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KCNQ1 Long QT Syndrome Patients Have Hyperinsulinemia and Symptomatic Hypoglycemia

Patients with loss-of-function mutations in *KCNQ1* have KCNQ1 long QT syndrome (LQTS). *KCNQ1* encodes a voltage-gated K^+ channel located in both cardiomyocytes and pancreatic β-cells. Inhibition of KCNQ1 in β-cells increases insulin secretion. Therefore *KCNQ1* LQTS patients may exhibit increased insulin secretion. Fourteen patients, from six families, diagnosed with KCNQ1 LQTS were individually matched to two randomly chosen BMI-, age-, and sex-matched control participants and underwent an oral glucose tolerance test (OGTT), a hypoglycemia questionnaire, and continuous glucose monitoring. *KCNQ1* mutation carriers showed increased insulin release (area under the curve 45.6 ± 6.3 vs. 26.0 ± 2.8 min · nmol/L insulin) and β-cell glucose sensitivity and had lower levels of plasma glucose and serum potassium upon oral glucose stimulation and increased hypoglycemic symptoms. Prolonged OGTT in four available patients and matched control subjects revealed hypoglycemia in carriers after 210 min (range 1.4–3.6 vs. 4.1–5.3 mmol/L glucose), and 24-h glucose profiles showed that the patients spent 77 ± 18 min per 24 h in hypoglycemic states (<3.9 mmol/L glucose) with 36 ± 10 min (<2.8 mmol/L glucose) vs. 0 min (<3.9 mmol/L glucose) for the control participants. The phenotype of patients with KCNQ1 LQTS, caused by mutations in *KCNQ1*, includes, besides long QT, hyperinsulinemia, clinically relevant symptomatic reactive hypoglycemia, and low potassium after an oral glucose challenge, suggesting that *KCNQ1* mutations may explain some cases of "essential" reactive hypoglycemia.

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The blood glucose level increases after a meal intake, leading to formation of ATP in the β-cell, closure of the ATP-dependent potassium channel, and thereby a reduction of the ATP-sensitive potassium current, depolarization of the β-cell, and increased insulin secretion. Kv7.1 is another potassium channel causing a voltage-gated repolarization current. Kv7.1 is encoded by *KCNQ1* and expressed both in β-cells (1) and in cardiomyocytes (2). Functional mutations in *KCNQ1* lead to KCNQ1 long QT syndrome (LQTS), with a predicted population prevalence of 1:2,000—a disease characterized by a delayed cardiac repolarization, prolonged QT interval on the electrocardiogram (ECG), syncope, malignant arrhythmias, and sudden death (3), but the clinical and

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physiological significance of functional *KCNQ1* mutations in β-cells is unknown. Interestingly, carriers of frequent intronic single nucleotide polymorphisms (SNPs) (rs2237892, rs2237895, and rs2237897) in *KCNQ1* have increased susceptibility for type 2 diabetes (4,5) and impaired β-cell function (6,7). Recent studies show that inhibition of Kv7.1 in β-cells increases exocytosis and insulin secretion (1), due to delayed repolarization (8), and *KCNQ1* knockdown with small interfering RNA similarly increases insulin exocytosis and secretion (1). Overexpression of *KCNQ1* in cultured MIN6 cells decreases both glucose and pyruvate- and tolbutamide-induced insulin secretion (9), and the intronic SNP risk allele of *KCNQ1* rs2237895 decreases exocytosis and insulin secretion (1), suggesting that the risk alleles of the intronic *KCNQ1* SNPs are gain-of-function polymorphisms and increase the susceptibility to type 2 diabetes due to increased *KCNQ1* expression and thereby decreased insulin exocytosis and secretion (1,9). Consequently, we hypothesize that LQTS patients with loss-of-function mutations of *KCNQ1* may exhibit increased insulin secretion due to delayed repolarization of the β-cell causing increased exocytosis. In this study, we show that *KCNQ1* LQTS patients have postprandial hyperinsulinemia and reactive hypoglycemia and experience symptoms of hypoglycemia.

RESEARCH DESIGN AND METHODS

Study Participants

Fourteen patients, from six nuclear families, diagnosed with *KCNQ1* LQTS (10) were recruited from the outpatient clinics at the cardiology departments at Gentofte and Aalborg hospitals, Denmark. Each patient was matched with respect to BMI, age, and sex with two randomly chosen control subjects from the Inter99 (11,12) or the Health (13) studies. A computer algorithm was applied to randomly select matching control subjects to be invited to participate in the study solely based on their best match with sex, BMI, and age. Six of 14 patients were in standard long QT therapy with β-blocking agents, and 6 of 14 patients had experienced syncope (of these 6, 3 were taking β-blocking agents). Before the examinations, all subjects were fasting overnight (including not taking any medication) and were free of any medication in the morning of examination. The characteristics of the two study groups are shown in Table 1. Informed written consent was obtained from all individuals before participation. The study was approved by the ethics committee of Copenhagen County (H-4-2010-036) and was in accordance with the principles of the Declaration of Helsinki.

Genetics

The LQTS patients were originally screened for mutations in genes known to cause LQTS, *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, and *SCN5A*, using published single-strand conformation polymorphism techniques followed by bidirectional sequencing of aberrant conformers, as previously described (14), and were found to be heterozygous carriers of the missense mutations p.H363N (15) (n = 4) and p.R366W (16) (n = 4), the frameshift/insertion mutation p.R401Pfs 62* (2) (n = 2), and the nonsense mutation p.Q530X (17) (n = 4). The protein reference sequence used was NP_000209.2.

Oral Glucose Tolerance Test, ECG, and Hypoglycemia Questionnaire

After an overnight fast, blood samples for measurements of plasma glucose, serum insulin, serum C-peptide, serum proinsulin, serum potassium, plasma glucagon-like peptide 1 (GLP-1), gastric inhibitory peptide (GIP), and plasma glucagon were taken prior to a standard 75-g oral glucose tolerance test (OGTT). Blood sampling was repeated every 15 min until 180 min after start of the OGTT. Before each blood sampling, ECG recordings were made with a MAC1600 ECG machine (GE Healthcare, Milwaukee, WI). QT intervals were corrected by heart rate with the Bazett correction method.

An adapted standard hypoglycemia questionnaire (18) (www.hypoglycemia.asn.au/2012/hypoglycemia-questionnaire; www.lisasaslove.com/uploads/8/4/4/9/8449235/hypoglycemia_questionnaire.pdf) (Supplementary Table 1) was filled at home by all participants except one patient (female, 56 years old) who died in between the physical examinations and answering the questionnaire. The patient smoked, lost consciousness, and set herself on fire and later died at the hospital owing

Table 1—Baseline characteristics of study participants

	KCNQ1 long QT patients	Patient subgroup for follow-up	Control group	Control subgroup for follow-up	$P_{patient\ vs.\ control}$	$P_{subgroup\ vs.\ whole\ group}$
N (men/women)	14 (5/9)	4 (2/2)	28 (10/18)	4 (2/2)		
Age (years)	44 ± 3	46 ± 7	46 ± 2	47 ± 7	1	0.9
BMI (kg/m^2)	28.5 ± 1.7	30.2 ± 1.4	29.0 ± 1.1	30.5 ± 1·0	1	0.7
Fat %	31.1 ± 1.6	32.4 ± 1.6	31.4 ± 1.3	31.7 ± 1.7	0.9	0.8
QT_B-interval (ms)	481 ± 8	480 ± 9	425 ± 4	423 ± 7	<0.0001	0.9

Data are means ± SEM unless otherwise indicated. QT_B-interval, QT intervals corrected by heart rate with Bazett correction method.

to her burns. Whether the unconsciousness was due to self-limiting arrhythmias, hypoglycemia, or other causes is unknown.

Prolonged OGTT and Continuous Blood Glucose Measurements

Follow-up studies were made in four available patients and four matched control individuals (Table 1) who underwent a prolonged OGTT (6 h) and continuous glucose measurements with IPro2 (Medtronic, Watford, U.K.) for 3–7 days. The group of 4 out of 14 patients represented those who agreed to participate in additional studies for up to 7 days' duration. The OGTTs were carried out as described above except that they were continued until 6 h after glucose ingestion. The continuous glucose measurements were conducted according to the manufacturer's manual (IPro2; Medtronic). IPro2 uses a retrospective algorithm to convert a sensor signal to glucose values based on self-monitored capillary blood glucose readings. Therefore, all participants received a glucose meter (Contour; Bayer Diabetes Care, Lyngby, Denmark) to ensure uniform measurements for conversion of sensor signals. Hypoglycemic time, normoglycemic time, and hyperglycemic time were calculated from the 24-h glucose profiles and defined as time spent with a blood glucose <3.9 mmol/L (and <2.8 mmol/L), between 3.9 and 7.8 mmol/L, and >7.8 mmol/L, respectively (Table 2). None of the four patients for the follow-up studies were taking β-blocking agents.

Biochemical and Anthropometric Measures

BMI was calculated as weight in kilograms divided by the square of height in meters. The percentage of fat was measured with bioimpedance analyzer, Biodynamics BIA 310e (Biodynamics, Seattle, WA). Plasma glucose was measured by a glucose oxidase method (Granutest; Merck, Darmstadt, Germany) with a detection limit of 0.11 mmol/L and intra- and interassay coefficients of variation of <1%. Radioimmunological determinations of fully processed glucagon and total plasma GLP-1 and GIP were performed as previously described (19–21). The analytical detection limit was 1 pmol/L, and intra- and interassay coefficients of variation were <6 and <15%, respectively. Serum insulin [excluding des (31,32) split products and intact proinsulin] was measured using the AutoDELFIA insulin kit (PerkinElmer Wallac, Turku, Finland). Serum C-peptide concentrations were measured by a time-resolved fluoroimmunoassay (AutoDELFIA C-peptide kit; PerkinElmer Wallac). Total intra- and interassay coefficients of variation were <3 and 4%, respectively. The analytical detection limit was 3 pmol/L. Proinsulin was measured by ELISA using Sunrise Touchscreen photometer (Tecan Austria GmbH, Salzburg, Austria). Total intra- and interassay coefficients of variation were <4 and 7%, respectively. The analytical detection limit was 0.3 pmol/L. Serum potassium was measured using Vitros 5600 (Ortho Clinical Diagnostics, Cedex, France). Total intra- and interassay coefficients of variation were <1%. The analytical detection limit was 1 mmol/L.

Table 2—Twenty-four-hour glucose profiles during continuous glucose measurements for 3–7 days

Blood glucose level (mmol/L)	KCNQ1 long QT patients	Control participants
>7.8	30 ± 10 (2.1 ± 0.7)	59 ± 18 (4.1 ± 1.4)
3.9–7.8	1,333 ± 199 (92.6 ± 14.1)	1,381 ± 251 (95.9 ± 17.4)
<3.9*	77 ± 19 (5.3 ± 1.3)	0
<2.8*	36 ± 10 (2.5 ± 0.7)	0

Data are means ± SEM minutes (means ± SEM percentage) per 24 h spent in different glucose states during continuous glucose measurements for 3–7 days in four patients and four matched control participants. *$P < 0.05$ when patients vs. control participants were analyzed for each blood glucose level state.

Sample Size

OGTT

The aim of the study was to test differences in insulin secretion among 14 KCNQ1 LQTS patients and 28 matched control subjects. With a mean difference in the maximal insulin response at 60 min of 212 pmol/L insulin, we will be able to reject the null hypothesis that this response difference is zero with a probability (power) >0.8.

Prolonged OGTT

The aim of the study was to test differences in glucose level among four KCNQ1 LQTS patients and four matched control subjects. With a mean difference in the minimum glucose level at 210 min of 1.5 mmol/L glucose, we will be able to reject the null hypothesis that this response difference is zero with a probability (power) >0.8. Sample size calculation was carried out with PS Power and sample size calculation 3.0.34 (22,23).

Data Calculations

Area under the curve (AUC) was calculated using GraphPad Prism 5. Insulinogenic index (serum insulin at 30-min [pmol/L] − fasting serum insulin [pmol/L]) / (plasma glucose at 30-min [mmol/L] − fasting plasma glucose [mmol/L]) was calculated as a measure of β-cell response to an oral glucose load (24). Whole-body insulin sensitivity was estimated from oral glucose tolerance data by applying the Matsuda insulin sensitivity index ($10{,}000/\sqrt{}$[(fasting plasma glucose × fasting serum insulin) × (mean plasma glucose × mean serum insulin during OGTT)] (25,26). Fasting homeostasis model assessment of insulin resistance index was calculated as follows: (fasting plasma glucose [mmol/L] × fasting serum insulin [pmol/L])/22.5. Prehepatic insulin secretion

rates (ISR) for each individual were calculated using a two-compartment model of C-peptide kinetics and population-based C-peptide kinetic parameters allowing calculations of values adjusted for clinical status, age, weight and height, and sex using the ISEC software (27,28). The individually calculated ISR values, for the time points 0 to maximum plasma glucose levels, were plotted against the individual plasma glucose concentrations to establish the dose-response relationship for each individual. The slopes of these approximately linear relationships were regarded as measures of β-cell responsiveness to glucose (29).

Statistics

Statistical analysis was carried out with a mixed-model ANOVA with repeated measurements (version 9.2, PROC MIXED; SAS, Cary, NC) contrasting patient results versus control subjects in matching pairs (version 9.2, PROC MIXED; SAS) and tested with the Scheffes post hoc test for multiple comparisons. Values of insulin were logarithmically transformed before analysis. The data are shown as means ± SEM. *P* values are given for the overall ANOVA. The total hypoglycemia frequency score and total hypoglycemia severity score of each participant were calculated from the sum of the point scores given for each question answer (Fig. 4; Supplementary Tables 1 and 2). The differences in scores between patients and control participants were tested with Student *t* test. Regression analyses between the QT interval and glucose, insulin, or potassium levels were made using SAS, version 9.2, PROC MIXED. A P value < 0.05 was considered significant.

RESULTS

The *KCNQ1* LQTS patients had lower plasma glucose levels compared with matched control participants 3 h after glucose ingestion (4.4 ± 0.3 vs. 5.4 ± 0.5 mmol/L glucose, $P = 0.03$ [Fig. 1]) and had significantly increased insulin responses to oral glucose stimulation (AUC 45.6 ± 6.3 vs. 26.0 ± 2.8 min · nmol/L insulin), $P < 10^{-5}$, as well as significantly increased serum levels of C-peptide and proinsulin and higher ISR, $P < 10^{-5}$ (Fig. 2). β-Cell sensitivity to glucose, evaluated as the slope of the relation between ISR and glucose, was significantly greater in mutation carriers (2.8 ± 0.31 vs. 1.9 ± 0.18, $P < 10^{-5}$ [Fig. 3]) and so was the insulinogenic index (116 ± 9 vs. 82 ± 5, $P < 10^{-3}$). Hypoglycemia frequency and severity score were significantly higher among patients compared with control participants, $P < 10^{-3}$ (Fig. 4; Supplementary Table 1). There was no difference in homeostasis model assessment of insulin resistance between the groups (10.0 ± 1.0 vs. 9.5 ± 1.3, $P > 0.05$), while the Matsuda index was significantly lower in the patients (11.0 ± 1.0 vs. 16.7 ± 1.5, $P < 10^{-3}$).

Serum potassium levels were significantly lower during the OGTT in mutation carriers compared with control participants ($P < 10^{-4}$). There were no significant differences between the groups in circulating levels of glucagon, GLP-1, and GIP ($P > 0.05$) (Fig. 5).

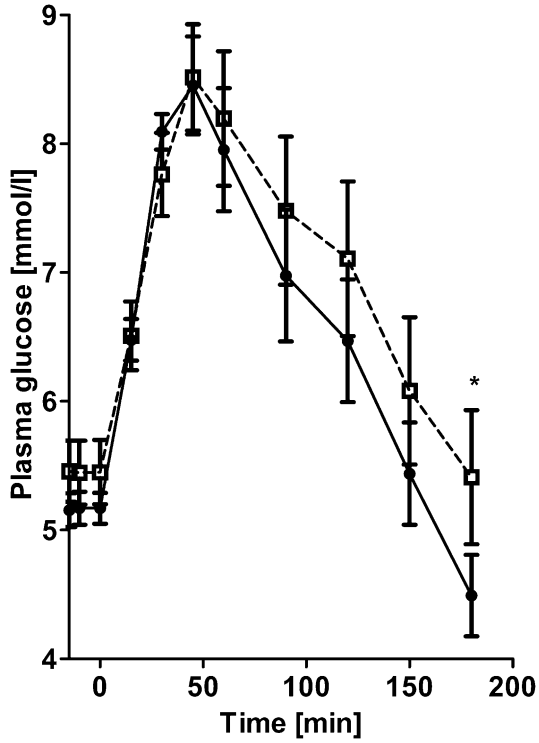

Figure 1—Plasma glucose levels during an OGTT in 14 patients with *KCNQ1* LQTS due to functional mutations in *KCNQ1* (●) and 28 randomly chosen BMI-, sex-, and age-matched control participants (□) (mean ± SEM); $P = 0.03$. *Significantly different time points.

During the OGTT at the time corresponding to insulin peak levels, the patients had significantly increased QT interval by 10 ± 2 ms compared with 5 ± 3 ms in control subjects ($P < 10^{-3}$). There was no significant correlation between the QT interval and circulating glucose, insulin, or potassium levels during the 3-h OGTT ($P > 0.05$).

Follow-up studies, including a prolonged OGTT for 6 h, undertaken in four available patients with matching control individuals, showed that all four patients were hypoglycemic (plasma glucose <3.9 mmol/L glucose) 3.5–5 h after glucose ingestion in contrast to control subjects who remained normoglycemic (plasma glucose nadir 1.4, 2.5, 3.3, and 3.6 mmol/L vs. nadir 4.1, 4.3, 4.7, and 5.0 mmol/L, $P < 10^{-4}$) (Fig. 6). Also, serum potassium levels were lower 3–6 h after oral glucose load among patients ($P < 10^{-3}$) (Fig. 6). The same participants underwent continuous glucose measurements for 3–7 days. Twenty-four-hour glucose profiles showed that the *KCNQ1* LQTS patients spent 77 ± 18 min/24 h in hypoglycemic states (<3.9 mmol/L glucose), with 36 ± 10 min of this period spent at <2.8 mmol/L glucose vs. 0 min (<3.9 mmol/L glucose) for the control participants ($P < 0.05$) (Table 2). The hypoglycemic episodes occurred

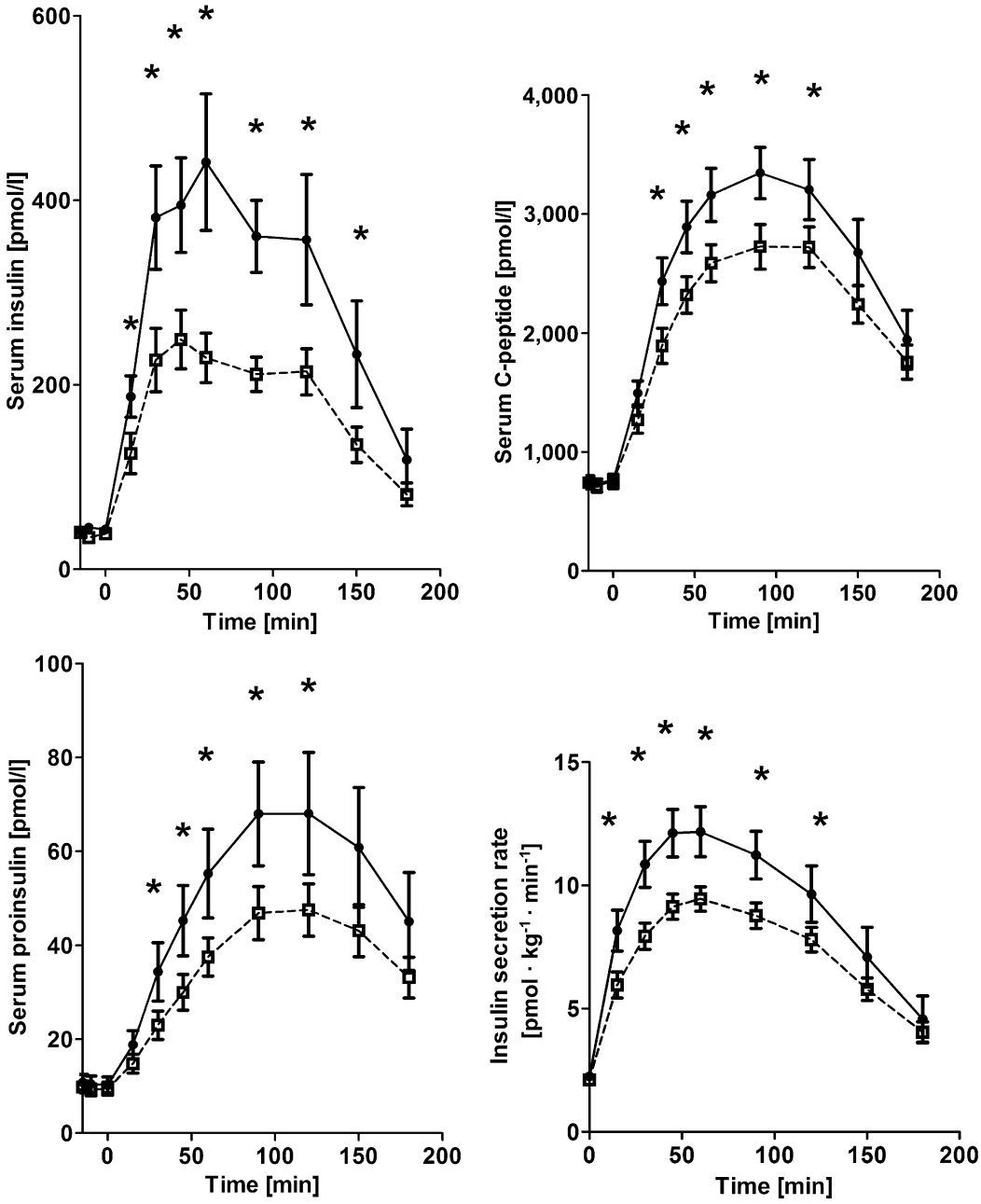

Figure 2—Serum insulin (*upper left panel*), C-peptide (*upper right panel*), and proinsulin (*lower left panel*) levels and ISR (*lower right panel*) during an OGTT in 14 patients with *KCNQ1* LQTS due to functional mutations in *KCNQ1* (●) and 28 randomly chosen BMI-, sex-, and age-matched control participants (□) (mean ± SEM); $P < 10^{-5}$. *Significantly different time points.

3–5 h after meals. The patient subgroup reported symptoms like extreme cravings for sweets, irritability, weakness, dizziness, shakiness, depression, or mood swings and anxiety or nervousness as well as night awakening and severe night sweats correlating to the exact time points of hypoglycemia, whereas the matching subgroup of control participants did not report any of the above-mentioned symptoms during the period of continuous glucose monitoring.

There were no differences in baseline observations (Table 1) or metabolic responses between the group of patients that participated in the follow-up studies and the whole patient group ($P > 0.7$). There were no differences in baseline observations or metabolic responses between the group of patients taking β-blocking agents (6 of 14 patients) or patients who had experienced syncope (6 of 14 patients) and the group that did not ($P > 0.8$). Furthermore, after exclusion of the group of patients taking β-blocking agents and their corresponding control participants from the analyses, a similar difference remained between patients and control participants with regard to

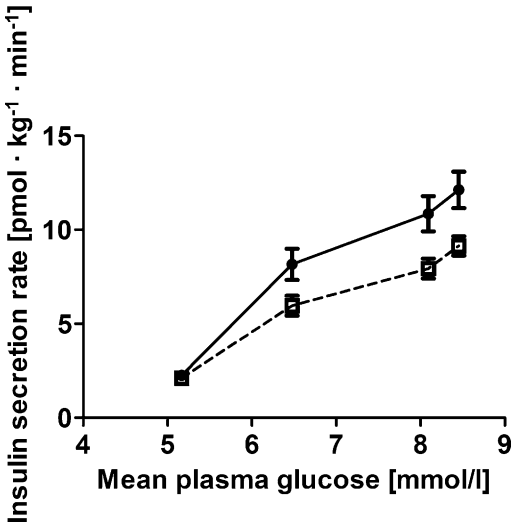

Figure 3—Illustration of the β-cell responsiveness to glucose. The calculated ISR values were plotted against plasma glucose to establish the dose-response relationship for each individual. The slopes of these approximately linear relations were regarded as measures of β-cell responsiveness to glucose. For illustrative purposes, the mean values of glucose at time 0, 15, 30, and 45 min were plotted on the *x*-axis; however, all data analysis and statistics were calculated for each individual. The slope was significantly greater among *KCNQ1* mutation carriers (●) vs. control individuals (□) (2.8 ± 0.31 vs. 1.9 ± 0.18, $P < 10^{-5}$).

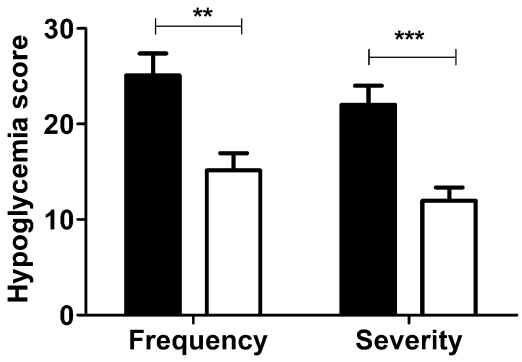

Figure 4—Hypoglycemia questionnaire score (see Supplementary Table 1) of 13 patients with *KCNQ1* LQTS due to functional mutations in *KCNQ1* (■) and 26 randomly chosen BMI-, sex-, and age-matched control participants (□). The total hypoglycemia frequency score and total hypoglycemia severity score of each participant were calculated from the sum of the point scores given for each question answer (Supplementary Tables 1 and 2). The differences in scores between patients and control participants were tested with Student *t* test (mean ± SEM); $^{**}P_{frequency} < 10^{-3}$, $^{***}P_{severity} < 10^{-4}$.

glucose, insulin, and potassium response ($P < 0.05$) (Supplementary Fig. 1).

DISCUSSION

We here report a novel extracardial phenotype in *KCNQ1* LQTS patients: postprandial hyperinsulinemic reactive hypoglycemia with clinically relevant symptoms of hypoglycemia. Until now, although *KCNQ1* is widely expressed, the only extracardial symptom reported in LQTS patients is sensorineural deafness in patients homozygous for *KCNQ1* loss-of-function mutations.

The lower postprandial glucose levels were recorded 3 h after glucose ingestion, indicating a delayed glycemic reaction to hyperinsulinemia, similar to what has been observed in genetically determined hyperinsulinemia owing to insulin receptor mutations (30). Therefore, we performed a prolonged OGTT for 6 h in four patients who were available for extended examination. Indeed, we observed that the patients became markedly hypoglycemic 3.5 h after glucose ingestion in contrast to the matched control participants. Consistent with this observation, 24-h glucose profiles showed that the *KCNQ1* LQTS patients had symptomatic hypoglycemic episodes in their own living environment 3-5 h after meal intake. Furthermore, the low circulating glucose levels observed in *KCNQ1* LQTS patients hours after an oral glucose load are in agreement with observations of low glucose levels in *KCNQ1* KO mice (31).

The patients also had lower serum potassium levels. This is presumably due to insulin activating the sodium-potassium ATPase to move potassium from the extracellular to the intracellular compartment (32). Other mechanisms leading to low potassium levels might include fecal loss of potassium as reported for *KCNQ1* KO mice (33).

Patients with KCNQ1 LQTS are characterized by rare episodes of syncope, ventricular tachyarrhythmia, and cardiac arrest, which hitherto have been ascribed to their long QT interval. However, we now show that these patients besides long QT interval also suffer from hyperinsulinemic reactive hypoglycemia along with low potassium levels and symptoms of hypoglycemia. Hypoglycemia may cause sympathetic activation, which is associated with increased propensity for arrhythmias and sudden death (34,35) in *KCNQ1* LQTS patients. The insulin-induced hypokalemia will also decrease the repolarization reserve by a reduction of the rapid delayed rectifier current I_{Kr} (36), thus further increasing the risk of cardiac arrhythmia and cardiac arrest (34). Furthermore, in a population-based study of 2,570 elderly people without diabetes it was demonstrated that hyperinsulinemia was associated with significantly increased QT interval and increased risk of sudden death (37). In addition, a recent study revealed that QT prolongation and hypokalemia were common in diabetic patients with severe hypoglycemia, which increased their risk of fatal arrhythmia and death (38). The combination of hyperinsulinemia, symptomatic postprandial hypoglycemia, and low serum potassium levels might thus further increase the propensity for cardiac events in *KCNQ1* LQTS patients.

None of the LQTS patients had previously been diagnosed with reactive hypoglycemia. The clinical symptoms characterizing this patient group, e.g., syncope, have previously been solely ascribed to their arrhythmia.

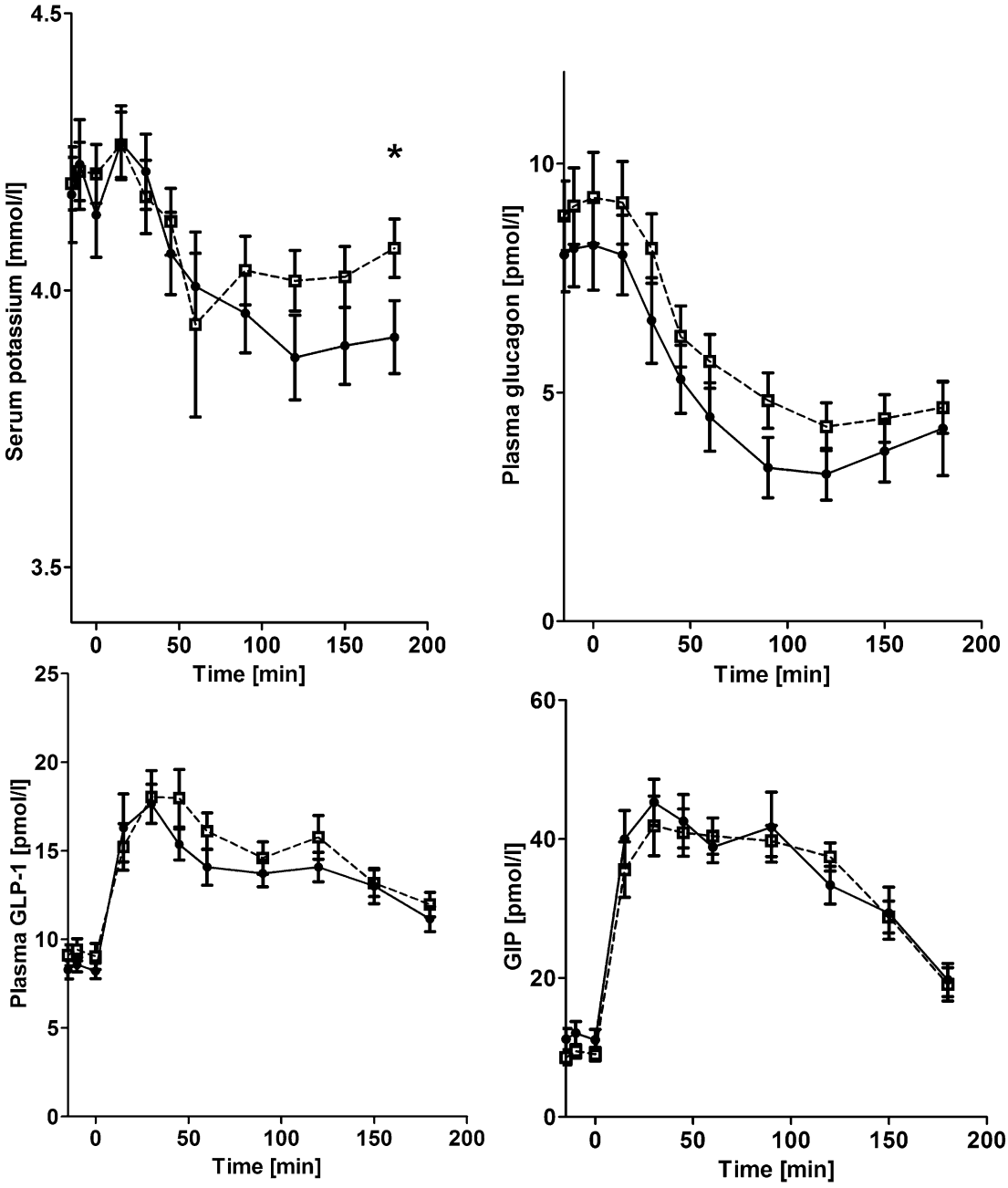

Figure 5—Serum potassium (*upper left panel*), plasma glucagon (*upper right panel*), plasma GLP-1 (*lower left panel*), and plasma GIP (*lower right panel*) levels during an OGTT in 14 patients with *KCNQ1* LQTS due to functional mutations in *KCNQ1* (●) and 28 randomly chosen BMI-, sex-, and age-matched control participants (□). $P_{potassium} < 10^{-4}$ and $P_{glucagon}$, $P_{GLP\text{-}1}$, and $P_{GIP} > 0.05$. *Significantly different time points.

However, syncope could also be a sign of hypoglycemia, and our study suggests that the *KCNQ1* LQTS patients also have symptoms related to hypoglycemia.

The higher sensitivity of the β-cell to increments in plasma glucose, which was a feature of the mutation carriers, is in agreement with the observations found in β-cell studies when blocking KCNQ1 (1,8). Thus, our findings confirm that blocking KCNQ1 increases insulin release in vivo, presumably due to prolonged depolarization of the β-cell causing calcium influx and insulin exocytosis. This observation also correlates with the genome-wide association study observations that intronic SNPs in *KCNQ1* are associated with type 2 diabetes (4,5) and reduced serum insulin levels (6,7), which could be secondary to increased expression of the channel and thereby decreased insulin exocytosis (1). Taken together, these findings underline that the voltage-gated K^+ channel encoded by *KCNQ1* is a key player in insulin secretion.

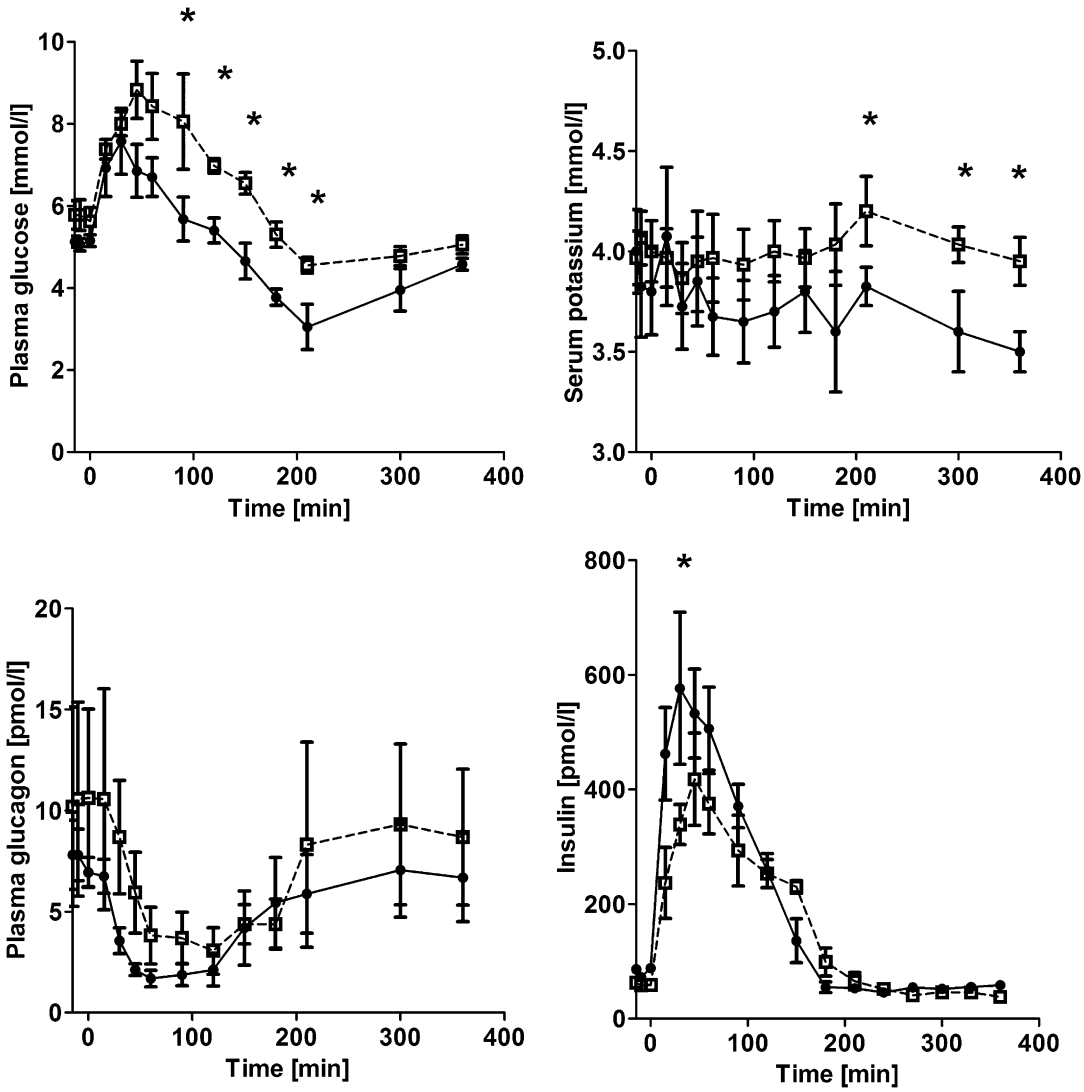

Figure 6—Plasma glucose (*upper left panel*), serum potassium (*upper right panel*), plasma glucagon (*lower left panel*), and serum insulin (*lower right panel*) levels during an extended glucose tolerance test for 6 h in four patients with *KCNQ1* LQTS with functional mutations in *KCNQ1* (●) and four randomly chosen BMI-, sex-, and age-matched control participants (□) (mean ± SEM); $P_{glucose} < 10^{-4}$, $P_{potassium}$ and $P_{insulin} < 10^{-3}$, and $P_{glucagon} > 0.05$. *Significantly different time points.

Pancreatic β-cells express a variety of K^+ channels regulated by voltage (Kv channels) and/or by the intracellular Ca^{2+} concentration (KCa channels). Inhibition of Kv channels with tetraethylammonium extends action potential duration. Consequently, blockade of Kv channels is a potent tool to augment insulin release (39,40). In the absence of KCNQ1 channels, the repolarization is delayed, leaving it to the other K^+ channels to repolarize the β-cell.

One question in the questionnaire included timing of the hypoglycemia event, whereas the other questions do not distinguish between postprandial or fasting symptoms. However, as activation of Kv channels requires membrane depolarization, targeting Kv channels affect insulin secretion only in the presence of elevated glucose concentrations or other depolarizing stimuli (39,40). This is in agreement with our finding that insulin secretion is only abnormally increased postprandially and not during fasting when glucose levels are low.

The mutation carriers exhibited moderately decreased insulin sensitivity during the first hours after glucose stimulation but not in the fasted state. This postprandial insulin resistance might be a protective mechanism against severe postprandial hyperinsulinemic hypoglycemia. The observation is reminiscent of the syndrome of autosomal-dominant hyperinsulinemic hypoglycemia linked to a mutation in the human insulin receptor gene where hyperinsulinemia coexists with a moderate insulin resistance (30).

Our findings suggest that functional *KCNQ1* mutations underlie some cases of "essential" postprandial

hypoglycemia. This syndrome is characterized by appearance of reactive hypoglycemia occurring up until 4 h after food intake without conspicuous cause. Thus, ECG monitoring and genetic testing should be considered when other causes of reactive hypoglycemia (e.g., gastrointestinal surgery or medications) have been excluded.

The glucagon levels were not significantly different between patients and control participants at the time of hypoglycemia, although acute hypoglycemia under normal physiological circumstances will increase glucagon levels. This lack of appropriate glucagon response may be due to the counterregulatory response impairment observed when recurrent hypoglycemia occurs (41), as we indeed observed happened in the patients during the continuous glucose monitoring. Furthermore, a mutated KCNQ1 channel in pancreatic Δ-cells may increase somatostatin secretion, in the same manner as insulin is increased, and thereby inhibit glucagon. Both conditions may contribute to worsen the hypoglycemia. Also, a relatively elevated insulin secretion from pancreatic β-cells of KCNQ1 patients may have suppressed their α-cell glucagon response. Compared with control subjects, the relatively lower glucagon levels in KCNQ1 mutation patients during both OGTTs are consistent with a paracrine effect of β-cell hypersecretion suppressing α-cell glucagon release (42). In addition, studies have shown that lack of another K^+ channel, namely, the ATP-sensitive K^+ channel, causes β-cell depolarization and insulin secretion. This is in contrast to what happens in the α-cell, where the depolarization caused by lack of the ATP-sensitive K^+ channel is associated with reduced rather than increased electrical activity and thereby low glucagon secretion (43). Because α-cells possess a different complement of voltage-gated ion channels involved in action potential generation than β-cells (43), it is likewise plausible that lack of the KCNQ1 channel causes insulin secretion in β-cells but inhibits glucagon secretion from α-cells.

A previous study of carriers of frequent intronic SNPs in *KCNQ1* indicated that impairment of incretin secretion might be involved in the reduced β-cell function among SNP carriers (44). In our study of carriers of functional *KCNQ1* mutations, we do not find any difference in circulating incretin levels between patients and control individuals. This is in agreement with recent studies of L cells (45) and recent human genetic studies (46), indicating that KCNQ1 does not have a major influence on incretin secretion.

Six of 14 patients were in standard long QT type 1 treatment with β-blocking agents. However, all subjects were fasting overnight and free of any medication for at least 24 h before the morning of examination. Furthermore, since there was no difference in patient baseline characteristics or metabolic responses among the group of patients taking β-blocking agents and the group that did not and since studies have shown that β-adrenergic blockade has little effect on glucose regulation (47), the β-blockers are unlikely to explain the metabolic differences between patients and control individuals. In addition, excluding the group of patients taking β-blocking agents and their corresponding control participants from the analyses did not affect the highly significant difference between patients and control participants with regard to all measured metabolic responses.

We did not test stimuli other than glucose or study patients with other known LQTS-causing gene alterations, both of which would be of interest for future studies.

In conclusion, besides having prolonged QT interval, patients with *KCNQ1* LQTS were characterized by hyperinsulinemia upon an oral glucose load, postprandial hypoglycemia, symptoms of clinical hypoglycemia, and lower potassium levels, all of which increase risk of cardiac events. We confirm that the voltage-gated K^+ channel encoded by *KCNQ1* is involved in insulin secretion and suggest that *KCNQ1* mutations may explain some cases of "essential" reactive hypoglycemia.

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Author Contributions. S.S.T. and J.J.H. were responsible for study conception and design, analysis and interpretation of data, drafting the manuscript, revising the manuscript critically for important intellectual content, and final approval of the version to be published. E.I. was responsible for analysis and interpretation of data, revising the manuscript critically for important intellectual content, and final approval of the version to be published. M.C. and A.L. were responsible for study conception and design, revising the manuscript critically for important intellectual content, and final approval of the version to be published. O.P., J.K.K., and T.H. were responsible for study conception and design, analysis and interpretation of data, revising the manuscript critically for important intellectual content, and final approval of the version to be published. S.S.T., O.P., J.J.H., J.K.K., and T.H. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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