# Association of Type 2 Diabetes Candidate Polymorphisms in *KCNQ1* With Incretin and Insulin Secretion

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**OBJECTIVE—***KCNQ1* gene polymorphisms are associated with type 2 diabetes. This linkage appears to be mediated by altered β-cell function. In an attempt to study underlying mechanisms, we examined the effect of four *KCNQ1* single nucleotide polymorphisms (SNPs) on insulin secretion upon different stimuli.

**RESEARCH DESIGN AND METHODS—**We genotyped 1,578 nondiabetic subjects at increased risk of type 2 diabetes for rs151290, rs2237892, rs2237895, and rs2237897. All participants underwent an oral glucose tolerance test (OGTT); glucagon-like peptide (GLP)-1 and gastric inhibitory peptide secretion was measured in 170 participants. In 519 participants, a hyperinsulinemic-euglycemic clamp was performed, in 314 participants an intravenous glucose tolerance test (IVGTT), and in 102 subjects a hyperglycemic clamp combined with GLP-1 and arginine stimuli.

**RESULTS—**rs151290 was nominally associated with 30-min C-peptide levels during OGTT, first-phase insulin secretion, and insulinogenic index after adjustment in the dominant model (all $P \leq 0.01$). rs2237892, rs2237895, and rs2237897 were nominally associated with OGTT-derived insulin secretion indexes (all $P < 0.05$). No SNPs were associated with β-cell function during intravenous glucose or GLP-1 administration. However, rs151290 was associated with glucose-stimulated gastric inhibitory polypeptide and GLP-1 increase after adjustment in the dominant model ($P = 0.0042$ and $P = 0.0198$, respectively). No associations were detected between the other SNPs and basal or stimulated incretin levels (all $P \geq 0.05$).

**CONCLUSIONS—**Common genetic variation in *KCNQ1* is associated with insulin secretion upon oral glucose load in a German population at increased risk of type 2 diabetes. The discrepancy between orally and intravenously administered glucose seems to be explained not by altered incretin signaling but most likely by changes in incretin secretion. ***Diabetes*** **57:1715–1720, 2009**

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Recent genome-wide association (GWA) studies confirmed the significance of established candidate gene regions for type 2 diabetes, i.e., *PPARã*, *KCNJ11*, *TCF7L2*, and *WFS1*, and also revealed several novel type 2 diabetes susceptibility loci, i.e., *SLC30A8*, *HHEX*, *CDKAL1*, *IGF2BP2*, and *CDKN2A/B*, none of which were considered as functional candidates (1–5). Comprehensive metabolic analysis of genotyped cohorts, comprising measurement of insulin sensitivity and insulin secretion with state-of-the-art methods, revealed that the novel variants influence insulin secretion but show little, if any, impact on insulin sensitivity (6–11).

Two recent GWA studies identified *KCNQ1* as a novel diabetes susceptibility gene (12–13). Similar to the other novel gene variants that are associated with type 2 diabetes, the *KCNQ1* risk alleles for type 2 diabetes also appear to be associated with impaired pancreatic β-cell function as assessed by fasting state– and oral glucose tolerance test (OGTT)-derived indexes of insulin secretion (13).

*KCNQ1* contains 19 exons and spans more than 400 kb on chromosome 11p15.5 (14). The *KCNQ1* gene encodes the pore-forming α-subunit of the voltage-gated $K^+$ channel (KvLQT1), which plays an important role in controlling the ventricular repolarization process (15). Mutations in *KCNQ1* have been associated with inherited cardiac disorders, such as long QT syndrome and familial atrial fibrillation. The long QT syndrome may occur in a recessive form that is associated with deafness (Jervell and Lange-Nielsen syndrome) or in an autosomal dominant variant not associated with deafness (Romano-Ward syndrome) (16). In addition to the heart and cochlea, KCNQ1 is ubiquitously expressed in epithelial cells, including the exocrine and endocrine pancreas (17). KCNQ1 was reported previously to be expressed in insulin-secreting INS-1 cells, and inhibition of this potassium channel by the sulfonamide analog 293B was found to significantly increase insulin secretion in the presence of tolbutamide (18).

The aim of the present study was to investigate the influence of common type 2 diabetes–associated *KCNQ1* single nucleotide polymorphisms (SNPs) on insulin secretion kinetics in response to orally and intravenously administered glucose during an OGTT and intravenous glucose tolerance test (IVGTT) as well as a hyperglycemic clamp combined with glucagon-like peptide (GLP)-1 and arginine administration.

## RESEARCH DESIGN AND METHODS

We studied 1,578 nondiabetic participants at an increased risk for type 2 diabetes due to family history of diabetes (first-degree relatives of type 2 diabetic patients), history of gestational diabetes, overweight, impaired fast-

TABLE 1
Clinical characteristics of the study population

| | |
|---|---|
| Sex (female/male) | 1,044/534 |
| IFG/IGT/IFG and IGT | 164/152/123 |
| Age (years) | 40 ± 13 |
| BMI (kg/m$^2$) | 28.9 ± 8.2 |
| Waist circumference (cm) | 94 ± 17 |
| Fasting glucose (mmol/l) | 5.11 ± 0.55 |
| Glucose: 120-min OGTT (mmol/l) | 6.27 ± 1.66 |
| Fasting insulin (pmol/l) | 63.7 ± 52.9 |
| Insulin: 30-min OGTT (pmol/l) | 493.5 ± 392.7 |

Data are $n$ or means ± SD. IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

ing glucose, or impaired glucose tolerance determined in an OGTT (Table 1). Subjects were recruited from an ongoing study on the pathophysiology of type 2 diabetes (19). A subset of 519 participants was studied by a hyperinsulinemic-euglycemic clamp, 314 participants by an IVGTT, and 102 subjects by a hyperglycemic clamp combined with GLP-1 and arginine stimuli. First-degree relatedness among subjects was less than 1%. Informed written consent for all studies was obtained from all participants, and the local ethics committee approved the protocols.

Subjects were genotyped for rs151290, rs2237892, rs2237895, and rs2237897 (all located in intron 15) in the *KCNQ1* gene. rs2237897 and rs2237895 showed the strongest association with type 2 diabetes in a recent study (12). In the third screening of another study, rs151290 and rs2237895 were found to be most significantly associated with type 2 diabetes (13). As rs2237895 was already included in the SNPs chosen from the study by Unoki et al. (12), the SNP with the strongest association with type 2 diabetes in the replication study by Yasuda et al. (13), rs2237892, was additionally picked.

Genotyping was done using the TaqMan assay (Applied Biosystems, Forster City, CA). The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 7000, and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems). Genotyping for rs2237897 using the TaqMan assay was successful only in major allele carriers. Therefore, in those subjects for whom the TaqMan assay failed, genotypes were directly determined by bidirectional sequencing.

Quality control was performed as described previously (19). Overall, genotyping success rate was 99.9% (100% for rs151290 and rs2237895 and 99.9% for rs2237892 and rs2237897), and the error rate was 0% (3.2% of all samples were regenotyped by bidirectional sequencing). For OGTT, IVGTT, and hyperinsulinemic-euglycemic clamp, the assays were performed as previously described in detail (10).

The hyperglycemic clamp, combined with GLP-1 and arginine administration, was performed as described previously (20). After 120 min of hyperglycemic clamp at 10 mmol/l, a bolus of GLP-1 (4.5 pmol/kg) was given [human GLP-1 (7–36)amide; Poly Peptide, Wolfenbüttel, Germany], followed by a continuous GLP-1 infusion (1.5 pmol · kg$^{-1}$ · min$^{-1}$) during the next 80 min. At 180 min, a bolus of 5 g arginine hydrochloride (Pharmacia & Upjohn, Erlangen, Germany) was injected over 45 s while the GLP-1 infusion was continued. Blood for the measurement of glucose, insulin, and C-peptide was taken at −30, −15, 0, 2.5, 5, 7.5, 10, 20, 40, 60, 80, 100, 120, 125, 130, 140, 150, 160, 170, 180, 182.5, 185, 187.5, 190, and 200 min. This clamp allows measurement of different aspects of stimulus-secretion coupling: first and second phases of glucose-induced insulin secretion, GLP-1–induced insulin secretion, and the response to additional arginine administration.

Plasma glucose, insulin, and C-peptide concentrations were measured as described previously (19). GLP-1 and gastric inhibitory polypeptide (GIP) immunoreactivitiy were determined using radioimmunoassays specific for the COOH-terminal of the peptides (21,22). To avoid incretin degradation, venous blood was drawn into chilled tubes containing EDTA and aprotinin (Trasylol; 20,000 kallikrein inhibitor units/ml, 200 μl per 10 ml blood; Bayer, Leverkusen, Germany) and kept on ice. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at −20°C. BMI and waist and hip circumferences were measured as described earlier (19).

First-phase insulin secretion (picomoles per liter), insulin sensitivity from the OGTT (arbitrary units), and clamp-derived insulin sensitivity (arbitrary units) were calculated as reported previously (19). Insulinogenic index was assessed by (insulin at 30 min − insulin at 0 min)/(glucose at 30 min − glucose at 0 min). Insulin secretion during the IVGTT was assessed as the sum of C-peptide levels and insulin levels, respectively, during the first 10 min after glucose administration. Insulin secretion during the hyperglycemic clamp was calculated as reported previously using insulin levels determined during the clamp (20). Fold increase of incretins during OGTT was assessed by the ratio of the 30-min incretin value to the basal incretin value.

**Statistical analyses.** Data are means ± SD. Log-transformation of metabolic variables was performed before simple and multivariate linear regression analyses. Distribution was tested for normality using the Shapiro-Wilk W test. The secretion indexes were compared using multivariate regression models. In these models, the trait was the dependent variable, whereas age, sex, BMI, insulin sensitivity, and genotype were the independent variables. To account for the number of SNPs tested and the number of independent traits analyzed (anthropometrics, insulin sensitivity, and insulin secretion) in the OGTT study, a Bonferroni-corrected α-level of $P < 0.00425$ was considered statistically significant. Given that the IVGTT study, the hyperglycemic clamp, and measurement of incretin levels were hypothesis driven, we considered only the number of SNPs tested resulting in a Bonferroni-corrected α-level of $P = 0.0127$. The statistical software package JMP 7.0 (SAS Institue, Cary, NC) was used. In the dominant model, dependent on the SNP tested, the OGTT study was sufficiently powered (1-β > 0.8) to detect effect sizes as small as 0.13–0.24 (one-tailed $t$ test), the hyperinsulinemic-euglycemic clamp 0.23–0.49, the IVGTT study 0.29–0.62, and the combined hyperglycemic clamp 0.53–0.98. Power calculation was performed using G*power software available at http://www.psycho.uni-duesseldorf.de/aap/projects/gpower. Hardy-Weinberg equilibrium was tested using the $\chi^2$ test.

## RESULTS

**Characterization and genotyping of a German population at increased risk for type 2 diabetes.** We genotyped 1,578 nondiabetic subjects from the southwest of Germany whose clinical characteristics are presented in Table 1. Of these subjects, 68.1% had a family history of diabetes, i.e., at least one second-degree relative with type 2 diabetes. The observed minor allele frequency (MAF) and the MAF published by HapMap were 0.208 and 0.217, respectively, for rs151290, 0.064 and 0.075 for rs2237892, and 0.037 and 0.051 for rs2237897. Whereas the observed MAF for rs2237895 was 0.427, an MAF for this SNP was not published by HapMap. All allele frequencies were in Hardy-Weinberg equilibrium ($\chi^2$ test, $P > 0.05$).

**Association of genetic variation in *KCNQ1* with anthropometric and metabolic data.** The four SNPs were not associated with anthropometric data, such as BMI, waist circumference, and body fat content, except for a nominal association between rs2237895 and BMI in the additive model only ($P = 0.0252$; Table 2). rs151290 was nominally associated with 30-min C-peptide levels during OGTT, first-phase insulin secretion, and the insulinogenic index ($P = 0.0072$, $P = 0.0072$, and $P = 0.0104$, respectively) after adjustment for sex, age, BMI, and insulin sensitivity (Table 2 and Fig. 1*A*). rs2237892 was significantly associated with 30-min insulin levels during OGTT ($P = 0.0010$) and nominally with 30-min C-peptide concentrations during OGTT and the insulinogenic index ($P = 0.0330$ and 0.0472, respectively) after adjustment for sex, age, BMI, and insulin sensitivity in the dominant model. rs2237895 was nominally associated with 30-min C-peptide levels during OGTT, first-phase insulin secretion, and the insulinogenic index ($P = 0.0442$, $P = 0.0410$, and $P = 0.0409$, respectively) after adjustment for sex, age, BMI, and insulin sensitivity in the dominant model. rs2237897 was nominally associated with 30-min C-peptide levels during OGTT ($P = 0.0478$) after adjustment for sex, age, BMI, and insulin sensitivity in the dominant model. Whereas indexes of insulin secretion were improved in minor allele carriers of rs151290, rs2237892, and rs2237897, minor allele carriers of rs2237895 depicted reduced insulin secretion. Nominal associations were found between rs2237897 and fasting insulin and OGTT-derived insulin sensitivity ($P = 0.0388$ and 0.0340, respectively) after adjustment for age, sex, and BMI in the dominant model.

TABLE 2
Associations of *KCNQ1* SNPs rs151290, rs2237892, rs2237895, and rs2237897 with anthropometric and metabolic traits

| | rs151290 (0.208) | | | | | rs2237892 (0.064) | | | | | rs2237895 (0.427) | | | | | rs2237897 (0.037) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CC | CA | AA | $P_{add}$ | $P_{dom}$ | CC | AA | TT | $P_{add}$ | $P_{dom}$ | AA | AC | CC | $P_{add}$ | $P_{dom}$ | CC | CT | TT | $P_{add}$ | $P_{dom}$ |
| *n* | 995 | 508 | 75 | — | — | 1,384 | 183 | 10 | — | — | 521 | 765 | 292 | — | — | 1,463 | 111 | 3 | — | — |
| Age (years) | 39 ± 13 | 40 ± 14 | 38 ± 14 | 0.5 | 0.7 | 40 ± 13 | 39 ± 14 | 34 ± 12 | 0.4 | 0.5 | 39 ± 13 | 40 ± 13 | 39 ± 14 | 0.0091 | 0.05 | 40 ± 13 | 38 ± 15 | 37 ± 15 | 0.2 | 0.11 |
| BMI (kg/m$^2$) | 28.7 ± 8.1 | 29.1 ± 8.5 | 29.1 ± 7.3 | 0.6 | 0.4 | 28.8 ± 8.3 | 29.1 ± 7.7 | 31.2 ± 6.6 | 0.3 | 0.3 | 29.2 ± 8.1 | 29.0 ± 8.5 | 27.8 ± 7.6 | 0.0252 | 0.08 | 28.8 ± 8.2 | 29.6 ± 8.1 | 34.7 ± 6.8 | 0.16 | 0.15 |
| Waist circumference (cm) | 93 ± 17 | 95 ± 18 | 95 ± 17 | 0.4 | 0.17 | 94 ± 17 | 94 ± 17 | 99 ± 15 | 0.4 | 0.4 | 94 ± 17 | 94 ± 18 | 92 ± 17 | 0.09 | 0.10 | 94 ± 17 | 95 ± 17 | 103 ± 23 | 0.2 | 0.13 |
| Body fat (%) | 30.7 ± 10.8 | 31.4 ± 11.0 | 31.9 ± 11.0 | 0.2 | 0.3 | 30.8 ± 10.9 | 32.0 ± 10.7 | 32.8 ± 8.7 | 0.17 | 0.2 | 31.6 ± 10.8 | 30.8 ± 11.0 | 30.5 ± 10.5 | 0.17 | 0.07 | 30.9 ± 10.8 | 32.6 ± 11.0 | 38.3 ± 5.2 | 0.19 | 0.4 |
| Fasting glucose (mmol/l) | 5.11 ± 0.56 | 5.09 ± 0.52 | 5.15 ± 0.54 | 0.5 | 0.6 | 5.10 ± 0.55 | 5.12 ± 0.52 | 5.05 ± 0.52 | 0.0457 | 0.7 | 5.09 ± 0.54 | 5.10 ± 0.53 | 5.13 ± 0.61 | 0.9 | 0.8 | 5.11 ± 0.55 | 5.07 ± 0.54 | 5.45 ± 0.71 | 0.6 | 0.7 |
| Glucose: 120-min OGTT (mmol/l) | 6.32 ± 1.66 | 6.14 ± 1.64 | 6.52 ± 1.64 | 0.0035 | 0.0303 | 6.26 ± 1.65 | 6.31 ± 1.66 | 6.96 ± 2.20 | 0.0121 | 0.14 | 6.18 ± 1.63 | 6.27 ± 1.66 | 6.41 ± 1.70 | 0.5 | 0.6 | 6.26 ± 1.65 | 6.31 ± 1.72 | 7.39 ± 2.60 | 0.7 | 0.9 |
| Fasting insulin (pmol/l) | 62.8 ± 48.8 | 64.2 ± 59.0 | 72.6 ± 61.3 | 0.13 | 0.5 | 62.6 ± 51.4 | 70.8 ± 62.54 | 82.7 ± 67.0 | 0.18 | 0.7 | 65.4 ± 51.4 | 63.3 ± 55.7 | 61.8 ± 47.9 | 0.06 | 0.0198 | 62.9 ± 52.8 | 73.6 ± 54.4 | 101 ± 48.5 | 0.11 | 0.0388 |
| Insulin: 30-min OGTT (pmol/l) | 477 ± 369 | 512 ± 416 | 584 ± 508 | 0.18 | 0.2 | 480 ± 379 | 589 ± 473 | 565 ± 433 | 0.0027 | 0.0010 | 528 ± 424 | 487 ± 380 | 449 ± 362 | 0.18 | 0.08 | 484 ± 384 | 616 ± 486 | 592 ± 368 | 0.12 | 0.09 |
| First-phase insulin secretion (pmol/l) | 1,235 ± 782 | 1,322 ± 906 | 1,433 ± 1,085 | 0.0203 | 0.0072 | 1,245 ± 811 | 1,470 ± 1,024 | 1,445 ± 900 | 0.12 | 0.11 | 1,343 ± 899 | 1,250 ± 824 | 1,205 ± 771 | 0.09 | 0.0410 | 1,253 ± 824 | 1,532 ± 1,027 | 1,536 ± 526 | 0.2 | 0.10 |
| Insulinogenic index (pmol/mmol) | 137 ± 398 | 157 ± 174 | 160 ± 142 | 0.0246 | 0.0104 | 140 ± 350 | 174 ± 160 | 136 ± 68 | 0.14 | 0.0472 | 171 ± 263 | 126 ± 413 | 144 ± 153 | 0.06 | 0.0409 | 145 ± 323 | 136 ± 443 | 139 ± 42 | 0.4 | 0.3 |
| C-peptide$_{sum}$ 0–10 min IVGTT (pmol/l)* | 8,357 ± 3,544 | 8,355 ± 3,321 | 9,508 ± 7,534 | 0.9 | 0.8 | 8,299 ± 3,423 | 9,189 ± 5,624 | — | 0.9 | 0.9 | 8,776 ± 4,646 | 8,208 ± 3,313 | 8,432 ± 3,426 | 0.5 | 0.4 | 8,286 ± 3,399 | 10772 ± 7,904 | — | 0.4 | 0.4 |
| ISI: OGTT (U) | 16.3 ± 10.7 | 16.6 ± 10.9 | 14.3 ± 9.6 | 0.0330 | 0.4 | 16.6 ± 10.9 | 14.5 ± 9.2 | 15.0 ± 14.5 | 0.3 | 1.0 | 16.1 ± 10.7 | 16.3 ± 10.8 | 16.6 ± 10.7 | 0.08 | 0.0245 | 16.5 ± 10.8 | 14.4 ± 10.5 | 9.3 ± 8.2 | 0.10 | 0.0340 |
| ISI: clamp (U)† | 0.089 ± 0.060 | 0.080 ± 0.047 | 0.068 ± 0.027 | 0.3 | 0.14 | 0.087 ± 0.055 | 0.075 ± 0.056 | 0.134 | 0.4 | 0.3 | 0.088 ± 0.055 | 0.080 ± 0.056 | 0.095 ± 0.050 | 0.4 | 0.3 | 0.086 ± 0.054 | 0.082 ± 0.064 | — | 0.5 | 0.5 |

Data are means ± SD unless otherwise indicated. For statistical analysis, data were log transformed. Anthropometric data were adjusted for sex and age. Indexes of insulin sensitivity were adjusted for sex, age, and BMI. Indexes of insulin secretion were adjusted for sex, age, BMI, and insulin sensitivity. ISI, insulin sensitivity index; $P_{add}$, additive model; $P_{dom}$, dominant model. *IVGTT data were available from 314 subjects. †ISI (clamp) data were available from 519 subjects.

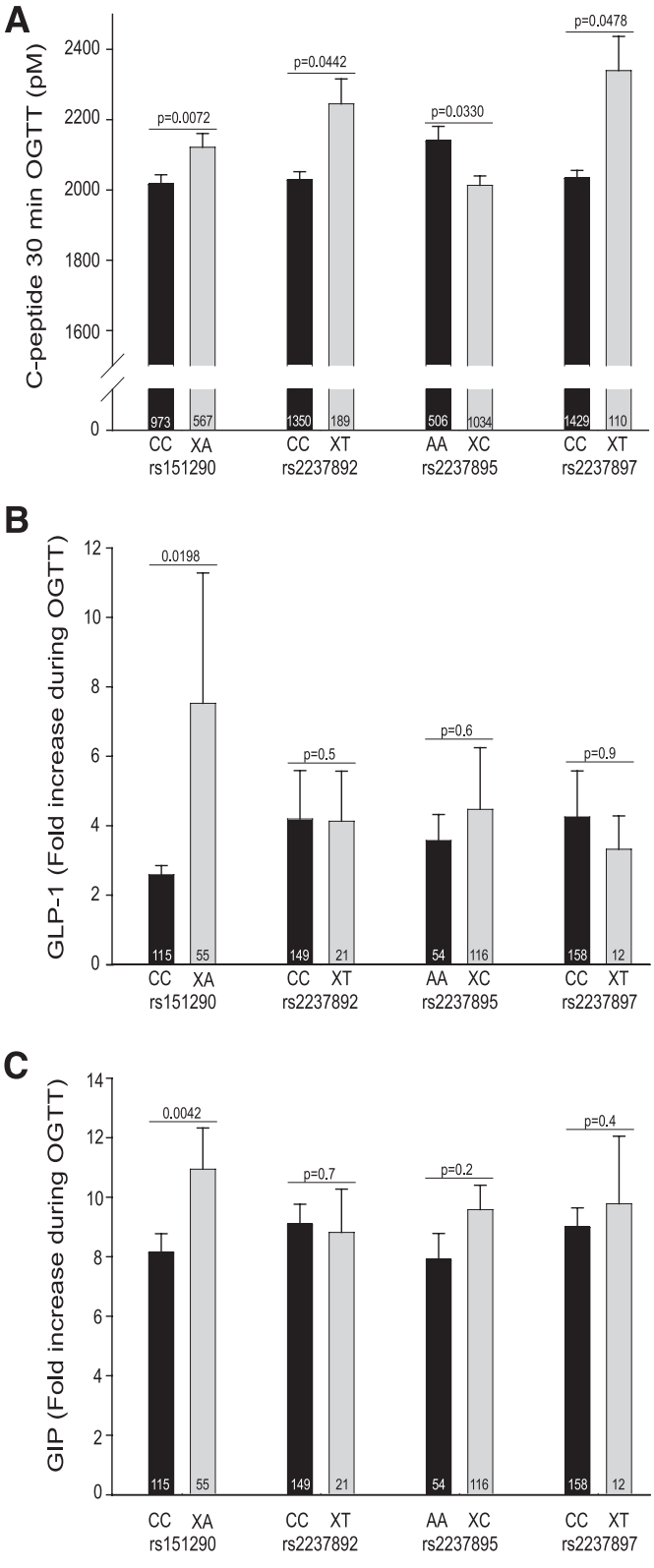


**FIG. 1. *A*: Associations of *KCNQ1* SNPs rs151290, rs2237892, rs2237895, and rs2237897 with insulin secretion. Insulin secretion was assessed by C-peptide levels at 30 min during an OGTT. Unadjusted data from 1,578 subjects are presented. *B*: Association of *KCNQ1* SNPs rs151290, rs2237892, rs2237895, and rs2237897 with increase of GLP-1 levels during an OGTT. *C*: Association of *KCNQ1* SNPs rs151290, rs2237892, rs2237895, and rs2237897 with increase of GIP levels during OGTT. Incretin increase was assessed by the ratio of levels at 30 min during OGTT to fasting levels. Unadjusted data from 170 subjects are presented. Before multivariate linear regression analysis in the dominant model, non–normally distributed data were log–transformed. C-peptide levels were adjusted for sex, age, BMI, and insulin sensitivity. Incretin increase was adjusted for sex, age, and BMI. *P* values are given above the columns. Sample sizes are given at the bottom of the columns.**

rs2237895 was also nominally associated with OGTT-derived insulin sensitivity ($P = 0.0245$) after adjustment for age, sex, and BMI in the dominant model. rs151290 was nominally associated with OGTT-derived insulin sensitivity ($P = 0.0330$) after appropriate adjustment in the additive model. However, such an association was not found in the dominant model ($P = 0.4$). rs2237892 was not associated with OGTT-derived insulin sensitivity ($P \geq 0.3$).

None of the four SNPs were associated with IVGTT-derived indexes of insulin secretion (all $P \geq 0.4$), and insulin sensitivity measured with the clamp technique was not affected by any of the genotypes (all $P \geq 0.14$). The discrepancy between OGTT- and IVGTT-derived insulin secretion pointed to an influence of common genetic variation in the *KCNQ1* gene on incretin production or incretin signaling. Recently, the two diabetes susceptibility loci *TCF7L2* and *WFS1* were found to be associated with impaired GLP-1–induced insulin secretion (23,24). Therefore, we also studied the influence of the four *KCNQ1* variants on a hyperglycemic clamp combined with GLP-1 administration. However, no associations were found between the *KCNQ1* variants and glucose-, GLP-1–, and arginine-induced insulin secretion during the hyperglycemic clamp after appropriate adjustment (all $P > 0.05$; supplementary Table 1, available in the online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db08-1589/DC1). To test the influence of genetic variation in *KCNQ1* on incretin secretion, in a subset GLP-1 and GIP levels were measured during OGTT. rs151290 was significantly associated with the glucose-stimulated GIP increase and nominally associated with the GLP-1 increase after adjustment for sex, age, and BMI in the dominant model ($P = 0.0042$ and $P = 0.0198$, respectively; Fig. 1*B* and *C*). The reason for the large SEM values of the fold increase of GLP-1 during OGTT appears to be an outlier with an extremely high 200-fold increase. After exclusion of this outlier, the difference between homozygous major allele carriers and risk allele carriers remains nominally significant (CC 2.6 ± 0.3 vs. XA 3.9 ± 0.7; $P < 0.05$). No associations were detected between the other three SNPs and basal or stimulated incretin levels (all $P \geq 0.05$; Table 3).

## DISCUSSION

Two recent GWA studies showed that common genetic variation in *KCNQ1* is associated with type 2 diabetes (12,13). One SNP, rs2237892, has been found to be associated with a fasting parameter of insulin secretion (homeostasis model assessment of β-cell function) in a Japanese population and with an OGTT-derived insulin secretion parameter (corrected insulin response) in a European cohort (13).

In a German population at increased risked for type 2 diabetes, we detected nominal associations of *KCNQ1* SNPs rs151290, rs2237892, rs2237895, and rs2237897 with several OGTT-derived indexes of insulin secretion, including C-peptide at 30 min during OGTT, first-phase insulin secretion, and insulinogenic index. Whereas insulin secretion was lower in homozygous major allele carriers of

rs151290 (CC), rs2237892 (CC), and rs2237897 (CC), β-cell function was improved in homozygous major allele carriers of rs2237895 (AA). Thus, our data confirm the previous study reporting an association between rs2237892 and indexes of insulin secretion (13). Furthermore, our findings are in agreement with the two previous studies that identified the C allele as the type 2 diabetes risk allele for rs151290, rs2237892, rs2237895, and rs2237897 (12,13).

None of the SNPs was associated with insulin secretion during IVGTT, pointing to an influence of common genetic variation in *KCNQ1* on incretin secretion or incretin signaling. Recently, we found that SNPs of the two diabetes susceptibility genes *TCF7L2* and *WFS1* were associated with impaired GLP-1 signaling that contributed to the pathogenetic mechanism (23,24). In contrast, none of the *KCNQ1* variants were associated with GLP-1–induced insulin secretion. However, we found an association between rs151290, the SNP with the most prominent effect on insulin secretion after an oral glucose load, and glucose-stimulated GLP-1 and GIP levels. These results may indicate that altered incretin secretion after food intake provides a potential link between *KCNQ1* gene variants and impaired β-cell function. In line with this assumption, *KCNQ1* is expressed along the entire gastrointestinal tract (25) and is involved in transport mechanisms in gastrointestinal epithelia (26).

It is worth noting that associations with alterations of glucose-stimulated incretin secretion were found only for rs151290, though rs2237892, rs2237895, and rs2237897 were also associated with indexes of insulin secretion during OGTT. The reason for these inconsistent results could be either that the effects of rs2237892, rs2237895, and rs2237897 on incretin secretion may be too small to be detected in our limited sample size or that these *KCNQ1* variants regulate insulin secretion differently than rs151290.

We are aware that the SNPs presented are located within intronic noncoding regions and that, therefore, the mechanisms of their actions remain elusive. The NCBI Reference Sequence (RefSeq) of *KCNQ1* contains 14 missense mutations, two frame-shift mutations, one nonsense mutation, and one SNP in the 5′-untranslated region. Only 4 of these 18 mutations are captured by the HapMap data. None of these SNPs are in linkage disequilibrium with any of the three chosen SNPs rs151290, rs2237892, and rs2237897. SNP rs2237895 is also not captured by the HapMap data. However, we cannot rule out that the chosen SNPs may be in linkage disequilibrium with a functional candidate that is not captured by the HapMap data. Alternatively, given that none of the chosen SNPs are located in coding regions, common genetic variants in KCNQ1 may affect gene expression and not the function of the gene product.

The present study has certain limitations that need to be taken into account. First, our study comprised subjects at an increased risk for type 2 diabetes, which may affect the phenotype of incretin secretion or mask some other effects of *KCNQ1* SNPs. Second, we were not able to detect effect sizes smaller than 53% with sufficient power (80%) in the combined hyperglycemic clamp study. Thus, effects sizes of *KCNQ1* SNPs below 53% possibly remained undetected in this study. Therefore, we cannot rule out that genetic variation in *KCNQ1* may, in addition to its effects on glucose-stimulated incretin secretion, also alter GLP-1–induced insulin secretion.

TABLE 3
Associations of *KCNQ1* SNPs rs151290, rs2237892, rs2237895, and rs2237897 with the incretins GLP-1 and GIP

| | rs151290 (0.208) | | | | | rs2237892 (0.064) | | | | | rs2237895 (0.427) | | | | | rs2237897 (0.037) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CC | CA | AA | $P_{add}$ | $P_{dom}$ | CC | CT | TT | $P_{add}$ | $P_{dom}$ | AA | AC | CC | $P_{add}$ | $P_{dom}$ | CC | CT | TT | $P_{add}$ | $P_{dom}$ |
| *n* | 116 | 47 | 8 | — | — | 150 | 21 | 0 | — | — | 55 | 92 | 24 | — | — | 159 | 12 | 0 | — | — |
| GLP-1 (pmol/l) | | | | | | | | | | | | | | | | | | | | |
| 0 min | 18.1 ± 9.1 | 15.0 ± 7.1 | 15.9 ± 8.6 | 0.2 | 0.10 | 17.2 ± 8.7 | 16.8 ± 9.0 | | 0.9 | 0.9 | 16.1 ± 8.2 | 17.8 ± 8.9 | 16.8 ± 8.8 | 0.4 | 0.3 | 17.3 ± 8.8 | 15.5 ± 6.8 | | 0.9 | 0.9 |
| 30 min | 35.4 ± 19.1 | 41.7 ± 35.2 | 95 ± 17 | 0.7 | 0.5 | 36.9 ± 25.6 | 36.5 ± 14.9 | | 0.4 | 0.4 | 31.9 ± 14.6 | 40.3 ± 28.7 | 34.5 ± 23.1 | 0.3 | 0.3 | 37.0 ± 25.0 | 34.1 ± 15.7 | | 1.0 | 1.0 |
| 120 min | 30.4 ± 13.5 | 29.0 ± 13.4 | 28.6 ± 9.3 | 0.4 | 0.18 | 29.7 ± 13.7 | 29.8 ± 10.9 | | 0.5 | 0.5 | 26.3 ± 12.3 | 31.4 ± 13.9 | 31.0 ± 13.0 | 0.15 | 0.05 | 29.8 ± 13.7 | 29.3 ± 8.5 | | 0.7 | 0.7 |
| GIP (pmol/l) | | | | | | | | | | | | | | | | | | | | |
| 0 min | 15.5 ± 11.3 | 12.5 ± 7.3 | 12.1 ± 4.6 | 0.3 | 0.12 | 14.8 ± 10.7 | 12.1 ± 5.7 | | 0.6 | 0.6 | 16.4 ± 14.2 | 13.9 ± 7.8 | 12.5 ± 6.6 | 0.2 | 0.09 | 14.7 ± 10.5 | 12.4 ± 5.2 | | 0.8 | 0.8 |
| 30 min | 84.5 ± 30.3 | 101.2 ± 47.8 | 71.8 ± 28.4 | 0.10 | 0.10 | 89.0 ± 37.1 | 84.7 ± 33.9 | | 0.8 | 0.8 | 87.6 ± 31.7 | 90.1 ± 41.3 | 84.1 ± 27.4 | 0.8 | 0.5 | 88.1 ± 37.3 | 93.6 ± 27.1 | | 0.5 | 0.5 |
| 120 min | 71.4 ± 29.1 | 76.1 ± 35.3 | 60.1 ± 26.0 | 0.8 | 0.9 | 72.0 ± 31.1 | 73.3 ± 29.5 | | 0.9 | 0.9 | 74.7 ± 34.2 | 70.2 ± 30.3 | 73.7 ± 24.8 | 0.7 | 0.8 | 71.8 ± 31.2 | 76.2 ± 26.9 | | 0.5 | 0.5 |

Data are means ± SD unless otherwise indicated. For statistical analysis, data were log transformed. Incretin data were adjusted for sex, age, and BMI. $P_{add}$, additive model; $P_{dom}$, dominant model.

In summary, common genetic variation in the *KCNQ1* gene is associated with β-cell function in our German population at increased risk of type 2 diabetes, confirming previous data in Japanese and European cohorts. The discrepancy between orally and intravenously administered glucose seems to be explained not by altered incretin signaling but most likely by changes in incretin secretion.

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