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 an intravenous 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 (all \( P \leq 0.05 \)). No associations were detected between the other SNPs and basal or stimulated incretin levels (all \( P > 0.05 \)).

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
glucose administration. Insulin secretion during the hyperglycemic clamp was assessed as the sum of insulin at 30 min (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 Institute, 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://wwwpsycho.uni-duesseldorf.de/aap/projects/gpower. Hardy-Weinberg equilibrium was tested using the χ² 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 (χ² test, P > 0.05).

Association of genetic variation in KCNQ1 with antrhopometric 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. 1A). 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 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/m²)         | 28.0 ± 6.0  |
| 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.


### TABLE 2

| SNP   | rs151290 | rs2237892 | rs2237895 | rs2237897 |
|-------|----------|-----------|-----------|-----------|
| Fasting insulin (pmol/l) | 6.26 | 6.14 | 6.14 | 6.14 |
| 10 min OGTT (mmol/l) | 7.70 | 7.52 | 7.52 | 7.52 |
| 15 min OGTT (mmol/l) | 8.20 | 8.02 | 8.02 | 8.02 |
| 30 min OGTT (mmol/l) | 9.60 | 9.42 | 9.42 | 9.42 |
| BMI (kg/m²) | 28.7 | 28.7 | 28.7 | 28.7 |
| Age (years) | 39 | 39 | 39 | 39 |
| Body fat (%) | 30.7 | 30.7 | 30.7 | 30.7 |
| Waist circumference (cm) | 93 | 93 | 93 | 93 |
| HOMA-IR | 1.70 | 1.66 | 1.66 | 1.66 |
| Insulinogenic index | 526 | 526 | 526 | 526 |

Note: Significant differences are indicated by an asterisk. The results are presented as mean ± standard deviation.
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. 1B 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 $\beta$-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 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.
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

| SNP       | GIP (pmol/l) | GLP-1 (pmol/l) |
|-----------|-------------|---------------|
| rs151290  |             |               |
| rs2237892 |             |               |
| rs2237895 |             |               |

| rs151290 | rs2237892 | rs2237895 |
|----------|----------|----------|
| CC       | CC       | CC       |
| CA       | CA       | CA       |
| TT       | TT       | TT       |
| TC       | TC       | TC       |
| CT       | CT       | CT       |

| rs151290 | rs2237892 | rs2237895 |
|----------|----------|----------|
| AA       | AA       | AA       |
| AC       | AC       | AC       |
| CC       | CC       | CC       |
| TT       | TT       | TT       |

| rs2237895 | rs2237897 |
|----------|----------|
| CC       | CC       |
| CA       | CA       |
| TT       | TT       |

| rs2237897 | rs2237895 |
|----------|----------|
| CC       | CC       |
| CA       | CA       |
| TT       | TT       |

| rs2237897 | rs2237895 |
|----------|----------|
| CC       | CC       |
| CA       | CA       |
| TT       | TT       |

| rs2237897 | rs2237895 |
|----------|----------|
| CC       | CC       |
| CA       | CA       |
| TT       | TT       |

| rs2237897 | rs2237895 |
|----------|----------|
| CC       | CC       |
| CA       | CA       |
| TT       | TT       |

| rs2237897 | rs2237895 |
|----------|----------|
| CC       | CC       |
| CA       | CA       |
| TT       | TT       |

| rs2237897 | rs2237895 |
|----------|----------|
| CC       | CC       |
| CA       | CA       |
| TT       | TT       |
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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