KCNQ1 Long QT syndrome patients have hyperinsulinemia and symptomatic hypoglycemia

Running title: Hyperinsulinemia and hypoglycemia in KCNQ1 LQTS

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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 beta cells. Inhibition of KCNQ1 in beta 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-gender-matched control participants and underwent oral glucose tolerance test, hypoglycemia questionnaire and continuous glucose monitoring.

*KCNQ1* mutation carriers showed increased insulin release (AUC:45.6±6.3 vs. 26.0±2.8 min*nmol/l insulin), and beta cell glucose sensitivity and had lower levels of plasma glucose and serum potassium upon oral glucose stimulation and increased hypoglycemic symptoms. Prolonged oral glucose tolerance test in four available patients and matched controls revealed hypoglycemia in carriers after 210 min. (range:1.4–3.6 vs. 4.1–5.3 mmol/l glucose), and 24-hour glucose profiles showed that the patients spent 77±18 min. per 24-hours 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 following an oral glucose challenge, suggesting that *KCNQ1* mutations may explain some cases of “essential” reactive hypoglycemia.
The blood glucose level increases after a meal intake, leading to formation of ATP in the beta-cell, closure of the ATP-dependent potassium channel and thereby a reduction of the ATP-sensitive potassium current, depolarization of the beta-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 beta cells(1) and cardiomyocytes(2). Functional mutations in KCNQ1 lead to KCNQ1 Long QT syndrome (LQTS) with a predicted population prevalence of 1:2000, a disease characterized by a delayed cardiac repolarization, prolonged QT interval on the ECG, syncope, malignant arrhythmias and sudden death(3), but the clinical and physiological significance of functional KCNQ1 mutations in beta cells is unknown. Interestingly, carriers of frequent intronic single nucleotide polymorphisms (SNP)s (rs2237892, rs2237895, rs2237897) in KCNQ1, have increased susceptibility for type 2 diabetes(4;5) and impaired beta cell function(6;7). Recent studies show that inhibition of Kv7.1 in beta cells increases exocytosis and insulin secretion(1), due to delayed repolarization(8) and KCNQ1 knock-down with siRNA similarly increases insulin exocytosis and secretion(1). Over-expression of KCNQ1 in cultured MIN6 cells decreases both glucose, 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 of 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 beta-cell causing increased exocytosis. In this study we show that KCNQ1 LQTS patients have postprandial hyperinsulinemia, 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 gender 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 gender, BMI and age. Six out of fourteen patients were in standard long QT therapy with beta-blocking agents and six out of fourteen patients had experienced syncope (of these six, three were taking beta-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 Ethical Committee of Copenhagen County (H-4-2010-036) and was in accordance with the principles of the Helsinki Declaration.

*Genetics*

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

*Oral glucose tolerance test, electrocardiogram and hypoglycemia questionnaire*
After an overnight fast, blood samples for measurements of plasma glucose, serum insulin, serum C-peptide, serum proinsulin, serum potassium, plasma GLP-1, plasma GIP and plasma glucagon were taken prior to a standard 75 g oral glucose tolerance test (OGTT). Blood sampling was repeated every 15 minutes until 180 minutes 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 Bazett’s 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) (see supplementary table 1) was filled at home by all participants except one patient (F, 56 years) 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, due 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 hours) and continuous glucose measurements with Ipro2 (Medtronic, Watford, UK) for 3 up to 7 days. The group of four out of 14 patients represented those that agreed to participate in additional studies for up to 7 days duration. The OGTT’s were carried out as described above except that they were continued until 6 hours after glucose ingestion. The continuous glucose measurements were conducted according to the manufacturer’s manual (Ipro2, Medtronic, Watford, UK). IPro2 uses a retrospective algorithm to convert 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 (and <2.8), between 3.9 and 7.8, and >7.8 mmol/L, respectively (Table 2). None of the four patients for the follow-up studies were taking beta-blocking agents.

**Biochemical and anthropometric measures**

BMI was calculated as weight (kg) / height (m)$^2$. The percentage of fat was measured with bioimpedance analyzer, Biodynamics BIA 310e (Biodynamics, Seattle, Washington, USA). 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 inter-assay coefficients of variation of <1%. Radioimmunological determinations of fully processed glucagon and total plasma glucagon-like-peptide (GLP) -1 and gastric inhibitory peptide (GIP) were performed as described(19-21). The analytical detection limit was 1 pmol/l and intra- and inter-assay 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 (Perkin-Elmer, Wallac, Turku, Finland). Serum C-peptide concentrations were measured by a time-resolved fluoroimmunoassay (Auto-DELFIA C-peptide kit; Perkin-Elmer/Wallac, Turku, Finland). Total intra- and inter-assay 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 inter-assay coefficients of variation were <4% and 7%, respectively. The analytical detection limit was 0.3 pmol/l. Serum potassium was measured using Vitros 5600 (Otho Clinical Diagnostics, Cedex, France). Total intra- and inter-assay coefficients of variation were <1%. The analytical detection limit was 1 mmol/l.

**Sample size**
OGTT: The aim of the study was to test differences in insulin secretion among 14 KCNQ1 LQTS patients and 28 matched controls. 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 4 KCNQ1 LQTS patients and 4 matched controls. 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 GradPad 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 beta-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 \( \frac{10,000}{\sqrt{(\text{fasting plasma glucose} \times \text{fasting serum insulin}) \times (\text{mean plasma glucose} \times \text{mean serum insulin during OGTT})}} \) (25;26). Fasting homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as \( \frac{(\text{fasting plasma glucose (mmol/l)} \times \text{fasting serum insulin (pmol/l)})}{22.5} \). 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 - 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 beta cell responsiveness to glucose(29).

Statistics

Statistical analysis was carried out with a mixed model ANOVA with repeated measurements (SAS version 9.2 PROC MIXED, Cary, NC) contrasting patient results versus control subjects in matching pairs (SAS version 9.2 PROC MIXED, Cary, NC) and tested with Scheffes post-Hoc test for multiple comparison. Values of insulin were logarithmically transformed before analysis. The data are shown as mean ± SEM. P values are given for the overall ANOVA with asterisks in figures indicating significantly different time points. 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 (Figure 4, supplementary table 1 and 2). The differences in scores between patients and control participants were tested with Students t-test. Regression analyses between the QT interval and glucose, insulin or potassium levels were made using SAS version 9.2 PROC MIXED, Cary, NC A p-value < 0.05 was considered significant.

Results

The KCNQ1 LQTS patients had lower plasma glucose levels compared to matched control participants three hours after glucose ingestion (4.4 ± 0.3 vs. 5.4 ± 0.5 mmol/l glucose, P = 0.03, Figure 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 pro-insulin and higher ISR, P < 10^{-5} (Figure 2). Beta 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}, Figure 3) and so was the insulinogenic index (41.5 ± 6.6
vs. 24.3 ± 3.7, P < 10^{-3}). Hypoglycemia frequency and severity score was significantly higher among patients compared to control participants, P < 10^{-3} (Figure 4 and Supplementary table 1).

There was no difference in HOMA-IR 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 to 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 (Figure 5).

During the OGTT at the time corresponding to insulin peak levels, the patients significantly increased their QT interval by 10 ± 2 ms, as compared to 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-hour OGTT (P > 0.05).

Follow-up studies, including a prolonged oral glucose tolerance test for 6 hours, 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 hours 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} (Figure 6). Also serum potassium levels were lower 3-6 hours after oral glucose load among patients, P < 10^{-3} (Figure 6). The same participants underwent continuous glucose measurements for 3 up to 7 days. Twenty-four hour glucose profiles showed that the KCNQ1 LQTS patients spent 77 ± 18 min. per 24 hours 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 3-5 hours 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 beta-blocking agents (6/14 patients) or patients who had experienced syncope (6/14 patients) and the group that did not (P > 0.8). Furthermore, after exclusion of the group of patients taking beta-blocking agents and their corresponding control participants from the analyses, a similar difference remained between patients and control participants with regards to glucose, insulin and potassium response (P < 0.05, Supplementary figure 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 three hours after glucose ingestion indicating a delayed glycemic reaction to hyperinsulinemia, similar to what has been observed in genetically determined hyperinsulinaemia due to insulin receptor mutations (30). Therefore, we performed a prolonged oral glucose tolerance test for 6 hours in four patients that were available for extended examination. Indeed, we observed that the patients became markedly hypoglycemic three and a half hours after glucose ingestion in contrast to the matched control participants. Consistent with this observation, twenty-four hour glucose profiles showed that the KCNQ1 LQTS patients had
symptomatic hypoglycemic episodes in their own living environment, three to five hours 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 repolarisation 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 2570 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 diabetes 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. However, e.g. 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 beta cell to increments in plasma glucose which was a feature of the mutation carriers is in agreement with the observations found in beta 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 beta-cell causing calcium influx and insulin exocytosis. This observation also correlate 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.

Pancreatic β-cells express a variety of K⁺ channels regulated by voltage (Kv channels) and/or by the intracellular Ca²⁺ concentration (KCa channels). Inhibition of Kv channels with TEA+ 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 beta-cell.

One question in the questionnaire included timing of the hypoglycemia event, whereas the other questions do not distinguish between post-prandial 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 post-prandially 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 co-exists 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 till four hours 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 counter-regulatory 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 delta 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 beta-cells of KCNQ1 patients may have suppressed their alpha-cell glucagon response. As compared to controls, the relatively lower glucagon levels in KCNQ1 mutation patients during both OGTTs are consistent with a paracrine effect of beta-cell hypersecretion suppressing alpha-cell glucagon release (42). In addition, studies have shown that lack of another K+ channel, namely the K_{ATP} channel causes beta-cell depolarization and insulin secretion. This is in contrast to what happens in
the alpha cell, where the depolarization caused by lack of the \( K_{ATP} \) channel, is associated with reduced rather than increased electrical activity and thereby low glucagon secretion\(^{(43)}\). Because alpha-cells possess a different complement of voltage-gated ion channels involved in action potential generation than beta cells \(^{(43)}\), it is likewise plausible that lack of the KCNQ1 channel causes insulin secretion in beta cells, but inhibits glucagon secretion from alpha cells.

A previous study of carriers of frequent intronic SNPs in \( KCNQ1 \) indicated that impairment of incretin secretion might be involved in the reduced beta-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 out of 14 patients were in standard long QT type 1 treatment with beta-blocking agents. However, all subjects were fasting overnight and free of any medication for at least 24 hours before the morning of examination. Furthermore, since there was no difference in patient baseline characteristics or metabolic responses among the group of patients taking beta-blocking agents and the group that did not, and since studies have shown that beta-adrenergic blockade has little effect on glucose regulation\(^{(47)}\), the beta-blockers are unlikely to explain the metabolic differences between patients and control individuals. In addition, excluding the group of patients taking beta-blocking agents and their corresponding control participants from the analyses did not affect the highly significant difference between patients and control participants with regards to all measured metabolic responses.

We did not test other stimuli 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 prolonged QT interval, patients with \textit{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 \textit{KCNQ1} is involved in insulin secretion and suggest that \textit{KCNQ1} mutations may explain some cases of “essential” reactive hypoglycemia.

\textbf{Acknowledgements}

\textit{Author contributions}

SS Torekov 1-5; E Iepsen 2,4,5, M Christiansen 1,4,5; A Linneberg 1,4,5; O Pedersen 1,2,4,5; JJ Holst 1-5; JK. Kanters 1,2,4,5; Torben Hansen 1,2,4,5 were responsible for (1) conception and design, (2) analysis and interpretation of data, (3) drafting the article, (4) revising it critically for important intellectual content; and (5) final approval of the version to be published.

\textit{Statements of assistance}

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\textit{Guarantors}

SS Torekov, O Pedersen, JJ Holst, JK. Kanter and Torben Hansen

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Conflict of interests

No conflicts of interest relevant for the present studies.

Disclosure

The study was presented at EADS, Barcelona, September 2013
### Table 1 Baseline characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>KCNQ1 Long QT patients</th>
<th>Patient subgroup for follow up</th>
<th>Control subgroup for follow up</th>
<th>Control subgroup vs whole group</th>
<th>$P_{patient}$</th>
<th>$P_{subgroup}$</th>
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<tbody>
<tr>
<td>N (m/w)</td>
<td>14 (5/9)</td>
<td>4 (2/2)</td>
<td>28 (10/18)</td>
<td>4 (2/2)</td>
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<tr>
<td>Age (years)</td>
<td>44 ± 3</td>
<td>46 ± 7</td>
<td>46 ± 2</td>
<td>47 ± 7</td>
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<td>0.9</td>
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<tr>
<td>BMI (kg/m$^2$)</td>
<td>28.5 ± 1.7</td>
<td>30.2 ± 1.4</td>
<td>29.0 ± 1.1</td>
<td>30.5 ± 1.0</td>
<td>1</td>
<td>0.7</td>
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<tr>
<td>Fat %</td>
<td>31.1 ± 1.6</td>
<td>32.4 ± 1.6</td>
<td>31.4 ± 1.3</td>
<td>31.7 ± 1.7</td>
<td>0.9</td>
<td>0.8</td>
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<tr>
<td>QT$_B$-interval (ms)</td>
<td>481 ± 8</td>
<td>480 ± 9</td>
<td>425 ± 4</td>
<td>423 ± 7</td>
<td>&lt;0.0001</td>
<td>0.9</td>
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</table>
Table 2 Twenty-four hour glucose profiles during continuous glucose measurements for 3 up to 7 days

<table>
<thead>
<tr>
<th>Blood glucose level</th>
<th>KCNQ1 Long QT patients</th>
<th>Control participants</th>
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</thead>
<tbody>
<tr>
<td>mmol/l</td>
<td>Minutes ± SEM per 24 hours (Percentage ± SEM per 24 hours)</td>
<td>Minutes ± SEM per 24 hours (Percentage ± SEM per 24 hours)</td>
</tr>
<tr>
<td>&gt;7.8</td>
<td>30 ± 10 (2.1 ± 0.7%)</td>
<td>59 ± 18 (4.1 ± 1.4%)</td>
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<tr>
<td>3.9-7.8</td>
<td>1,333 ± 199 (92.6 ± 14.1%)</td>
<td>1,381 ± 251 (95.9 ± 17.4%)</td>
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<tr>
<td>&lt;3.9*</td>
<td>77 ± 19 (5.3 ± 1.3%)</td>
<td>0</td>
</tr>
<tr>
<td>&lt;2.8*</td>
<td>36 ± 10 (2.5 ± 0.7%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Mean minutes ± SEM and mean percentage ± SEM per 24 hours spent in different glucose states during continuous glucose measurements for 3 up to 7 days in four patients and four matched control participants. *P<0.05 when analyzed patients vs. control participants for each blood glucose level state.
Figure text

Figure 1
Plasma glucose levels during an oral glucose tolerance test in 14 patients with KCNQ1 long QT syndrome due to functional mutations in KCNQ1 (closed circles) and 28 randomly chosen BMI-, gender and age-matched control participants (open squares) (Mean±SEM), P = 0.03.

Figure 2
Serum insulin (upper left panel), C-peptide (upper right panel), pro-insulin (lower left panel) levels and insulin secretion rate (ISR) (lower right panel) during an oral glucose tolerance test in 14 patients with KCNQ1 long QT syndrome due to functional mutations in KCNQ1 (closed circles) and 28 randomly chosen BMI-, gender-, and age-matched control participants (open squares) (Mean±SEM), P < $10^{-5}$.

Figure 3
Illustration of the beta cell responsiveness to glucose. The calculated insulin secretion rates (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 beta cell responsiveness to glucose. For illustrative purposes the mean values of glucose at time 0, 15, 30 and 45 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 (closed circles) vs. control individuals (open squares) (2.8 ± 0.3 vs. 1.9 ± 0.1, P < $10^{-5}$).
Figure 4

Hypoglycemia questionnaire score (see supplementary table 1) of 13 patients with KCNQ1 long QT syndrome due to functional mutations in KCNQ1 (black bars) and 26 randomly chosen BMI-gender and age-matched control participants (white bars). 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 table 1 and 2). The differences in scores between patients and control participants were tested with Students t-test. (Mean±SEM), $P_{\text{frequency}} < 10^{-3}$, $P_{\text{severity}} < 10^{-4}$

Figure 5

Serum potassium (upper left panel), plasma glucagon (upper right panel), plasma GIP (lower left panel) and plasma GLP-1 (lower right panel) levels, during an oral glucose tolerance test in 14 patients with KCNQ1 long QT syndrome due to functional mutations in KCNQ1 (closed circles) and 28 randomly chosen BMI-gender and age-matched control participants (open squares). $P_{\text{potassium}} < 10^{-4}$ and $P_{\text{glucagon}}, P_{\text{GLP-1}}$ and $P_{\text{GIP}} > 0.05$.

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 six hours in 4 patients with KCNQ1 long QT syndrome with functional mutations in KCNQ1 (closed circles) and 4 randomly chosen BMI-gender and age-matched control participants (open squares) (Mean±SEM), $P_{\text{glucose}} < 10^{-4}$ and $P_{\text{potassium}}$ and $P_{\text{insulin}} < 10^{-3}$, $P_{\text{glucagon}} > 0.05$. 
Figure 1

Plasma glucose [mmol/l] vs. Time [min]

- Solid line: Control group
- Dashed line: Diabetes group

* Significant difference at 0.05 level
Figure 2

![Graphs showing changes in serum insulin, C-peptide, proinsulin, and insulin secretion rate over time.](image)

- Serum insulin [pmol/l]
- Serum C-peptide [pmol/l]
- Serum proinsulin [pmol/l]
- Insulin secretion rate [pmol/kg\(^{-1}\) min\(^{-1}\)]

Time [min]:
- 0 50 100 150 200

Values are presented with error bars indicating variability.
Figure 3

Mean plasma glucose [mmol/l] vs. Insulin secretion rate [pmol/kg⁻¹·min⁻¹].

- Solid line: Insulin secretion rate in healthy individuals.
- Dashed line: Insulin secretion rate in diabetic patients.
Figure 4

Hypoglycemia score

- Frequency
- Severity

**

***

Diabetes
Figure 5
Figure 6

- Plasma glucose [mmol/l]
- Serum potassium [mmol/l]
- Plasma glucagon [pmol/l]
- Insulin [pmol/l]
Reference List


Supplementary appendix KCNQ1 Long QT syndrome patients have hyperinsulinemia and symptomatic hypoglycemia

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### Supplementary table 1 Hypoglycemia questionnaire.

<table>
<thead>
<tr>
<th>Question</th>
<th>% KCNQ1 patients / % Control participant&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Frequency&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>Severity&lt;sup&gt;§&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never (0)</td>
<td>Rarely (1)</td>
<td>Occasionally (2)</td>
</tr>
<tr>
<td>1. Cravings for sweets</td>
<td>0/8</td>
<td>8/12</td>
<td>62/50</td>
</tr>
<tr>
<td>2. Irritability if a meal is missed*</td>
<td>15/58</td>
<td>23/23</td>
<td>23/12</td>
</tr>
<tr>
<td>3. Tired or weak if a meal is missed*</td>
<td>8/46</td>
<td>23/19</td>
<td>31/23</td>
</tr>
<tr>
<td>4. Often hungry*</td>
<td>0/8</td>
<td>31/19</td>
<td>46/15</td>
</tr>
<tr>
<td>5. Dizziness when standing suddenly*</td>
<td>8/50</td>
<td>38/35</td>
<td>38/15</td>
</tr>
<tr>
<td>6. Generally dizzy*</td>
<td>31/81</td>
<td>38/15</td>
<td>8/4</td>
</tr>
<tr>
<td>7. Tired or uncomfortable some hours after a meal*</td>
<td>38/65</td>
<td>31/15</td>
<td>23/15</td>
</tr>
<tr>
<td>8. Trouble concentrating</td>
<td>15/35</td>
<td>77/38</td>
<td>0/27</td>
</tr>
<tr>
<td>9. Heart palpitations</td>
<td>23/62</td>
<td>62/19</td>
<td>8/19</td>
</tr>
<tr>
<td>10. Occasional shakiness</td>
<td>46/62</td>
<td>46/31</td>
<td>8/8</td>
</tr>
<tr>
<td>11. Occasional blurry vision*</td>
<td>15/62</td>
<td>54/23</td>
<td>8/15</td>
</tr>
<tr>
<td>12. Depression or mood swings*</td>
<td>46/46</td>
<td>38/31</td>
<td>0/23</td>
</tr>
<tr>
<td>13. Frequent anxiety or nervousness</td>
<td>54/73</td>
<td>38/23</td>
<td>0/4</td>
</tr>
<tr>
<td>14. Aggression*</td>
<td>31/69</td>
<td>54/27</td>
<td>0/4</td>
</tr>
<tr>
<td>15. Night awakening*</td>
<td>8/31</td>
<td>23/12</td>
<td>38/31</td>
</tr>
<tr>
<td>16. Night sweats*</td>
<td>15/38</td>
<td>8/27</td>
<td>38/12</td>
</tr>
<tr>
<td>17. Frequent sweating*</td>
<td>8/42</td>
<td>38/12</td>
<td>31/27</td>
</tr>
</tbody>
</table>

<sup>†</sup> Numbers in boxes indicate the percentage of 13 KCNQ1 LQTS patients and 26 control participants that gave the particular answer with percentage of patients written before / and the percentage of control participants written after /.

<sup>‡</sup> Frequency point score: Never: 0 point; Rarely: 1 point; Occasionally: 2 points; Usually: 3 points; Always: 4 points. The total frequency score of each participant was calculated from the sum of the point scores given for each question.

<sup>§</sup> Severity point score: Mild: 1 point; Moderate: 2 points; Severe: 3 points. The total severity score of each participant was calculated from the sum of the point scores given for each question.

*P<0.05. For each specific question, the difference in scores between patients and control participants was tested with Student’s t-test (see supplementary table 2). The total mean frequency and severity point score of patients and control participants, calculated from the sum of the point score given for each question, are shown in Figure 4.
Supplementary table 2. Hypoglycaemia scores for each specific question

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Cravings for sweets</td>
<td>2.5 (0.3)</td>
<td>2.1 (0.2)</td>
</tr>
<tr>
<td>2. Irritability if a meal is missed*</td>
<td>2.2 (0.4)</td>
<td>0.7 (0.2)</td>
</tr>
<tr>
<td>3. Tired or weak if a meal is missed*</td>
<td>2.1 (0.3)</td>
<td>1.1 (0.2)</td>
</tr>
<tr>
<td>4. Often hungry*</td>
<td>2.0 (0.2)</td>
<td>1.8 (0.1)</td>
</tr>
<tr>
<td>5. Dizziness when standing suddenly*</td>
<td>1.7 (0.3)</td>
<td>0.7 (0.1)</td>
</tr>
<tr>
<td>6. Generally dizzy*</td>
<td>1.3 (0.4)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>7. Tired or uncomfortable some hours after a meal*</td>
<td>1.0 (0.3)</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>8. Trouble concentrating</td>
<td>1.0 (0.2)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>9. Heart palpitations</td>
<td>1.0 (0.3)</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>10. Occasional shakiness</td>
<td>0.6 (0.2)</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>11. Occasional blurry vision*</td>
<td>1.4 (0.3)</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>12. Depression or mood swings</td>
<td>0.9 (0.4)</td>
<td>0.8 (0.2)</td>
</tr>
<tr>
<td>13. Frequent anxiety or nervousness</td>
<td>0.6 (0.3)</td>
<td>0.3 (0.1)</td>
</tr>
<tr>
<td>14. Aggression*</td>
<td>1.1 (0.4)</td>
<td>0.4 (0.1)</td>
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<td>15. Night awakening*</td>
<td>2.0 (0.3)</td>
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</tr>
</tbody>
</table>

*P<0.05. For each specific question the difference in scores between patients and control participants were tested with Students t-test (mean ±SEM).
The total mean frequency and severity point score of patients and control participants, calculated from the sum of the point score given for each question, are shown in Figure 4.
Supplementary figure 1

Plasma glucose, serum insulin and serum potassium levels during an oral glucose tolerance test in 8 treatment naïve patients with *KCNQ1* long QT syndrome due to functional mutations in *KCNQ1* (closed circles) and 16 randomly chosen BMI-gender and age-matched control participants (open squares) (Mean±SEM), *P* < 0.05.
Supplementary figure 1

**Plasma glucose [mmol/l]**

**Serum insulin [pmol/l]**

**Serum potassium [mmol/l]**