUR1 and mutated SUR1 can be grouped within one K_{ATP} channel complex and whether such mixed-type channels behave in the same way as K_{ATP} channels comprising four wild-type SUR1 subunits. Moreover, it is possible that defective subunits are not even incorporated into K_{ATP} channels when there

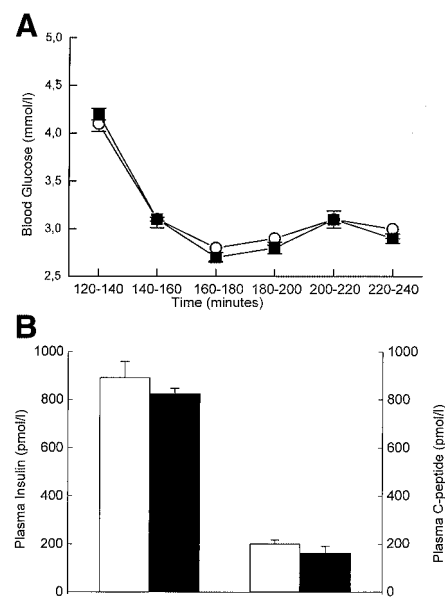


Figure 3—Blood glucose levels during the hypoglycemic clamp study (A) and plasma insulin and plasma C-peptide concentrations at the end of the hypoglycemic clamp study (mean values of concentrations at 225 and 240 min) (B). ○ And □, control subjects; ■, SUR1 V187D heterozygotes.

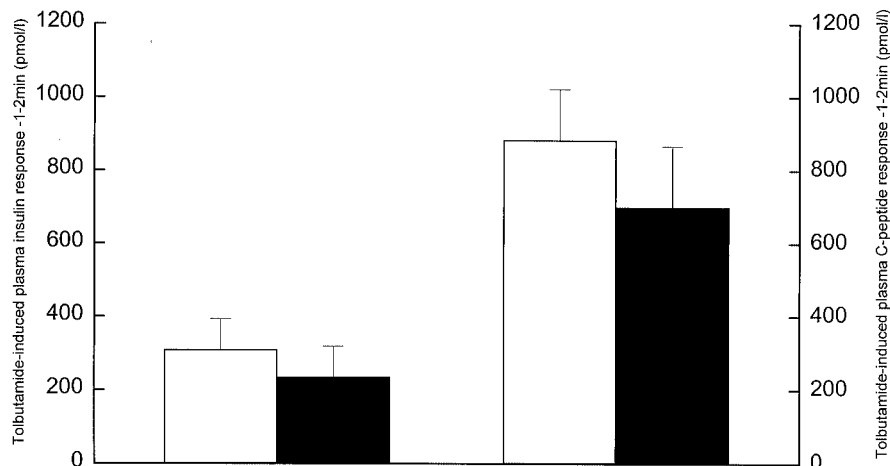


Figure 4—Tolbutamide-induced plasma insulin and plasma C-peptide responses, expressed as the difference between measurements obtained at 2 min after and 1 min before tolbutamide injection. □, Control subjects; ■, SUR1 V187D heterozygotes.

are normal subunits available. A recent study demonstrated in vitro that when four identical noncooperative ATP sites are grouped within one K_{ATP} channel complex, occupation of one site is sufficient to induce channel closure (19). Our present findings, demonstrating normal insulin and C-peptide responses after tolbutamide injection, indicate that normal K_{ATP} channel function is maintained in the β -cells of the SUR1 V187D heterozygotes.

Transgenic expression of a dominant-negative K_{ATP} channel in pancreatic β -cells leads to significantly impaired K_{ATP} channel function (20). Interestingly, these mice develop hypoglycemia as neonates and hyperglycemia as adults. Observations in the transgenic mice, and recently also in human patients with K_{ATP} channel mutations (21,22), suggest increased apoptosis of β -cells, which could gradually lead to decreased insulin secretory capacity. Theoretically, heterozygous mutations could also lead to impaired β -cell mass in the long term. Hansen et al. (23) found that young healthy carriers of the combined genotype of a silent variant in exon 18 (T759T) and an intronic variant in exon 16 (nt-3) in the SUR1 gene had decreased insulin and C-peptide levels after tolbutamide administration, whereas the responses after an intravenous glucose load were normal. Some early studies have suggested a high frequency of type 2 diabetes in families with hyperinsulinemia (6,7). In our study, only one of the mothers of V187D homozygous patients had transiently ele-

vated blood glucose levels during pregnancy. All V187D mutation carriers had normal glucose tolerance and normal rates of whole-body glucose uptake. In addition, first-phase insulin secretion, which is known to be impaired in individuals at high risk for type 1 (24) and type 2 (25,26) diabetes, was not impaired in subjects with the V187D substitution. Our results indicate that the carriers of the V187D substitution do not have any features of type 2 diabetes. However, long-term follow-up would be needed to determine whether insulin secretion decompensates with age in these individuals.

Many parents of CHI patients report symptoms of hypoglycemia, including tiredness, shaking, sweating, and headache, during fasting in their normal daily life. In our study, 7 of the 8 V187D carriers but only 4 of the 10 control individuals had suffered from such symptoms. Therefore, we determined whether insulin levels of the V187D carriers were higher after overnight fasting and during hypoglycemia. The results clearly indicate that this is not the case. Furthermore, the levels of circulating insulin and C-peptide were also similar during the hypoglycemic clamp, and the prevalence of hypoglycemic symptoms did not differ between the study groups. This demonstrates that the capacity to turn off insulin release is not impaired in individuals carrying the inactivating SUR1 mutation in one allele.

Activation of glucose counterregulatory systems plays an important role in

the prevention and correction of hypoglycemia (27). Glucagon plays a primary role in the counterregulatory system. Adrenaline becomes critical when glucagon is deficient. Growth hormone and cortisol are less critical in acute glucose counterregulation but are important in the defense against prolonged hypoglycemia. According to previous studies, the threshold for the activation of counterregulatory hormone secretion occurs at higher blood glucose levels than for the initiation of autonomic and neuroglycopenic warning symptoms (12,14). In the present study, carriers of the V187D substitution had quite normal counterregulatory system function. This finding is in accordance with their appropriate responses to glucose and tolbutamide.

Electrophysiological studies of the variants in the SUR1 gene have shown that there are differences between the functional effects of different CHI-associated SUR1 mutations, leading to clinically variable severity of CHI (28). Some mutations impair the function of K_{ATP} channels only slightly, whereas the V187D mutation leads to total inactivation of pancreatic β -cell K_{ATP} channels. Our results indicate that despite the heterozygosity of this severe mutation, carriers have normal glucose metabolism and appropriate insulin secretion. Therefore, the finding that SUR1 mutation carriers are not at increased risk of hypoglycemia or other disturbances of glucose metabolism has important clinical implications, especially in families with healthy children carrying CHI-associated SUR1 mutations. Furthermore, these results confirm the totally recessive nature of CHI caused by this—and probably most other—recessively inherited SUR1 mutations.

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