

Genetic Regulation of Birth Weight and Fasting Glucose by a Common Polymorphism in the Islet Cell Promoter of the Glucokinase Gene

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Rare mutations in the glucokinase (*GCK*) gene cause fasting hyperglycemia and considerably influence birth weight when present in a mother or her offspring. The role of common variation of *GCK* is uncertain. A polymorphism at position -30 of the *GCK* β -cell-specific promoter, present in 30% of the population, has been variably associated with type 2 diabetes and diabetes-related quantitative traits. Using 1,763 U.K. Caucasian normoglycemic adult subjects, we demonstrated that the A allele at *GCK*(-30) is associated with a 0.06-mmol/l increase in fasting plasma glucose (FPG) ($P = 0.003$). The A allele was also associated with an increase in FPG in 755 women who were 28 weeks pregnant (0.075 mmol/l, $P = 0.003$). We then went on to analyze the effect of *GCK*(-30) on birth weight using 2,689 mother/child pairs. The presence of the A allele in the mother was associated with a 64-g (25–102 g) increase in offspring birth weight ($P = 0.001$). We did not detect a fetal genotype effect. The increase in offspring birth weight in the 30% of mothers carrying an A allele at *GCK*(-30) is likely to reflect an elevated FPG during pregnancy. This study establishes that common genetic variation, in addition to rare mutations and environmental factors, can affect both FPG and birth weight. *Diabetes* 54:576–581, 2005

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ALSPAC, Avon Longitudinal Study of Parents and Children; BCG, Barry Caerphilly Growth; EFS, Exeter Family Study; FPG, fasting plasma glucose; *GCK*, glucokinase; NGT, normal glucose tolerant; PEB, Plymouth EarlyBird.

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In pancreatic β -cells and hepatocytes, glucokinase (*GCK*) catalyzes the first rate-limiting step in glucose metabolism. Its key regulatory role in the β -cell has led to it being described as the "pancreatic β -cell glucose sensor" (1). Mutations in the *GCK* gene cause maturity-onset diabetes of the young, a dominantly inherited young-onset subtype of diabetes (2–4). The *GCK* maturity-onset diabetes of the young phenotype is characterized by lifelong mild fasting hyperglycemia (usually between 5.5 and 8.5 mmol/l) that deteriorates little with age (5). Fetal insulin is a critical regulator of fetal growth and is secreted in response to maternal glucose. Babies born to mothers with a *GCK* mutation have increased birth weight (~600 g) due to increased fetal insulin secretion in response to maternal hyperglycemia (6). Conversely, if the fetus inherits a *GCK* mutation, this reduces its ability to sense glucose, and fetal insulin secretion is reduced and these babies are lighter by an average of 500 g (6). When both the mother and the fetus have a *GCK* mutation, the two effects cancel out and the baby is of normal birth weight (6).

We hypothesized that common variants in *GCK* may explain some of the variation in fasting plasma glucose (FPG) and birth weight within a population. Among U.K. Caucasians, there are no common polymorphisms in the coding region of *GCK*; however, an A to G variant, minor allele frequency 18%, in the islet cell promoter region is likely to have some influence on *GCK* expression, as it occurs 30 bp upstream of *GCK* in a region of strong homology among humans, mice, and rats (available from the Santa Cruz Genome Website <http://genome.ucsc.edu>).

In view of the inconsistent results of previously published *GCK*(-30) association studies (7–14), we hypothesized that *GCK*(-30) had only a small effect on FPG that would require combined analysis from multiple large cohorts to be reliably detected. Using 2,518 subjects from three large U.K. Caucasian population cohorts, the initial part of our study established that the A allele at *GCK*(-30) was associated with a modest elevation in FPG in both normal glucose tolerant (NGT) and pregnant subjects. Having shown that the polymorphism was associated with a phenotype consistent with reduced *GCK* activity, we

TABLE 1
Characteristics of subjects used in the fasting glucose study

| | BCG | Plymouth adults | Exeter male subjects | Exeter pregnant female subjects | Plymouth offspring |
|--------------------------------|------------------|------------------|----------------------|---------------------------------|--------------------|
| <i>n</i> (% males) | 626 (53) | 356 (52) | 781 (100) | 755 (0) | 203 (51) |
| Age (years) | 25.0 ± 0.8 | 34.6 ± 5.3 | 32.7 ± 5.8 | 30.4 ± 5.1 | 4.9 ± 0.3 |
| BMI (kg/m ²) | 25.1 ± 4.7 | 26.5 ± 4.7 | 26.7 ± 3.8 | 27.9 ± 4.7 | 16.2 ± 1.9 |
| FPG (mmol/l) | 4.60 ± 0.38 | 4.70 ± 0.43 | 4.68 ± 0.42 | 4.36 ± 0.38 | 4.31 ± 0.40 |
| Fasting blood insulin (pmol/l) | 38.6 (29.4–55.2) | 56.7 (42.7–79.5) | 59.8 (40.0–67.0) | 58.8 (46.3–81.7) | 30.8 (18.9–42.7) |

Data are means ± SD, means (interquartile range), or *n* (%).

went on to examine the effect of maternal and fetal GCK(–30) variation on birth weight.

RESEARCH DESIGN AND METHODS

Association of GCK(–30) genotype with FPG in nonpregnant subjects. To assess the association of GCK(–30) with FPG in nonpregnant subjects, we used 1,763 people from three large population-based cohorts of normoglycemic adult subjects on whom fasting glucose data and a GCK(–30) genotype were available. Clinical characteristics of the subjects in the FPG study are presented in Table 1. Brief descriptions of each of the cohorts are provided below.

The Exeter Family Study (EFS) is an ongoing prospective study of newborn babies and their parents from a geographically defined region of Exeter, U.K., and has been described previously (15). The EFS was originally set up to study genetic and environmental factors influencing fetal growth and development. The mothers were 28 weeks pregnant at the time of study. The EFS, therefore, provided 781 NGT fathers for this part of the study. Fasting blood glucose was measured in the local clinical chemistry laboratory at the Royal Devon and Exeter Hospital using dry slide technology on Vitros 950 analyzers (Ortho Clinical) before December 2001 and manufacturer's standard reagents on Modular analyzers (Roche Diagnostics, Lewes, East Sussex, U.K.) thereafter. Both methods demonstrated analytical coefficients of variation of <5%.

The Plymouth EarlyBird (PEB) study (16) is a nonintervention prospective study of school-age children and their parents that aims to identify causes of childhood insulin resistance. All Plymouth primary schools were identified, and 56 schools consented to participate, from which a random selection (after stratification by socioeconomic status) was made. With the parents' consent, 307 children and their parents became part of the EarlyBird cohort. Various anthropometric and biochemical measurements were made on the children and their parents. Glucose was measured on a Cobas Integra 700 analyzer (Roche Diagnostics, Lewes, East Sussex, U.K.). A total of 356 parents were provided by this study.

The Barry Caerphilly Growth (BCG) study is a longitudinal study that has been described in detail previously (17) and provided 626 adult subjects. Briefly, the BCG study was initially undertaken between 1972 and 1974 as a randomized control trial on the effects of milk supplementation during pregnancy and up to the age of 5 years on childhood growth. Between 1997 and 1999, all the original children who had completed the 5-year follow-up were traced, and they completed a questionnaire and attended a screening clinic where growth and oral glucose tolerance test measurements were taken.

All subjects were healthy U.K. Caucasians with normal glycemia (FPG between 3.0 and 6.0 mmol/l and, where data were available, HbA_{1c} levels <6.5%). All subjects in the FPG and birth weight studies gave their informed consent, and ethical approval was obtained from the relevant local committee for each study.

Association of GCK(–30) genotype with FPG in pregnant women. To assess the impact of the GCK(–30) polymorphism on fasting glucose in pregnancy, we studied 755 pregnant mothers from the EFS who had had their

fasting blood glucose measured at 28 weeks gestation. All subjects were healthy U.K. Caucasians with FPG between 3.0 and 6.0 mmol/l.

Association of maternal GCK(–30) genotype with birth weight. The effect of maternal GCK(–30) variation on birth weight was assessed using the subjects shown in Table 2. For this part of the study, we required a maternal GCK(–30) genotype and her child's birth weight. The EFS study provided 661 mother/child pairs, the PEB 203, and the BCG 111. In addition, we analyzed 555 families from the Uppsala study, a Swedish study of siblings and their parents chosen to examine genetic and intrauterine influences on the association between birth weight and blood pressure. A further 1,159 informative mother/child pairs were available from the "Children In Focus" Avon Longitudinal Study of Parents and Children (ALSPAC) cohort (18) (available from <http://www.alspac.bris.ac.uk>). When birth weights were available from more than one offspring, to keep observations independent, we only used the older sibling on whom data was available for this study. Overall, we therefore examined 2,689 mother/child pairs. In the EFS, PEB, ALSPAC, and Uppsala studies birth weights were taken from hospital records, and gestational age was estimated from the last menstrual period and ultrasound data. For the BCG study parental birth weights were obtained from hospital records, but offspring birth weights were from maternal report. Subjects born <36 weeks gestation were excluded from the analysis. All subjects were European Caucasians.

Association of fetal GCK(–30) with birth weight. To examine the fetal GCK(–30) genotype effect on birth weight, we analyzed 3,350 subjects who had a GCK(–30) genotype and a birth weight measurement. These comprised 581 EFS, 154 PEB, 636 BCG, 552 Uppsala, and 1,427 ALSPAC subjects. If maternal GCK(–30) genotype alters birth weight, it may mask any fetal genotype effect. Therefore, in addition to looking for overall association with fetal genotype, we analyzed our data stratifying by differences in fetal and maternal genotype in the mother/child pairs. Both maternal and fetal genotype and birth weight data were available for 2,102 mother/child pairs (546 EFS, 154 PEB, 525 Uppsala, and 876 ALSPAC subjects). The BCG study was not used for this analysis, as DNA was not available from offspring. In addition, we performed a discordant sibpair analysis using the 138 sibpairs from the Uppsala study who were discordant for GCK(–30).

Genotyping. For the EFS, PEB, and BCG studies, the GCK(–30) polymorphism was genotyped as previously described (10). We used a Taqman assay (Applied Biosystems) to genotype the ALSPAC cohort for GCK(–30). For the Uppsala study, the polymorphism was genotyped by a homogeneous minisequencing assay with fluorescence polarization detection (19,20). Primer details and reaction conditions are available from the authors. All cohorts, both separately and combined, were in Hardy-Weinberg equilibrium. Genotyping accuracy was demonstrated by showing 100% concordance with results obtained by direct sequencing and by randomly retyping 15% of subjects, which were >99.5% concordant for all methods.

Statistical analysis. Within each cohort, we assessed the association of GCK(–30) with FPG using multiple linear regression, with sex (coded 0 for men and 1 for women), age, and BMI as covariates. The women from the EFS study were analyzed separately, as they were 28 weeks pregnant at the time of study. For the combined analysis of nonpregnant adults, "study" was included as a random covariate in the regression model by using separate dummy

TABLE 2
Characteristics of subjects used in the birth weight study

| | Exeter | Plymouth | Uppsala | BCG | ALSPAC |
|-------------------------|-------------|-------------|-------------|-------------|-------------|
| <i>n</i> (% Male) | 661 (51) | 203 (51) | 555 (51) | 636 (53) | 1,447 (52) |
| Birth weight (g) | 3,561 ± 472 | 3,476 ± 474 | 3,594 ± 575 | 3,372 ± 499 | 3,497 ± 486 |
| Gestational age (weeks) | 40 (36–43) | 40 (36–42) | 40 (38–41)* | 40 (36–44) | 40 (36–43) |

Data are *n* (%), means ± SD, or median (range). *This study only included subjects with gestational ages between 38 and 41 weeks.

variables coded 0 and 1 for the PEB men, PEB women, BCG men, and BCG women.

We assessed the association of GCK(-30) with birth weight using multiple linear regression within each cohort, adjusting for sex and gestational age. To produce an estimated combined effect size across studies, we used a weighted mean-difference meta-analysis method (StatsDirect V2.2.6, StatsDirect, Sale, U.K.). For the maternal/fetal interaction analysis, we stratified mother/child pairs by the relative number of fetal-to-maternal A alleles. This analysis is based on the monogenic model where only children with an additional mutant GCK allele compared with their mother demonstrate low birth weight. When the mother and child both have a mutant GCK gene, the effect of maternal hyperglycemia and reduced fetal insulin secretion cancel out. To estimate the combined-study fetal and maternal A allele effect size and the associated *P* values, we again used the weighted mean-difference approach. Analysis of the discordant-for-GCK(-30) sibs was as follows: birth weight, gestational age, and parity data were available from all 584 sibpairs from the Uppsala study, so a regression equation of birth weight against sex (men coded 0 and women 1), gestational age (dummy variables coded 0 and 1, for 38, 39, and 41 weeks), and parity (dichotomized to older [1] vs. younger [0]) was obtained. Individual birth weight residuals were then added to the overall mean for birth weight, which provided an adjusted birth weight for each subject. Sibs discordant for GCK(-30) were analyzed by a paired *t* test based on the corrected birth weights. For all analyses, unadjusted and equivalent nonparametric analyses produced essentially identical results. All *P* values are two sided.

RESULTS

Fasting glucose in NGT nonpregnant subjects. Table 3 and Fig. 1 present the results of our FPG study for the 1,763 normoglycemic nonpregnant subjects. There was no evidence for heterogeneity among the three cohorts (*Q* test, *P* = 0.75). Carriers of the A allele at GCK(-30) had a plasma glucose concentration 0.061 mmol/l higher than common homozygotes subjects (*P* = 0.003). Genotype analysis did not show an allele dosage trend (GG = 4.65 mmol/l, GA = 4.71 mmol/l, and AA = 4.68 mmol/l).

Fasting glucose in NGT pregnant female subjects. The A allele at GCK(-30) was associated with FPG in the 755 pregnant women from the EFS study (0.075 mmol/l, *P* = 0.003; Table 3). Genotype analysis did not show an allele dosage trend (GG = 4.31 mmol/l, GA = 4.39 mmol/l, and AA = 4.35 mmol/l). Combining the pregnant women with the NGT subjects produced an estimated effect size for the A allele of 0.067 mmol/l, *P* = 0.00005.

Birth weight

Maternal GCK(-30) genotype effect. The effect of maternal GCK(-30) variation on offspring birth weight in five large cohorts is shown in Fig. 2. The presence of a maternal A allele was associated with a mean increase of 64 g (25–102 g) for A allele carriers over GG homozygotes (combined *P* = 0.001). There was weak evidence for a maternal A allele dosage effect, with AA homozygotes being 98 g (3–195 g) heavier (*P* = 0.04) and AG heterozygotes being 58 g (19–97 g) (*P* = 0.004) heavier than GG homozygotes.

Pregnancy FPG data were only available from the EFS cohort. Adjusting birth weight for FPG in the EFS cohort, the association of the maternal GCK(-30) genotype with birth weight became nonsignificant (*P* = 0.13). This demonstrates that the effect of GCK(-30) on FPG can, at least partially, explain the increase in birth weight associated with maternal GCK(-30) genotype.

Fetal GCK(-30) genotype effect. A simple combined analysis of all studies where fetal genotype was available did not provide any evidence that fetal GCK(-30) genotype affects birth weight (AG/AA vs. GG effect size = -6 g, *P* = 0.75). However, this may be due to confounding by maternal genotype. In the four studies where we had

TABLE 3
Fasting glucose concentration, adjusted for sex, age, and BMI by cohort, by GCK(-30) genotype

| | BCG | Plymouth adults | Exeter male subjects | Combined nonpregnant/NGT subjects | Exeter pregnant female subjects |
|-----------------------------|------------------------|------------------------|------------------------|-----------------------------------|---------------------------------|
| GG | 4.57 (4.54–4.60) (426) | 4.66 (4.60–4.71) (232) | 4.69 (4.65–4.73) (508) | 4.65 (4.63–4.67) (1,166) | 4.31 (4.28–4.34) (502) |
| GA | 4.64 (4.59–4.69) (180) | 4.74 (4.66–4.82) (111) | 4.75 (4.69–4.81) (239) | 4.71 (4.67–4.75) (530) | 4.39 (4.35–4.43) (226) |
| AA | 4.50 (4.35–4.65) (20) | 4.78 (4.54–5.01) (13) | 4.76 (4.60–4.95) (34) | 4.68 (4.58–4.78) (67) | 4.35 (4.22–4.47) (27) |
| GG vs. GA (<i>P</i> value) | 0.04 | 0.09 | 0.08 | 0.002 | 0.003 |
| GG vs. AA (<i>P</i> value) | 0.33 | 0.36 | 0.33 | 0.57 | 0.57 |
| GG vs. A (<i>P</i> value)* | 0.10 | 0.06 | 0.07 | 0.003 | 0.003 |

Data are means (95% CI) (*n*). EFS female subjects were pregnant at time of study so were analyzed separately. *GA and AA combined.

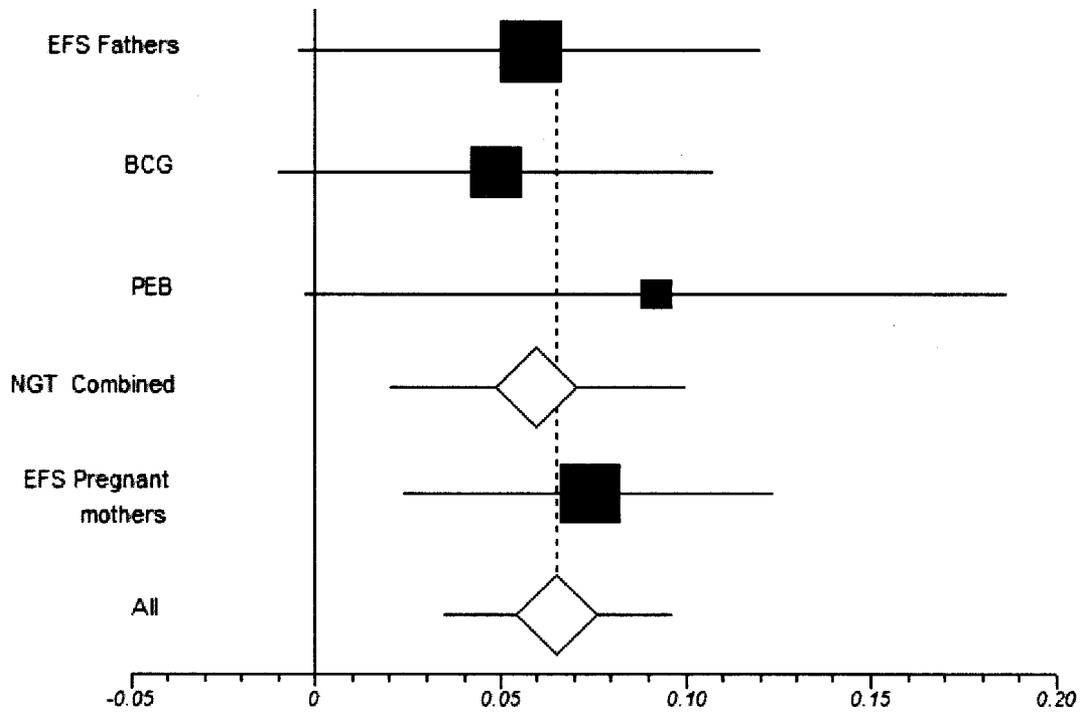


FIG. 1. Forest plot of the effect on FPG subjects carrying an A allele at GCK(-30). The mean difference in FPG for subjects carrying at least one A allele at GCK(-30) vs. GG homozygote subjects.

access to maternal and fetal DNA, we were able to analyze the effect of fetal genotype after stratifying by maternal genotype. Children who had an additional A allele relative to their mother were not significantly lighter than offspring

who had an equivalent number of A alleles to their mother (18 g lighter, $P = 0.43$). In the Uppsala study, 138 of 552 sibpairs were discordant for GCK(-30) genotype. Sibs with at least one extra A allele were not significantly

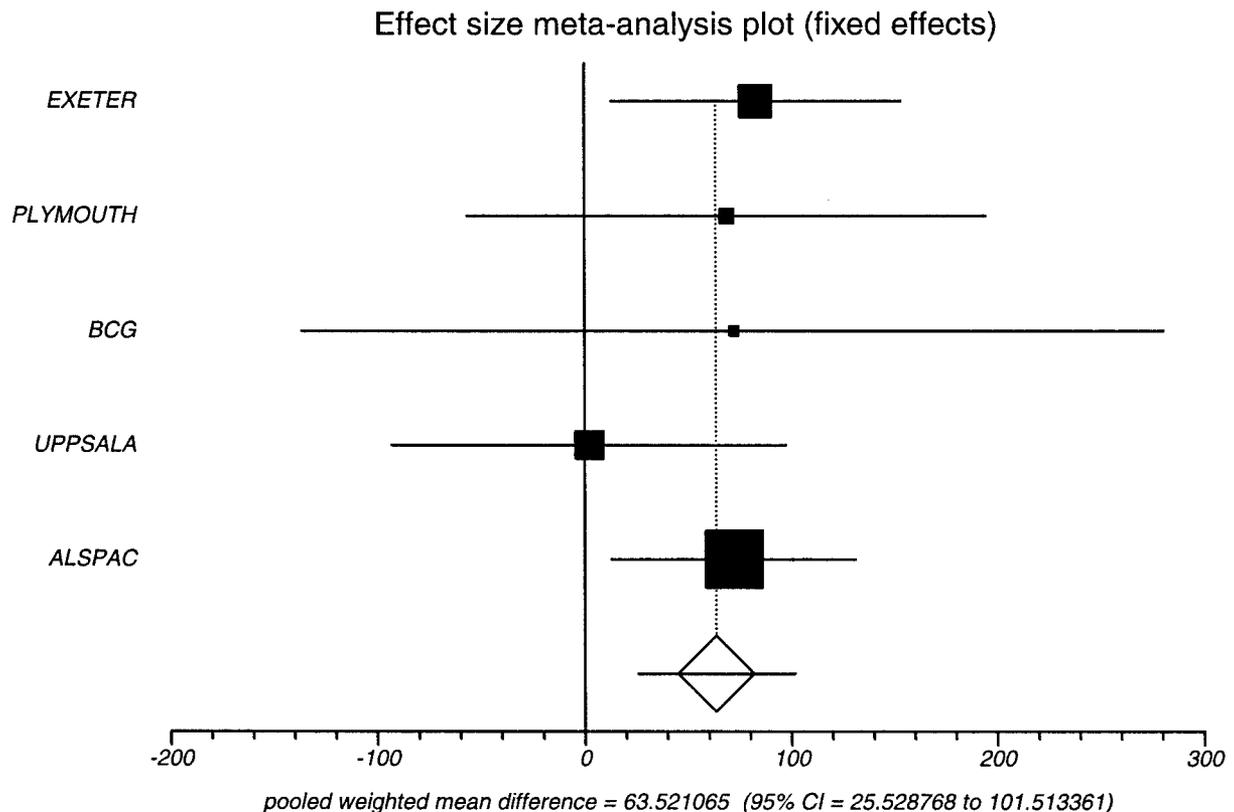


FIG. 2. The effect on offspring birth weight for mothers carrying an A allele at GCK(-30). Birth weights were adjusted for sex and gestational age. The plots show the mean difference in offspring birth weight for mothers carrying at least one A allele at GCK(-30) vs. GG homozygote subjects.

lighter than their siblings (61 g [-25 to 147 g] lighter, $P = 0.17$).

DISCUSSION

Using five large population-based cohorts, we have demonstrated that a common variant in the *GCK* islet cell promoter, which is present in ~30% of the U.K. population, affects FPG and birth weight. This is, to our knowledge, the first common genetic variant to be associated, in large studies, with FPG and birth weight.

GCK(-30) increases FPG in the NGT population and in pregnancy. We found a small (0.06 mmol/l), but highly significant, increase in FPG associated with the presence of an A allele at the GCK(-30) β -cell promoter polymorphism. This association was consistent across three adult normoglycemic nonpregnant populations ($P = 0.003$). The A allele at GCK(-30) was also associated with elevated FPG (0.075 mmol/l) in women who were 28 weeks pregnant. The relatively small size of the effect explains why previous association studies (7–14) of GCK(-30) have not all been “positive.” Of the eight previous studies, four have presented FPG data. Three of these studies (8–10) showed nonsignificant trends of elevated FPG with the A allele at GCK(-30). The fourth and smallest study (7) ($n = 65$) demonstrated a nominally significant association of the A allele with FPG (GG = 5.3 mmol/l vs. AG/AA = 5.8 mmol/l, $P < 0.05$) in NGT subjects at baseline, but this was not replicated in a 5-year follow-up. There was no evidence to support an allele dosage effect, suggesting that the polymorphism has a similar impact when heterozygous and homozygous. This result had not been anticipated because for severe mutations, the homozygous phenotype is more severe than the heterozygous phenotype (21,22).

Maternal GCK(-30) genotype is associated with birth weight. Our study demonstrates that maternal GCK(-30) genotype is associated with fetal growth. Although small, the effect size of 64 g for A allele carriers is similar to the effect of nutritional supplementation demonstrated in randomized trials (23,24). It is likely this result reflects increased fetal growth as a result of the increase in maternal glucose. In keeping with this, the significant association of maternal GCK(-30) genotype with birth weight is removed when we include maternal 28-week glucose concentration as a covariate in the analysis. Our study provides no evidence for a fetal GCK(-30) effect on fetal growth. This is unexpected, as for rare mutations, the reduction associated with a fetal GCK mutation was only slightly smaller than the increase in birth weight seen with a maternal *GCK* mutation (6).

Is GCK(-30) the functional variant? The association of GCK(-30) with FPG strongly suggests that the A allele, or the genetic variation in strong linkage disequilibrium with it, is reducing the activity of *GCK*. Direct sequencing of 100 subjects identified no common polymorphisms (minor allele frequency >5%) in the *GCK* coding region. Therefore, reduced expression of *GCK* is the most likely explanation for the observed associations. GCK(-30) occurs in the *GCK* β -cell-specific promoter, in a region strongly conserved among humans, mice, and rats, and transversional mutagenesis of a 10-bp sequence, including GCK(-30), reduced *GCK* transcription by 22% (25). Therefore, it is possible that GCK(-30) is the functional variant

explaining its association with FPG and birth weight. However, further haplotype and functional studies are required to exclude the possibility that the causal variant is another noncoding variant in strong linkage disequilibrium with GCK(-30).

The small effect size on FPG and birth weight means that even large individual cohorts have low power to detect an effect of GCK(-30). For example, in our FPG study, only the pregnant women demonstrated a significant association individually. In the absence of sufficiently large individual cohorts, this study illustrates the value of combining cohorts for polygenic analyses.

This is the first study to reproducibly demonstrate an association between a common polymorphism and the quantitative traits of FPG and birth weight. Further, GCK(-30) is the first common genetic variant to be associated in a large study with altered birth weight through an effect on a maternal phenotype. Our study, therefore, establishes that common genetic variation of *GCK* can affect FPG and, by altering the intrauterine environment, fetal growth.

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