

# Additive Effects of Genetic Variation in *GCK* and *G6PC2* on Insulin Secretion and Fasting Glucose

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**OBJECTIVE**—Glucokinase (*GCK*) and glucose-6-phosphatase catalytic subunit 2 (*G6PC2*) regulate the glucose-cycling step in pancreatic  $\beta$ -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 METabolic Syndrome in Men (METSIM) Study.

**RESULTS**—rs1799884 was not associated with any quantitative trait (corrected  $P > 0.1$ ), whereas rs560887 was significantly associated with the oral glucose tolerance test 30-min incremental insulin response (30'  $\Delta$ insulin, 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 single nucleotide polymorphisms was associated with fasting glucose (corrected  $P = 0.03$ ) and 30'  $\Delta$ insulin (corrected  $P = 0.027$ ). This additive association was replicated in METSIM (fasting glucose,  $P = 3.5 \times 10^{-10}$  30'  $\Delta$ insulin,  $P = 0.028$ ). When we examined the relationship between fasting glucose and 30'  $\Delta$ insulin stratified by *GCK* and *G6PC2*, we noted divergent changes in these quantitative traits for *GCK* but parallel changes for *G6PC2*. We observed a similar pattern in METSIM.

**CONCLUSIONS**—Our data suggest that variation in *GCK* and *G6PC2* have additive effects on both fasting glucose and insulin secretion. *Diabetes* 58:2946–2953, 2009

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Genome-wide association (GWA) studies have identified several loci for type 2 diabetes (1–5) and type 2 diabetes-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  $\beta$ -cells. Mutations in *GCK* confer susceptibility to maturity-onset diabetes of the young (MODY)-2 (24–26), and a  $-30$  *GCK* promoter variant (rs1799884) has been shown to be associated with  $\beta$ -cell function (21), fasting glucose, and birth weight (23). Chen et al. (9) demonstrated an association between the *G6PC2* region and fasting glucose, an observation replicated by Bouatia-Naji et al. (10). Although fasting glucose levels are associated with both *GCK* and *G6PC2*, there has been no evidence that genetic variation at these two loci contribute a risk for type 2 diabetes, suggesting contribution to mild elevations in glycemia.

*GCK* phosphorylates glucose to glucose-6-phosphate, whereas *G6PC2* dephosphorylates glucose-6-phosphate back to glucose, forming a glucose cycle previously demonstrated to exist within pancreatic  $\beta$ -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 validated by a 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  $\beta$ -cell compensation (disposition index) with the goal of identifying genes influencing variations in type 2 diabetes-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  $\beta$ -cells, we hypothesized that interaction between these loci may be associated not just with fasting glucose but also with measures of insulin secretion or  $\beta$ -cell function. We tested this hypothesis in the BetaGene Study and, for replication, in a separate sample of Finnish men participating in the METabolic Syndrome in Men (METSIM) Study (36).

## RESEARCH DESIGN AND METHODS

### The BetaGene Study

**Subject recruitment.** Subject recruitment for BetaGene was ongoing at the time of these analyses. Subjects are Mexican American (both parents and  $\geq 3$  grandparents Mexican or of Mexican descent) who are either probands with GDM diagnosed within the previous 5 years and diagnosed in their family members or non-GDM probands with normal glucose levels in pregnancy in the past 5 years. Subject recruitment has been previously detailed (33–35). The institutional review boards of participating institutions approved all protocols for BetaGene, and all participants provided written informed consent before 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 an oral glucose tolerance test (OGTT; 75 g) as previously described (33–35). Participants with fasting glucose  $< 126$  mg/dl were invited back for a second visit, which consisted of a dual-energy X-ray absorptiometry scan for body composition (percent of body fat) and an insulin-modified intravenous glucose tolerance test (IVGTT) performed as previously described (37).

**Assays.** Plasma glucose is measured on an autoanalyzer using the glucose oxidase method (YSI Model 2300; Yellow Springs Instruments, Yellow Springs, OH). Insulin is measured by two-site immunoenzymometric assay that has  $< 0.1\%$  crossreactivity with proinsulin and intermediate split products.

### The METSIM Study

**Subject recruitment.** We attempted to replicate our findings in subjects participating in the ongoing METSIM Study (36). The primary goal of the METSIM Study was to investigate the effect of genetic variation on risk for type 2 diabetes and cardiovascular disease in a random sample of Finnish men (aged 50–70 years) living in the town of Kuopio, eastern Finland (population 95,000). There were 5,327 men from this ongoing population-based study included in this report. The ethics committee of the University of Kuopio and in accordance with the Helsinki Declaration approved the METSIM Study.

**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 min postload.

**Molecular analysis.** In BetaGene, GKC  $-30G \rightarrow A$  polymorphism (rs1799884) and G6PC2 rs560887 were genotyped using the TaqMan system (Applied Biosystems) (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, GKC rs4607517 and G6PC2 rs560887 were genotyped by the homogeneous MassEXTEND reaction using the MassARRAY System (Sequenom) (9). In the HapMap CEU data (40), which provide a good representation of linkage disequilibrium in Finns (41), rs4607517 is a perfect proxy for rs1799884 ( $D' = 1.0$ ,  $r^2 = 1.0$ ). 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 and for non-Mendelian inheritance using PEDSTATS version 0.6.4 (42). Allele frequencies for BetaGene samples were estimated using all available data taking into account relatedness using SOLAR version 2.1.4 (43,44), whereas 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'  $\Delta$ Insulin) and the incremental area under the insulin curve for the first 10 min of the IVGTT (AIR). IVGTT glucose and insulin data were analyzed using the minimal model (MINMOD Millennium version 5.18) to derive measures of glucose effectiveness and  $S_1$  (45). Disposition index, a measure of  $\beta$ -cell compensation for insulin resistance, was computed as the product of  $S_1$  and AIR. We also computed an OGTT-derived measure of  $\beta$ -cell compensation as the product of  $S_1$  and 30'  $\Delta$ insulin (DI30).

We initially examined the association between the two single nucleotide polymorphisms (SNPs) and fasting glucose, fasting insulin, 30'  $\Delta$ 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 recognized that these variants could also underlie variation in other type 2 diabetes-related quantitative traits. We tested these SNPs for association with 2-h glucose, 2-h insulin,  $S_p$ ,  $S_c$ , BMI, and percent of body fat as a series of secondary analyses.

Quantitative trait data were statistically transformed to approximate univariate normality before analyses. We first tested each SNP for association with type 2 diabetes-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 because of 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 (four traits in the primary analysis, six traits in the secondary analysis). We used  $P_{ACT}$  to control for the number of tests performed and to account for the correlation among the quantitative traits tested for association (46).  $P_{ACT}$  compares the observed test statistic with their asymptotic distribution using numeric integration and directly accounts for correlation among both dependent and independent variables.

Next, we performed a test of the association between type 2 diabetes-related quantitative traits and the interaction between GKC 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-d.f. test and performed a Bonferroni correction to account for the number of traits tested because  $P_{ACT}$  is not valid for multi-d.f. 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 GKC 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-d.f. test to test the joint effect of rs1799884 and rs560887 for association with type 2 diabetes-related quantitative traits.

**METSIM Study.** Specific results observed in the BetaGene Study were tested in the METSIM samples. Standard regression methods were used to test for association between genetic variants and type 2 diabetes-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 means and SD, whereas METSIM data are reported as age-adjusted means and SD 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 of 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 ongoing 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.

GKC rs1799884 (G>A) had a minor allele frequency of 18.0% and was marginally associated with fasting glucose (Table 2,  $P = 0.052$ ) that modestly increased ( $P = 0.021$ ) after adjustment for body fat. Individuals homozygous for the G allele had a lower average fasting glucose compared with those with at least one A allele ( $5.13 \pm 0.52$  vs.  $5.22 \pm 0.59$  mmol/l). However, the association between GKC 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 type 2 diabetes-related quantitative traits (Table 3), although there was a tendency for mean fasting insulin, 30'  $\Delta$ insulin, 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'  $\Delta$ insulin ( $P = 0.0038$ ) and marginally associated with AIR ( $P = 0.088$ ). These associ-

TABLE 1  
Subject characteristics

	GDM probands	Siblings	Cousins	Non-GDM probands
Men/women	164/0	239/154	133/101	67/0
Age (years)	35.5 (5.6)	35.1 (8.6)	33.2 (9.3)	34.6 (4.9)
BMI (kg/m <sup>2</sup> )	38.8 (5.5)	32.8 (8.4)	31.0 (8.5)	36.9 (5.7)
Body fat (%)	31.4 (6.7)	29.8 (5.9)	28.4 (6.4)	28.9 (6.0)
Fasting glucose (mM)	5.3 (0.6)	5.2 (0.5)	5.1 (0.6)	4.8 (0.4)
2-h glucose (mM)	8.6 (2.4)	7.7 (2.2)	6.9 (2.0)	6.3 (1.2)
Fasting insulin (pM)	74 (53)	62 (47)	59 (52)	49 (37)
30' insulin (pM)	494 (316)	522 (342)	547 (373)	506 (353)
2-h insulin (pM)	647 (475)	523 (410)	453 (380)	362 (252)
30' Δinsulin (pM)	420 (288)	461 (320)	488 (345)	458 (325)
S <sub>G</sub> (×10 <sup>-2</sup> min <sup>-1</sup> )	1.58 (0.55)	1.73 (0.68)	1.83 (0.71)	2.01 (0.69)
S <sub>I</sub> (×10 <sup>-3</sup> min <sup>-1</sup> per pM)	2.98 (1.77)	2.95 (1.64)	3.14 (1.72)	3.32 (1.46)
AIR (pM × 10 min)	4,540 (4,280)	5,771 (5,565)	6,313 (5,690)	7,082 (5,079)
Disposition index	10,933 (8,882)	14,002 (9,519)	15,518 (9,712)	19,173 (9,267)

Data are unadjusted median (interquartile range).

ations 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 ( $5.19 \pm 0.52$  vs.  $5.08 \pm 0.61$  mmol/l). 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 ( $P_{ACT} = 0.021$ ). Although *G6PC2* rs560887 was not associated with most type 2 diabetes-related quantitative traits, we did observe an association between this SNP and percent body fat ( $P_{ACT} = 0.035$ ), 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 type 2 diabetes-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 type 2 diabetes-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 observed a similar linear relationship (data not shown) when we stratified *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 Fig. 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 Fig. 1.

When we stratified individuals by *GCK* and *G6PC2* genotypes as depicted in Fig. 1, a linear trend in 30' Δinsulin (Fig. 2, top;  $P = 0.0016$ ) is observed where on average, 30' Δinsulin decreased ~5.6 pmol/l among genotype groups. We do not see a clear linear relationship when the same stratification is used to examine the relationship with fasting glucose (Fig. 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 examined fasting

TABLE 2  
Univariate tests of associations between genetic variants and fasting glucose and measures of insulin secretion

	<i>GCK</i> (rs1799884)			<i>G6PC2</i> (rs560887)		
	GG ( <i>n</i> = 405)	GA or AA ( <i>n</i> = 207)	Uncorrected <i>P</i> *	GG ( <i>n</i> = 458)	GA or AA ( <i>n</i> = 165)	Uncorrected <i>P</i> *
Fasting glucose (mM)	5.13 ± 0.52	5.22 ± 0.59	0.052	5.19 ± 0.52	5.08 ± 0.61	0.015
Fasting insulin (pM)	63.2 ± 49.7	57.5 ± 47.3	0.412	60.5 ± 45.3	63.0 ± 57.0	0.833
30' Δinsulin (pM)	489 ± 338	447 ± 314	0.119	488 ± 339	427 ± 300	0.004
AIR (pM × 10 min)	6,197 ± 6,238	5,721 ± 4,258	0.770	6,196 ± 5,673	5,650 ± 5,485	0.088

Data are unadjusted means ± SD unless otherwise indicated. \*Age- and sex-adjusted *P* value not corrected for multiple testing.

TABLE 3

Univariate tests of associations between genetic variants and additional type 2 diabetes-related quantitative traits

	<i>GCK</i> (rs1799884)			<i>G6PC2</i> (rs560887)		
	GG ( <i>n</i> = 405)	GA or AA ( <i>n</i> = 207)	Uncorrected <i>P</i> *	GG ( <i>n</i> = 458)	GA or AA ( <i>n</i> = 165)	Uncorrected <i>P</i> *
BMI (kg/m <sup>2</sup> )	29.6 ± 6.4	28.7 ± 5.6	0.150	29.7 ± 6.2	28.3 ± 5.7	0.012
Body fat (%)	32.2 ± 8.6	31.8 ± 8.3	0.121	32.5 ± 8.5	31.4 ± 8.4	0.007
2-h glucose (mM)	7.39 ± 2.24	7.40 ± 2.07	0.477	7.38 ± 2.08	7.45 ± 2.43	0.805
2-h insulin (pM)	505 ± 416	485 ± 374	0.764	502 ± 409	488 ± 377	0.871
S <sub>G</sub> (×10 <sup>-2</sup> min <sup>-1</sup> )	1.78 ± 0.71	1.73 ± 0.66	0.340	1.78 ± 0.67	1.73 ± 0.74	0.218
S <sub>I</sub> (×10 <sup>-3</sup> min <sup>-1</sup> per pM)	3.02 ± 1.78	3.00 ± 1.48	0.944	2.96 ± 1.68	3.12 ± 1.66	0.469

Data are unadjusted means ± SD unless otherwise indicated. \*Age- and sex-adjusted *P* value not corrected for multiple testing.

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, whereas 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 (Fig. 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 with variation in *G6PC2*. For *GCK* rs1799884, individuals homozygous for the G allele (Fig. 3, circles) had lower fasting glucose and higher 30' Δinsulin than individuals with at least one A allele (Fig. 3, squares). For *G6PC2* rs560887, individuals homozygous for the G allele (Fig. 3, open symbols) had higher fasting glucose and higher 30' Δinsulin than individuals with at least one A allele (Fig. 3, solid symbols). In other words, variation in *GCK* led to inverse changes in fasting glucose and 30' Δinsulin, whereas 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 linkage disequilibrium 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-d.f. test of the joint additive effect of the two SNPs was significant for both fasting glucose (*P* = 3.5 × 10<sup>-10</sup>) 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<sup>-11</sup>); the association with 30' Δinsulin became stronger when BMI was included as a

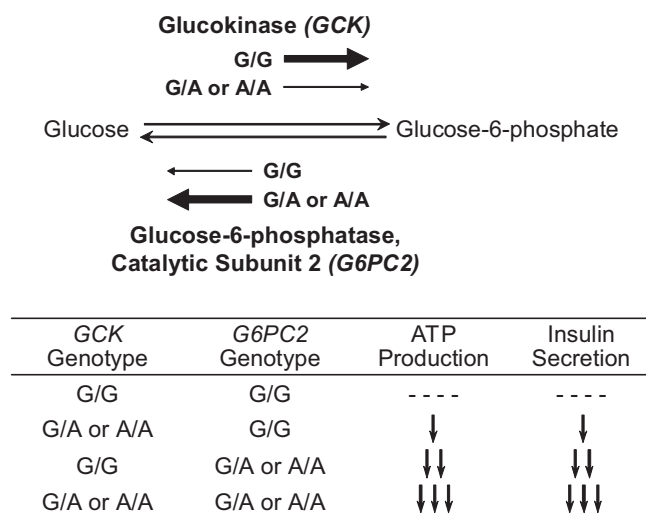


FIG. 1. Hypothesized effect of *GCK* rs1799884 and *G6PC2* rs560887 genotype combinations on ATP production and insulin secretion in pancreatic β-cells. Upper portion of the figure shows the glucose-cycling step and the effect of specific alleles in *GCK* and *G6PC2* on activity of each enzyme: thick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

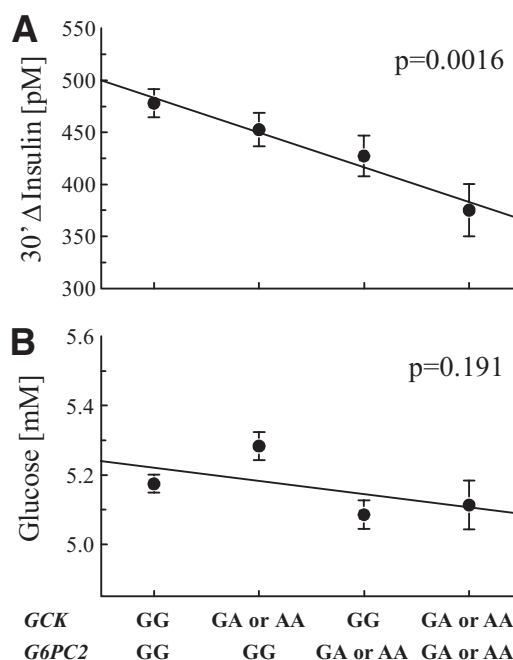
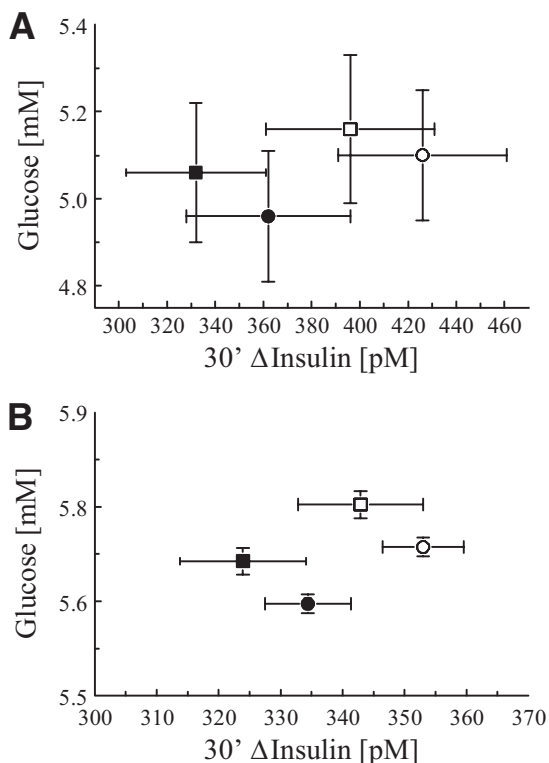


FIG. 2. The 30' Δinsulin and fasting glucose stratified by *GCK* rs1799884 and *G6PC2* rs560887 genotypes. A: Age- and sex-adjusted 30' Δinsulin (means ± SE) stratified by *GCK* rs1799884 and *G6PC2* rs560887 genotype combination. Genotype groups are ordered by hypothesized effect on glucose cycling 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. B: Age- and sex-adjusted fasting plasma glucose (means ± SE) stratified by genotype. Details are as described for top panel. Fasting glucose was not linearly related to genotype group (*P* = 0.191).



**FIG. 3.** The 30'  $\Delta$ insulin vs. fasting plasma glucose stratified by *GCK* rs1799884 and *G6PC2* rs560887 genotype. **A:** Shows results from the BetaGene Study. Model-predicted age- and sex-adjusted 30'  $\Delta$ insulin (means  $\pm$  SD) stratified by genotype is plotted against model-predicted age- and sex-adjusted fasting plasma glucose (means  $\pm$  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. **B:** 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.

covariate ( $P = 0.0028$ ). When we examined the relationship between fasting glucose and 30'  $\Delta$ insulin stratified by *GCK* rs4607517 and *G6PC2* rs560887 genotype groupings, an identical pattern of relationship to that seen in BetaGene was observed (Fig. 3, bottom). For *GCK* rs4607517, individuals homozygous for the G allele had lower fasting glucose and higher 30'  $\Delta$ insulin than individuals with at least one A allele. For *G6PC2* rs560887, individuals homozygous for the G allele had higher fasting glucose and higher 30'  $\Delta$ insulin 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

**TABLE 4**

Univariate tests of associations between genetic variants and fasting glucose and 30'  $\Delta$ insulin in METSIM

	<i>GCK</i> (rs4607517)			<i>G6PC2</i> (rs560887)		
	GG ( $n = 4,185$ )	GA or AA ( $n = 962$ )	$P^*$	GG ( $n = 2,661$ )	GA or AA ( $n = 2,315$ )	$P^*$
Fasting glucose (mM)	$5.68 \pm 0.49$	$5.74 \pm 0.50$	0.0003	$5.72 \pm 0.49$	$5.64 \pm 0.50$	$1.2 \times 10^{-8}$
30' $\Delta$ insulin (pM)	$343 \pm 270$	$333 \pm 235$	0.7362	$351 \pm 280$	$332 \pm 244$	0.0517

Data are unadjusted means  $\pm$  SD unless otherwise indicated. \*Age-adjusted  $P$  value.

**TABLE 5**

Summary of modeling analysis

Model	BetaGene		METSIM	
	Fasting glucose	30' $\Delta$ insulin	Fasting glucose	30' $\Delta$ insulin
Age + sex + <u><i>GCK</i></u>	0.058	0.148	0.0003	0.736
Age + sex + <u><i>G6PC2</i></u>	0.012	0.012	$1.2 \times 10^{-8}$	0.052
Age + sex + <i>GCK</i> + <i>G6PC2</i> + <u><i>GCK</i> <math>\times</math> <i>G6PC2</i></u>	0.257	0.396	0.038	0.049
Age + sex + <u><i>GCK</i></u> + <u><i>G6PC2</i></u>	0.0075	0.0067	$3.5 \times 10^{-10}$	0.028

Data are uncorrected  $P$  values for the genetic effect being tested. Underline indicates the genetic effect being tested.

between 30'  $\Delta$ insulin 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'  $\Delta$ insulin. Although 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'  $\Delta$ insulin. 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'  $\Delta$ insulin ( $P = 0.148$  for BetaGene;  $P = 0.723$  for METSIM). Our a priori hypothesis tested whether the multiplicative interaction between rs1799884 and rs560887 was associated with type 2 diabetes-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  $\sim 1.8\%$  of the variability in fasting glucose or 30'  $\Delta$ insulin, assuming  $\alpha = 0.01$ , 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'  $\Delta$ insulin. 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'  $\Delta$ insulin against *GCK* rs1799884 and *G6PC2* rs560887 genotype combinations sorted by the hypothesized biologic effect on insulin secretion, we observed a linear relationship with 30'  $\Delta$ insulin. Recently, Pirot et al. (47) showed in mouse and human islets that *G6PC2* protein levels and enzymatic activity increased when islets were incubated in high-glucose media, whereas those for *GCK* did not change, suggesting that *G6PC2* activity may adapt to changes in glucose to alter insulin secretion. Because 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. (32) showed that altering glucose cycling by varying the *G6PC2*-to-*GCK* ratio leads to reductions in ATP production and reduced glucose-stimulated insulin secretion in MIN-6 cells. Taken together, these studies examining the balance between *GCK* and *G6PC2* support our hypothesized effects of variation in *GCK* and *G6PC2* on insulin secretion (compared with Fig. 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  $\beta$ -cell sensitivity to glucose should result in increased systemic insulin concentrations in relation to glucose levels. The fact that the linearity in 30'  $\Delta$ insulin we observed between *GCK* rs1799884 and *G6PC2* rs560887 genotype combinations did not extend to fasting glucose (compared with Fig. 2) is unexpected. We confirmed this initial observation by examining the relationship between fasting glucose and 30'  $\Delta$ insulin, stratifying on *GCK* and *G6PC2* genotype (compared with Fig. 3). In both the BetaGene and METSIM studies, the presence of the *GCK* rs1799884 A allele resulted in lower 30'  $\Delta$ insulin 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 both lower 30'  $\Delta$ insulin 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. This effect is unlikely because 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. (48,49) provide evidence for this possibility. Specifically, their studies show that in canines 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. Variation in *G6PC2* may disrupt pulsatility in insulin secretion, thereby reducing insulin-signaling efficiency between the pancreas and liver. The small increase in glucose because of 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 (compared with 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 because of 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 conclusion, 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 because of 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 to fully unravel the genetic architecture underlying glucose regulation and diabetes.

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