ADDITIVE EFFECTS OF GENETIC VARIATION IN GCK AND G6PC2 ON INSULIN SECRETION AND FASTING GLUCOSE

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Submitted 18 February 2009 and accepted 27 August 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes. The American Diabetes Association, publisher of Diabetes, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes in print and online at http://diabetes.diabetesjournals.org.
**Objective:** GCK and G6PC2 regulate the glucose cycling step in pancreatic β-cells and may regulate insulin secretion. *GCK* rs1799884 and *G6PC2* rs560887 have been independently associated with fasting glucose, but their interaction on glucose-insulin relationships is not well characterized.

**Research Design and Methods:** We tested whether these variants are associated with diabetes-related quantitative traits in Mexican Americans from the BetaGene study and attempted to replicate our findings in Finnish men from the METSIM study.

**Results:** rs1799884 was not associated with any quantitative trait (corrected p>0.1), whereas rs560887 was significantly associated with the OGTT 30-minute incremental insulin response (30’ ΔInsulin, corrected p=0.021). We found no association between quantitative traits and the multiplicative interaction between rs1799884 and rs560887 (p>0.26). However, the additive effect of these SNPs was associated with fasting glucose (corrected p=0.03) and 30’ ΔInsulin (corrected p=0.027). This additive association was replicated in METSIM (fasting glucose, p=3.5×10^-10 30’ ΔInsulin, p=0.028). When we examined the relationship between fasting glucose and 30’ ΔInsulin stratified by *GCK* and *G6PC2*, we noted divergent changes in these quantitative traits for *GCK*, but parallel changes for *G6PC2*. A similar pattern was observed in METSIM.

**Conclusions:** We conclude that variation in *GCK* and *G6PC2* have additive effects on both fasting glucose and insulin secretion.
genome-wide association (GWA) studies have identified several loci for type 2 diabetes mellitus (T2DM) (1-5) and T2DM-related quantitative traits (6-20). Two of these loci, glucokinase (*GCK*) (21-23) and glucose-6-phosphatase catalytic subunit 2 (*G6PC2*) (9;10), regulate the critical glucose sensing mechanism within pancreatic β-cells. Mutations in *GCK* confer susceptibility to MODY2 (24-26) and a -30 *GCK* promoter variant (rs1799884) has been shown to be associated with β-cell function (21), fasting glucose, and birth weight (23). Chen *et al.* demonstrated an association between the *G6PC2* region and fasting glucose (9), an observation replicated by Bouatia-Najji and colleagues (10). Although fasting glucose levels are associated with both *GCK* and *G6PC2*, to date there has been no evidence that genetic variation at these two loci contribute to risk for T2DM, suggesting contribution to mild elevations in glycemia.

GCK phosphorylates glucose to glucose-6-phosphate, while *G6PC2* dephosphorylates glucose-6-phosphate back to glucose, forming a glucose cycle previously demonstrated to exist within pancreatic β-cells (27;28). The important role of GCK in glucose sensing by pancreatic islets has been demonstrated by numerous studies and other studies suggest a role for glucose cycling in insulin secretion and diabetes (28-30), implying that the balance between GCK and G6PC2 activity is important for determining glycolytic flux, ATP production, and subsequent insulin secretion. This was further validated by demonstration that direct manipulation of glucose cycling alters insulin secretion (31;32).

BetaGene is a study in which we are performing detailed phenotyping of Mexican American probands with recent gestational diabetes mellitus (GDM) and their family members to obtain quantitative estimates of body composition, insulin sensitivity (*S_I*), acute insulin response (AIR), and β-cell compensation (disposition index, DI) with the goal of identifying genes influencing variation in T2DM-related quantitative traits (33-35). Based on the evidence that variation in *GCK* (rs1799884) and *G6PC2* (rs560887) are independently associated with fasting glucose concentrations and both are crucial to glucose cycling in β-cells, we hypothesized that interaction between these loci may be associated not just with fasting glucose, but also with measures of insulin secretion or β-cell function. We tested this hypothesis in the BetaGene sample and, for replication, in a separate sample of Finnish men participating in the METabolic Syndrome In Men (METSIM) Study (36).

**METHODS**

The BetaGene Sample: **Subject Recruitment**—Subject recruitment for BetaGene was on-going at the time of these analyses. Subjects are Mexican American (both parents and ≥3 grandparents Mexican or of Mexican descent) who are either probands with GDM diagnosed within the previous five years and their family members, or non-GDM probands with normal glucose levels in pregnancy in the past five years. Subject recruitment has been previously detailed (33-35). All protocols for BetaGene have been approved by the Institutional Review Boards of participating institutions and all participants provided written informed consent prior to participation.

**Clinical protocols.** Phenotyping was performed on two separate visits to the University of Southern California General Clinical Research Center. The first visit consisted of a physical examination, DNA collection, and a 75 g oral glucose tolerance test (OGTT) as previously described (33-35). Participants with fasting glucose <126 mg/ml were invited back for a second visit, which consisted of a dual-energy X-ray...
absorptiometry (DEXA) scan for body composition (percent body fat) and an insulin-modified intravenous glucose tolerance test (IVGTT) performed as previously described (37).

**Assays:** Plasma glucose is measured on an auto-analyzer using the glucose oxidase method (YSI Model 2300, Yellow Springs Instruments, Yellow Springs, OH). Insulin is measured by two-site immunoenzymometric assay (TOSOH) that has <0.1% cross-reactivity with proinsulin and intermediate split products.

**The METSIM Sample: Subject Recruitment**--We attempted to replicate our findings in subjects participating in the ongoing METSIM Study (36). The primary aim of the METSIM study is to investigate the effect of genetic variation on risk for T2DM and cardiovascular disease in a random sample of Finnish men, aged from 50 to 70 years and living in the town of Kuopio (population 95,000), eastern Finland. A total of 5,327 men from this on-going population-based study were included in this report.

The METSIM study was approved by the ethics committee of the University of Kuopio and was in accordance with the Helsinki Declaration.

**Clinical Protocols:** Phenotyping for METSIM was performed in a single visit to the Clinical Research Unit of the University of Kuopio. In addition to fasting blood samples for DNA, glucose, and insulin, all subjects underwent an OGTT with samples obtained at 30 and 120 minutes post-load.

**Molecular analysis:** In BetaGene, GKC -30G→A polymorphism (rs1799884) and G6PC2 rs560887 were genotyped using the Applied Biosystems, Inc. (ABI) TaqMan system (38,39). Genotyping discrepancy rate based on blinded duplicates was <0.1% and overall genotyping success rates were >97% for rs1799884 and >99% for rs560887.

In METSIM, GCK rs4607517 and G6PC2 rs560887 were genotyped by the homogeneous MassEXTEND reaction using the MassARRAY System (Sequenom) (9). In the HapMap CEU data (40), which provides a good representation of linkage disequilibrium (LD) in Finns (41), rs4607517 is a perfect proxy for rs1799884 (D'=1.0, r²=1.0). There were no genotype discrepancies based on >220 blinded duplicates and overall genotyping success rates were >96% for rs4607517 and >94% for rs560887.

**Data analysis:** Genotype data were tested for deviation from Hardy-Weinberg equilibrium (HWE) and for non-Mendelian inheritance using PEDSTATS V0.6.4 (42). Allele frequencies for BetaGene samples were estimated using all available data taking into account relatedness using SOLAR V2.1.4 (43,44), while for METSIM frequencies were estimated by gene counting.

We calculated two measures of insulin response to glucose: the difference between the 30’ and fasting plasma insulin concentration from OGTT (30’ ∆Insulin) and the incremental area under the insulin curve for the first 10 min of the IVGTT (acute insulin response, AIR). IVGTT glucose and insulin data were analyzed using the minimal model (MINMOD Millennium V5.18) to derive measures of glucose effectiveness and S₁ (45). DI, a measure of β-cell compensation for insulin resistance, was computed as the product of S₁ and AIR. We also computed an OGTT-derived measure of β-cell compensation as the product of S₁ and 30’ ∆Insulin (DI30).

We initially examined the association between the two SNPs and fasting glucose, fasting insulin, 30’ ∆Insulin, and AIR given our primary hypothesis that these variants may underlie variation in these specific quantitative traits. Details of the association analyses are described below. We also recognized these variants could also underlie variation in other T2DM-related quantitative traits. We tested these SNPs for association with 2-hour glucose, 2-hour insulin, S₁, Sgü,
BMI, and percent body fat as a series of secondary analyses.

Quantitative trait data were statistically transformed to approximate univariate normality prior to analyses. We first tested each SNP for association with T2DM-related quantitative traits using likelihood ratio testing under a variance components framework as implemented in SOLAR (43;44). Because families were ascertained through probands based on previous GDM status, we corrected for ascertainment bias by conditioning each model on the proband’s phenotype. Each SNP was tested for association with quantitative traits assuming a dominant genetic model due to the relatively low minor allele frequencies observed for both SNPs. All models were adjusted for age, sex, and where appropriate, percent body fat. P-values were corrected for multiple testing to account for the number of traits tested (4 traits in the primary analysis, 6 traits in the secondary analysis). We used PACT to control for the number of tests performed and to account for the correlation among the quantitative traits tested for association (46). PACT compares the observed test statistic with their asymptotic distribution using numeric integration and directly accounts for correlation among both dependent and independent variables.

We next performed a test of the association between T2DM-related quantitative traits and the interaction between GCK and G6PC2. We compared a model with age, sex, rs1799884, and rs560887 as main effects with a second model that included the multiplicative interaction between the two SNPs. As in the univariate analyses, we assumed dominant genetic models for both SNPs. We tested for a significant interaction effect using a 1-df test and performed a Bonferroni correction to account for the number of traits tested, because PACT is not valid for multi-degree-of-freedom tests (46). However, because many of the quantitative traits examined are correlated and Bonferroni assumes independence, the corrected p-values we report should be overly conservative.

Given that GCK and G6PC2 work in opposite directions on the glucose cycle, we hypothesized that additivity may best characterize their interaction. Therefore, we performed a second analysis in which we used a 2-df test to test the joint effect of rs1799884 and rs560887 for association with T2DM-related quantitative traits.

METSIM Analysis: Specific results observed in the betagene analysis were tested in the METSIM samples. Standard regression methods were used to test for association between genetic variants and T2DM-related traits as METSIM participants are unrelated individuals. Covariates included age and, when appropriate, BMI. Given that only specific a priori hypotheses were tested in the METSIM data, we did not apply a correction for multiple testing.

All data for BetaGene are reported as age- and sex-adjusted mean and standard deviation, while METSIM data are reported as age-adjusted mean and standard deviation unless noted otherwise.

RESULTS

The BetaGene Study: We report results from 861 BetaGene individuals in 251 families with available phenotype and genotype data (Table 1). Probands, siblings, and cousins were similar in median age, BMI, and percent body fat, although these characteristics tended to be highest in the GDM probands and lowest in cousins. Non-GDM probands were slightly younger and less obese compared with GDM probands, reflecting the fact that BetaGene participant accrual was on-going and recruitment of non-GDM probands was slightly lagging to allow for matching as previously described (33-35). Parameters of glucose metabolism tended to
be best in the non-GDM probands and worst in the GDM probands.

GCK rs1799884 (G>A) had a minor allele frequency of 18.0% and was marginally associated with fasting glucose (Table 2, p=0.052), which modestly increased (p=0.021) after adjustment for body fat. Individuals homozygous for the G allele had a lower average fasting glucose compared to those with at least one A allele (5.13±0.52 vs. 5.22±0.59 mM). However, the association between GCK rs1799884 and fasting glucose did not remain significant when corrected for multiple testing. In addition, we did not observe any evidence for association between this SNP and measures of insulin secretion (Table 2) or other T2DM-related quantitative traits (Table 3), although there was a tendency for mean fasting insulin, 30’ ΔInsulin, and AIR to be lower in individuals with at least one rs1799884 A allele (Table 2).

G6PC2 rs560887 (G>A) had a minor allele frequency of 14.5% and was nominally associated with fasting glucose (Table 2, p=0.015) and 30’ ΔInsulin (p=0.0038) and marginally associated with AIR (p=0.088). These associations became weaker when body fat was included in the model (p=0.059, p=0.014, and p=0.222, respectively). Individuals homozygous for the rs560887 G allele had an average fasting glucose higher than those with at least one A allele (5.19±0.52 vs. 5.08±0.61 mM). In parallel with these glucose differences, both 30’ ΔInsulin and AIR were, on average, higher in individuals homozygous for the G allele (Table 2). Only the association with 30’ ΔInsulin remained significant after correction for multiple testing (PACT=0.021). Although G6PC2 rs560887 was not associated with most T2DM-related quantitative traits, we did observe an association between this SNP and percent body fat (PACT=0.035), where individuals homozygous for the G allele had higher trait values (Table 3).

We found no evidence for association between the multiplicative interaction between GCK rs1799884 and G6PC2 rs560887 and T2DM-related quantitative traits (uncorrected p>0.26) and additional adjustment for body fat did not change the overall results. In contrast, when we tested whether the additive effects of GCK rs1799884 and G6PC2 rs560887 were associated with T2DM-related quantitative traits, we observed association with fasting glucose (Bonferroni p=0.03) and 30’ ΔInsulin (Bonferroni p=0.027).

Bouatia-Naji et al. reported a linear relationship between number of “glucose lowering alleles” for GCK, G6PC2, and GCKR and fasting glucose, which suggested additive effects of these alleles on fasting glucose (10). We observe a similar linear relationship (data not shown) when we stratified on GCK and G6PC2 genotypes, consistent with the results of our statistical analysis and the observations of Bouatia-Naji et al. (10). However, given the specificity of these two gene products in regulating the glucose sensing step in pancreatic β-cells, we hypothesized the primary effect of variation in GCK and G6PC2 to be at the level of insulin secretion, not directly upon glucose. We tested this hypothesis using the conceptual construct depicted in Figure 1. The presence of an A allele in the GCK rs1799884 promoter variant is hypothesized to reduce GCK gene expression and result in decreased GCK protein (23). This should result in modest reductions in glycolytic flux and ATP production, resulting in modest reductions in insulin secretion for any level of circulating glucose. G6PC2 rs560887 is located in intron 3, just 26 bp proximal to exon 4 (9;10). The presence of an A allele may result in a G6PC2 isoform with differential activity that favors increased conversion of glucose-6-phosphate to glucose, significantly reducing glycolytic flux and ATP generation, resulting in larger reductions
in insulin secretion for any glucose level. The balance between the effects of GCK and G6PC2 should differentially modulate insulin secretion, depending upon genotype combination as detailed in Figure 1.

When we stratified individuals by GCK and G6PC2 genotypes as depicted in Figure 1, a linear trend in 30’ ΔInsulin (Figure 2, top; p=0.0016) is observed where on average, 30’ ΔInsulin decreased ~5.6 pM among genotype groups. We do not see a clear linear relationship when the same stratification is used to examine the relationship with fasting glucose (Figure 2, bottom; p=0.191). Thus, the linearity between GCK and G6PC2 genotype combinations and insulin secretion does not directly translate into linear changes in fasting glucose. We observed a similar dichotomy when we examine fasting glucose and 30’ ΔInsulin stratified by total number of glucose “raising” alleles, as previously performed by Bouatia-Naji et al. (10); fasting glucose is linear with total number of glucose “raising” alleles, while 30’ ΔInsulin is not (data not shown).

Grouping and ordering by genotype as described above is semi-artificial. Therefore, we plotted fasting glucose against 30’ ΔInsulin from BetaGene stratified by GCK and G6PC2 genotypes (Figure 3, top) to better characterize the relationship between 30’ ΔInsulin and fasting glucose. We noted a different pattern of coordinate changes in fasting glucose and 30’ ΔInsulin associated with variation in GCK as compared to variation in G6PC2. For GCK rs1799884, individuals homozygous for the G allele (circles) had lower fasting glucose and higher 30’ ΔInsulin than individuals with at least one A allele (squares). For G6PC2 rs560887, individuals homozygous for the G allele (open symbols) had higher fasting glucose and higher 30’ ΔInsulin than individuals with at least one A allele (solid symbols). In other words, variation in GCK led to inverse changes in fasting glucose and 30’ ΔInsulin, while variation in G6PC2 led to parallel changes in these two traits.

**Replication in the METSIM Study:**

Given the observation of additivity between GCK rs1799884 and G6PC2 rs560887 on fasting glucose and 30’ ΔInsulin in BetaGene, we attempted to replicate these findings in the METSIM sample, in which GCK rs4607517 is in perfect LD with rs1799884. GCK rs4607517 was associated with fasting glucose, but not associated with 30’ ΔInsulin (Table 4). G6PC2 rs560887 was associated with fasting glucose and marginally associated with 30’ ΔInsulin (Table 4). The multiplicative interaction between GCK rs4607517 and G6PC2 rs560887 was weakly associated with fasting glucose (p=0.038) and marginally associated with 30’ ΔInsulin (p=0.049). However, the 2-df test of the joint additive effect of the two SNPs was significant for both fasting glucose (p=3.5×10^-10) and 30’ ΔInsulin (p=0.028). The evidence for association between the joint additive effect of both SNPs and fasting glucose was not affected when BMI was included as an additional covariate (p=3.3×10^-11), the association with 30’ ΔInsulin became stronger when BMI was included as a covariate (p=0.0028). When we examined the relationship between fasting glucose and 30’ ΔInsulin stratified by GCK rs4607517 and G6PC2 rs560887 genotype groupings, an identical pattern of relationship to that seen in BetaGene was observed (Figure 3, bottom). For GCK rs4607517, individuals homozygous for the G allele had lower fasting glucose and higher 30’ ΔInsulin than individuals with at least one A allele. For G6PC2 rs560887, individuals homozygous for the G allele had higher fasting glucose and higher 30’ ΔInsulin than individuals with at least one A allele.

**DISCUSSION**

Our results from Mexican Americans, replicated in a sample of Finnish men, show that variation in both GCK and G6PC2 have
additive effects on both fasting glucose and insulin secretion. Our examination of the relationship between 30' ΔInsulin and fasting glucose stratified by GCK and G6PC2 genotype reveals that the joint effect of genetic variation in these two genes may be through distinct mechanisms. Variation in GCK leads to inverse changes in fasting glucose and insulin secretion, consistent with a primary effect on insulin secretion. Variation in G6PC2 leads to parallel changes in fasting glucose and insulin secretion, suggesting a primary effect on glucose, with secondary changes in insulin secretion.

Table 5 summarizes our examination of the effects of GCK rs1799884 and G6PC2 rs560887 on fasting glucose and 30' ΔInsulin. While it is possible that our conclusion of these variants having an additive effect could be driven mainly by G6PC2 rs560887, the summary in Table 5 suggests otherwise. It is clear that in both BetaGene and METSIM, the model testing the joint effect of GCK and G6PC2 shows greater significance than the effect of each individual gene variant alone, which suggests both genes are contributing to the effect on fasting glucose and 30' ΔInsulin. In support of this observation, when we tested for the additional effect of GCK in the presence of G6PC2 we observed marginal or significant effects for fasting glucose (p=0.058 for Betagene; p=0.0009 for METSIM) and 30' ΔInsulin (p=0.148 for BetaGene; p=0.723 for METSIM). Our a priori hypothesis tested whether the multiplicative interaction between rs1799884 and rs560887 was associated with T2DM-related quantitative traits. In BetaGene we observed no evidence for a multiplicative interaction; our analysis favored a joint additive effect of these two loci. However, our relatively small size may limit our ability to exclude a multiplicative interaction. A priori power estimates indicated that a sample size of 600 should have provided 80% power to detect a multiplicative interaction effect that accounted for ~1.8% of the variability in fasting glucose or 30' ΔInsulin, assuming α=0.01, minor allele frequencies of 15% for both loci, and dominant genetic models for both loci. Thus, BetaGene cannot discount the possibility that a very weak multiplicative interaction exists between these two loci. In fact, the METSIM sample, which is ~6 times larger than BetaGene, showed modest evidence for a multiplicative interaction (Table 5) suggesting that a weak multiplicative interaction between GCK and G6PC2 could be contributing to variation in fasting glucose and 30' ΔInsulin. This interaction would need to be replicated in a sample of similar or larger size.

Our initial examination of the additive effect of these two genes was motivated by the fact that when we plotted 30' ΔInsulin against GCK rs1799884 and G6PC2 rs560887 genotype combinations sorted by the hypothesized biologic effect on insulin secretion, we observed a linear relationship with 30' ΔInsulin. Recently Pirot et al. showed in mouse and human islets that G6PC2 protein levels and enzymatic activity increased when islets were incubated in high glucose media, while those for GCK did not change (47), suggesting that G6PC2 activity may adapt to changes in glucose to alter insulin secretion. Since insulin secretion was not measured, it is unclear whether the changes observed translated into altered rates of insulin secretion. However, previous studies have demonstrated that manipulation of glucose cycling in insulin secreting cell lines does result in changes in insulin secretion (31;32). For example, Iizuka et al. showed that altering glucose cycling by varying the G6PC2:GCK ratio leads to reductions in ATP production and reduced glucose-stimulated insulin secretion in MIN-6 cells (32). Taken together, these studies examining the balance between GCK and G6PC2 support our hypothesized effects of variation in GCK and G6PC2 on insulin
secretion (cf. Figure 1). However, they do not account for the differences we observed in the relationship between fasting glucose and insulin secretion when stratified by these two loci.

Primary enhancement of β-cell sensitivity to glucose should result in increased systemic insulin concentrations in relation to glucose levels. The fact that the linearity in 30’ ΔInsulin we observed between GCK rs1799884 and G6PC2 rs560887 genotype combinations did not extend to fasting glucose (cf. Figure 2) is unexpected. We confirmed this initial observation by examining the relationship between fasting glucose and 30’ ΔInsulin, stratifying on GCK and G6PC2 genotype (cf. Figure 3). In both the BetaGene and METSIM studies, the presence of the GCK rs1799884 A allele resulted in lower 30’ ΔInsulin and higher fasting glucose, regardless of G6PC2 genotype. The results suggest that variation in GCK may alter insulin secretion and fasting glucose in the traditionally envisioned manner where changes in insulin secretion result in reciprocal changes in glucose concentration.

However, the presence of a G6PC2 A allele resulted in lower 30’ ΔInsulin and fasting glucose, regardless of GCK genotype. There are two possible mechanisms that may explain our observation. First, variation in G6PC2 could have a direct and independent effect to regulate hepatic glucose production. However, this effect is unlikely, since the hepatic isoform of glucose-6-phosphatase is encoded by an independent gene. Second, variation in G6PC2 could alter a characteristic of insulin secretion that disrupts the normal signaling between the pancreas and other tissues, resulting in both insulin secretion and glucose changing in tandem. Recent studies by Matveyenko et al. provide evidence for this possibility (48;49). Specifically, their studies show that in dogs with partial pancreas resection or in rats transgenic for human islet amyloid polypeptide disruption of pulsatile insulin secretion results in hepatic insulin resistance and impaired fasting glucose (A. Matveyenko, personal communication). They conclude that the loss of pulsatile insulin secretion results in a loss of efficiency in insulin action at the liver, leading to hepatic insulin resistance and increased glucose output, which subsequently leads to a gradual rise in glucose levels. It is possible that variation in G6PC2 may disrupt pulsatility in insulin secretion, thereby reducing insulin signaling efficiency between the pancreas and liver. The small increase in glucose due to hepatic insulin resistance may result in modest increases in absolute insulin secretion. Additional studies will be required to test this hypothesis.

Finally, we observed association between G6PC2 rs560887 and measures of adiposity that have not been previously reported. BMI and body fat were both lower in the presence of the A allele (cf. Table 3), but the biologic mechanism underlying this association is unclear. One possibility is that if variation in G6PC2 results in decreased insulin signaling efficiency due to the loss of pulsatility and leads to hepatic insulin resistance, then a similar loss of insulin signaling may occur in other tissues, such as adipose. Our observed association between variation in G6PC2 and adiposity will require replication in other populations and further study to elucidate the mechanism underlying the association.

In summary, we observed evidence for an additive effect of variation in GCK (rs1799884) and G6PC2 (rs560887) on insulin secretion and fasting glucose. When stratified by genotype, the effect of GCK rs1799884 follows an expected pattern of reduced insulin secretion and reciprocal increase in fasting glucose. By contrast, glucose levels and insulin secretion change in parallel with G6PC2 rs560887, suggesting a primary effect on insulin signaling to regulate hepatic glucose production. Our analyses
highlight the importance of considering biologic effects in assessing relationships between genotype and quantitative traits. Like previous studies, our analyses show that these variants account for only a small fraction of the variability in insulin secretion or glucose. The remaining variability is likely due to the effects of other common genetic variants of both modest and moderate effect and a variety of additional gene-gene and gene-environment interactions. Thus, additional studies of samples with detailed phenotyping will be necessary in order to fully unravel the genetic architecture underlying glucose regulation and diabetes.

ACKNOWLEDGEMENTS

We thank the families who participated in the BetaGene study, our recruiting and technical staff, and the support of the USC General Clinical Research Center (M01-RR-00043).

This work was supported by NIH grant R01-DK-61628 and an American Diabetes Association Distinguished Clinical Scientist Award to T.A.B. A portion of this work was conducted in a facility constructed with support from Research Facilities Improvement Program Grant Number C06 (RR10600-01, CA62528-01, RR14514-01) from the National Center for Research Resources.
REFERENCES


Table 1. Subject Characteristics. Values are unadjusted median and interquartile range.

<table>
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<tr>
<th></th>
<th>GDM Probands</th>
<th>Siblings</th>
<th>Cousins</th>
<th>Non-GDM Probands</th>
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<td>453 (380)</td>
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Table 2. Univariate tests of associations between genetic variants and fasting glucose and measures of insulin secretion. Values are unadjusted mean±SD.

<table>
<thead>
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<th>Trait</th>
<th>GCK (rs1799884)</th>
<th>G6PC2 (rs560887)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=405)</td>
<td>GA or AA (n=207)</td>
</tr>
<tr>
<td>Fasting Glucose [mM]</td>
<td>5.13±0.52</td>
<td>5.22±0.59</td>
</tr>
<tr>
<td>Fasting Insulin [pM]</td>
<td>63.2±49.7</td>
<td>57.5±47.3</td>
</tr>
<tr>
<td>30’ ΔInsulin [pM]</td>
<td>489±338</td>
<td>447±314</td>
</tr>
<tr>
<td>AIR [pM × 10 min]</td>
<td>6197±6238</td>
<td>5721±4258</td>
</tr>
</tbody>
</table>

* age- and sex-adjusted p-value not corrected for multiple testing.
Table 3. Univariate tests of associations between genetic variants and additional T2DM-related quantitative traits. Values are unadjusted mean±SD.

<table>
<thead>
<tr>
<th>Trait</th>
<th>GCK (rs1799884)</th>
<th>G6PC2 (rs560887)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=405)</td>
<td>GA or AA (n=207)</td>
</tr>
<tr>
<td>BMI [Kg/m²]</td>
<td>29.6±6.4</td>
<td>28.7±5.6</td>
</tr>
<tr>
<td>Body Fat [%]</td>
<td>32.2±8.6</td>
<td>31.8±8.3</td>
</tr>
<tr>
<td>2-hour Glucose [mM]</td>
<td>7.39±2.24</td>
<td>7.40±2.07</td>
</tr>
<tr>
<td>2-hour Insulin [pM]</td>
<td>505±416</td>
<td>485±374</td>
</tr>
<tr>
<td>S_G [×10² min⁻¹]</td>
<td>1.78±0.71</td>
<td>1.73±0.66</td>
</tr>
<tr>
<td>S_I [×10⁻³ min⁻¹ per pM]</td>
<td>3.02±1.78</td>
<td>3.00±1.48</td>
</tr>
</tbody>
</table>

* age- and sex-adjusted p-value not corrected for multiple testing.

Table 4. Univariate tests of associations between genetic variants and fasting glucose and 30’ ΔInsulin in METSIM. Values are unadjusted mean±SD.

<table>
<thead>
<tr>
<th>Trait</th>
<th>GCK (rs4607517)</th>
<th>G6PC2 (rs560887)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=4185)</td>
<td>GA or AA (n=962)</td>
</tr>
<tr>
<td>Fasting Glucose [mM]</td>
<td>5.68±0.49</td>
<td>5.74±0.50</td>
</tr>
<tr>
<td>30’ ΔInsulin [pM]</td>
<td>343±270</td>
<td>333±235</td>
</tr>
</tbody>
</table>

* age-adjusted p-value
Table 5. Summary of Modeling Analysis.

<table>
<thead>
<tr>
<th>Model*</th>
<th>BetaGene</th>
<th>METSIM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting Glucose**</td>
<td>30’ ΔInsulin**</td>
</tr>
<tr>
<td>Age + Sex + GCK</td>
<td>0.058</td>
<td>0.148</td>
</tr>
<tr>
<td>Age + Sex + G6PC2</td>
<td>0.012</td>
<td>0.012</td>
</tr>
<tr>
<td>Age + Sex + GCK + G6PC2 + GCK×G6PC2</td>
<td>0.257</td>
<td>0.396</td>
</tr>
<tr>
<td>Age + Sex + GCK + G6PC2</td>
<td>0.0075</td>
<td>0.0067</td>
</tr>
</tbody>
</table>

* Underline indicates the genetic effect being tested
** Uncorrected p-values for the genetic effect being tested.

FIGURE LEGENDS

Figure 1. Hypothesized effect of *GCK* rs1799884 and *G6PC2* rs560887 genotype combinations on ATP production and insulin secretion in pancreatic β-cells. Top half of the figure shows the glucose cycling step and the effect of specific alleles in *GCK* and *G6PC2* on activity of each enzyme: thick arrow depicts increased activity, thin arrow depicts reduced activity. Bottom half shows the specific genotype combinations and the hypothesized effect on ATP production and subsequent insulin secretion.

Figure 2. 30’ ΔInsulin and fasting glucose stratified by *GCK* rs1799884 and *G6PC2* rs560887 genotypes. Top Panel: Age- and sex-adjusted 30’ ΔInsulin (mean±SEM) stratified by *GCK* rs1799884 and *G6PC2* rs560887 genotype combination. Genotype groups are ordered by hypothesized effect on glucose cycling in the pancreatic β-cell (see text for details). Dominant genetic models are assumed for both loci. 30’ ΔInsulin changed linearly (p=0.0016) with genotype group. Bottom Panel: Age- and sex-adjusted fasting plasma glucose (mean±SEM) stratified by genotype. Details are as described for top panel. Fasting glucose was not linearly related to genotype group (p=0.191).

Figure 3. 30’ ΔInsulin versus fasting plasma glucose stratified by *GCK* rs1799884 and *G6PC2* rs560887 genotype. Top Panel: Shows results from the BetaGene study. Model-predicted age- and sex-adjusted 30’ ΔInsulin (mean±SD) stratified by genotype is plotted against model-predicted age- and sex-adjusted fasting plasma glucose (mean±SD). Solid symbols represent *G6PC2* rs560887 G/A or A/A genotypes and open symbols represent *G6PC2* rs560887 G/G genotype. Circles represent *GCK* rs1799884 G/G genotype and squares represent *GCK* rs1799884 G/A and A/A genotypes. Bottom Panel: Shows results from the METSIM study. Details are identical to that described for the top panel except that circles and squares represent *GCK* rs4607517 genotypes.
Figure 1

Glucokinase (GCK)
- G/G
- G/A or A/A

Glucose → Glucose-6-phosphate
- G/G
- G/A or A/A

Glucose-6-phosphatase, Catalytic Subunit 2 (G6PC2)

<table>
<thead>
<tr>
<th>GCK Genotype</th>
<th>G6PC2 Genotype</th>
<th>ATP Production</th>
<th>Insulin Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>G/G</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>G/A or A/A</td>
<td>G/G</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>G/G</td>
<td>G/A or A/A</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>G/A or A/A</td>
<td>G/A or A/A</td>
<td>↓</td>
<td>↑</td>
</tr>
</tbody>
</table>

Figure 2

30' ΔInsulin [pM]

Glucose [mM]

<table>
<thead>
<tr>
<th>GCK</th>
<th>G6PC2</th>
<th>GCK</th>
<th>G6PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>GG</td>
<td>GG</td>
<td>GG</td>
</tr>
<tr>
<td>GA or AA</td>
<td>GA or AA</td>
<td>GA or AA</td>
<td>GA or AA</td>
</tr>
</tbody>
</table>

p=0.0016

p=0.191
Figure 3