Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: Common Genetic Variants in *GCK* and *TCF7L2* Are Associated With Fasting and Postchallenge Glucose Levels in Pregnancy and With the New Consensus Definition of Gestational Diabetes Mellitus From the International Association of Diabetes and Pregnancy Study Groups

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OBJECTIVE—Common genetic variants in *GCK* and *TCF7L2* are associated with higher fasting glucose and type 2 diabetes in nonpregnant populations. However, their associations with glucose levels from oral glucose tolerance tests (OGTTs) in pregnancy have not been assessed in a large sample. We hypothesized that these variants are associated with quantitative measures of glycemia in pregnancy.

RESEARCH DESIGN AND METHODS—We analyzed the associations between variants rs1799884 (*GCK*) and rs7903146 (*TCF7L2*) and OGTT outcomes at 24–32 weeks' gestation in 3,811 mothers of European (U.K. and Australia) and 1,706 mothers of Asian (Thailand) ancestry from the HAPO cohort. We also tested associations with offspring birth anthropometrics.

RESULTS—The maternal *GCK* variant was associated with higher fasting glucose in Europeans ($P = 0.001$) and Thais ($P < 0.0001$), 1-h glucose in Europeans ($P = 0.001$), and 2-h glucose in Thais ($P = 0.005$). It was also associated with higher European offspring birth weight, fat mass, and skinfold thicknesses ($P < 0.05$). The *TCF7L2* variant was associated with all three maternal glucose outcomes ($P = 0.03$, $P < 0.0001$, and $P < 0.0001$ for fasting and 1-h and 2-h glucose, respectively) in the Europeans but not in the Thais ($P > 0.05$). In both populations, both variants were associated with higher odds of gestational diabetes mellitus according to the new International Association of Diabetes and Pregnancy Study Groups recommendations ($P = 0.001–0.08$).

CONCLUSIONS—Maternal *GCK* and *TCF7L2* variants are associated with glucose levels known to carry an increased risk of adverse pregnancy outcome in women without overt diabetes. Further studies will be important to determine the variance in maternal glucose explained by all known genetic variants. ***Diabetes*** **59:2682–2689, 2010**

Maternal glycemia in pregnancy is associated with adverse pregnancy outcomes including birth weight >90th percentile, delivery by cesarean section, neonatal hypoglycemia, and fetal hyperinsulinemia (1). These associations occur across the full range of maternal glucose levels below those classified as overt diabetes.

In healthy, nondiabetic, nonpregnant populations, approximately one-third of the variation in fasting glucose is genetic (2), and common genetic variants at multiple loci are now robustly associated with fasting glucose (3–10) and with type 2 diabetes and related glycemic traits (11–18). Thus, genetic factors are likely to contribute to variation in glucose levels in pregnancy. However, these variants have not been examined extensively in large studies of pregnant women.

Studies of birth weight in Europeans have provided indirect evidence that two common genetic variants influence maternal glycemia in pregnancy. The T-allele of the rs1799884 variant in the *GCK* gene is associated with higher fasting glucose in the general population (4) and with type 2 diabetes (10). Pregnant women who carry this allele give birth to babies that are, on average, 32 g (95% CI 11–53) heavier at birth (4). Similarly, each additional T-allele of rs7903146 in the *TCF7L2* gene—which is associated with reduced β-cell function, raised fasting glucose, and type 2 diabetes (10,13,19)—is also associated with a 30-g (95% CI 15–45) higher offspring birth weight when carried by the mother (20). We hypothesize that these associations with birth weight reflect higher levels of maternal glucose, which result in greater fetal insulin secretion and a consequent increase in fetal size at birth (21).

There is some evidence from small studies that the *GCK*

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Received 5 February 2010 and accepted 18 July 2010. Published ahead of print at http://diabetes.diabetesjournals.org on 3 August 2010. DOI: 10.2337/db10-0177.

*A complete list of the members of the HAPO Study Cooperative Research Group is available in the appendix of the following publication: HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991–2002.

and *TCF7L2* variants are associated with fasting glucose in pregnancy or gestational diabetes mellitus. The *GCK* variant was associated with higher fasting glucose in 755 European pregnant women from the U.K. (22) and with gestational diabetes mellitus in a Scandinavian sample (23). Variation at the *TCF7L2* locus was not associated with fasting glucose in 921 European pregnant women (20) but was associated with gestational diabetes mellitus in Scandinavian (24,25), Korean (26), and Mexican-American (27) samples.

The large sample size and detailed pregnancy and birth phenotype data available in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study provide a unique opportunity to investigate more thoroughly the associations of the *GCK* and *TCF7L2* variants with maternal glycemia, as measured in an oral glucose tolerance test (OGTT), during pregnancy as well as fetal size at birth and body composition. We used OGTT results from 5,517 pregnant women of European and South East Asian ancestry to assess associations both with quantitative measures of maternal glucose and with the new International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommendations for the diagnosis of gestational diabetes mellitus (28).

RESEARCH DESIGN AND METHODS

We studied 3,811 pregnant women of European ancestry (Manchester and Belfast, U.K., and Newcastle and Brisbane, Australia) and 1,706 pregnant women of South East Asian ancestry (Bangkok, Thailand). The protocol was approved by the institutional review board at each field center, and all participants gave written, informed consent.

Maternal phenotypes and exclusion criteria. The HAPO study methods have previously been described in detail (1,29,30). Briefly, eligible women (30) underwent a 75-g OGTT at 24–32 weeks' gestation (as close to 28 weeks as possible). Fasting, 1-h, and 2-h glucose levels were measured. Height, weight, and blood pressure were also measured using standardized procedures and calibrated equipment. A sample for random plasma glucose was collected at 34–37 weeks' gestation as a safety measure to identify cases with hyperglycemia above a predefined threshold. Gestational age was determined as previously described (30). Demographic and lifestyle characteristics, age, and parity were collected via questionnaire. Race/ethnicity was self-identified.

Participants, caregivers, and HAPO study staff (except laboratory personnel) remained blinded to glucose values unless fasting plasma glucose was >5.8 mmol/l, 2-h OGTT plasma glucose was >11.1 mmol/l, random plasma glucose was ≥8.9 mmol/l, or any plasma glucose value was <2.5 mmol/l. Although unblinded participants were excluded from the original HAPO study of pregnancy outcome (1), we did not exclude them from maternal glucose analyses in the current study because there was no intervention to alter maternal glucose levels before the OGTT.

Neonatal phenotypes. Cord blood samples for DNA, plasma glucose, and serum C-peptide were collected from the offspring at delivery. Neonatal anthropometric data were collected within 72 h of delivery. These have been described in detail previously (31). In the current study, we analyzed weight, length, head circumference, triceps, and flank and subscapular skinfold thicknesses. Birth weight, length, and head circumference were available from medical records in addition to the measurements taken by HAPO study personnel. To maximize sample size, we used the medical record birth weight because it was more widely available and highly correlated with the HAPO-measured birth weight ($r = 0.98$). We used the HAPO study measures for length and head circumference and added medical record values when these were unavailable. Fat mass at birth (grams) was derived using the following formula (32):

$$1{,}000 \times (0.39055 \times \mathrm{BW}/1{,}000 + 0.0453 \times \mathrm{FLS} - 0.03237 \times \mathrm{BL} + 0.54657) \quad (1)$$

where BW is birth weight in grams, FLS is flank skinfold thickness in millimeters, and BL is birth length in centimeters (all measured by HAPO study personnel). From this, percent body fat was derived as follows: 100 × fat mass/birth weight.

Genotyping. We selected the rs1799884 (*GCK*) and rs7903146 (*TCF7L2*) single nucleotide polymorphisms (SNPs) for association analysis. As well as being robustly associated with higher fasting glucose and type 2 diabetes in Europeans (4,10,19), these SNPs, or perfectly correlated ($r^2 = 1$) proxies, show similar associations in East Asian and South Asian populations (33–37). We therefore carried out our primary analyses using only these SNPs. However, we also selected *TCF7L2* rs290487 and rs11196218 for analysis in our Thai samples because they have shown independent evidence of association with type 2 diabetes in East Asians ($r^2 < 0.1$ with rs7903146 and $r^2 = 0.01$ with each other in the HapMap Han Chinese from Beijing and Japanese from Tokyo reference samples) (35,38–40) and are more common than rs7903146 in these samples, giving greater power to detect associations.

Genotyping was carried out as part of a larger candidate panel of 1,536 SNPs using the Illumina Golden Gate platform in the Northwestern University Genomics Core. We included DNA samples in our study that were successfully genotyped for over 94% of SNPs. Call rates for the *GCK* and *TCF7L2* SNPs exceeded 99% in these samples, and there was not deviation from Hardy-Weinberg equilibrium ($P > 0.01$ for the four SNPs in each of the five field centers). The frequency of the T-allele of *GCK* rs1799884 (associated with higher glucose levels) ranged from 17.1 to 18.7% in the European samples and was 10.3% in the Thai sample. The type 2 diabetes risk allele frequency of *TCF7L2* rs7903146 ranged from 29.2 to 30.6% in the Europeans and was 4.7% in the Thai. The rs290487 and rs11196218 risk allele frequencies (risk alleles defined by previous studies) (35,38–40) were 47.4 and 72.4%, respectively, in the Thai sample.

Statistical analyses: associations between maternal genotype and maternal glycemia. All analyses were carried out using Stata (version 10; Stata, College Station, TX). We analyzed the association between each of the three primary outcome measures (fasting plasma glucose, 1-h OGTT glucose, and 2-h OGTT glucose) and genotype using linear regression under an additive genetic model. We included maternal age, BMI, and mean arterial pressure as covariates in the model. We repeated the analysis including additional covariates (BMI squared, gestational age at OGTT, parity, sex of the baby, and maternal height) to check that they did not change the results. We analyzed the European and Thai samples separately and included field center as a covariate in all analyses of Europeans.

We also sought to investigate the association of each SNP with maternal glucose levels that carry a substantially higher risk of adverse pregnancy outcome. We created a high glucose variable with a value of 1 if the participant had one or more high values in the OGTT (fasting plasma glucose ≥5.1 mmol/l, 1-h glucose ≥10.0 mmol/l, or 2-h glucose ≥8.5 mmol/l) and a value of 0 if the glucose was below these thresholds. The high glucose threshold corresponds to an odds ratio (OR) of 1.75, relative to the mean glucose level, averaged across three outcomes in the HAPO study: offspring birth weight >90th percentile, cord C-peptide >90th percentile, offspring percent body fat >90th percentile, and is the IADPSG-recommended threshold for gestational diabetes mellitus (28). We analyzed the association between each SNP and the odds of high glucose using logistic regression (log-additive genetic model), adjusting for field center (European ancestry samples only) maternal age, BMI, and mean arterial pressure. Using all 5,515 study subjects with both *GCK* and *TCF7L2* genotype available (and adjusting for field center), we then analyzed the association between the odds of high glucose and the combined number of T-alleles at both loci (0, 1, 2, and 3 or 4), with the same covariates as before. We combined individuals with three or four T-alleles into one group because of low numbers in the final category. To test for deviation from a multiplicative trend across the four groups, we compared the results with a full model (including the allele score as indicator variables) using a likelihood ratio test.

To guard against possible population stratification, we generated principal components of ancestry using smartpca from the Eigensoft software package (41) based on 141 ancestry informative markers that were genotyped in the same panel of 1,536 SNPs as the *GCK* and *TCF7L2* SNPs. We repeated our analyses including the first two principal components as covariates.

Associations between maternal genotype and neonatal anthropometric traits. We analyzed the association between each neonatal outcome and maternal genotype using linear regression (additive genetic model). We performed the analysis twice: *1*) a minimally adjusted model with field center (European ancestry only), sex of the baby, and gestational age at delivery as covariates and *2*) a fully adjusted model including maternal age at OGTT, maternal BMI at OGTT, maternal BMI at OGTT squared, parity, maternal smoking (yes/no), mean arterial pressure, and maternal height as additional covariates. The first analysis was performed to enable comparison with previously published studies (4,20,22), and the second was performed for comparison with the first to verify that the additional covariates did not change the results. All analyses of neonatal outcomes excluded babies born preterm (before 37 full weeks of gestation) and pregnancies in which caregivers were unblinded to maternal glucose levels.

We performed inverse variance meta-analysis (fixed effects) of the association between each SNP and birth weight to combine the HAPO study data with data from previously published studies (4,20,22). Within each study sample, the association between genotype and birth weight was analyzed using linear regression under an additive genetic model, with sex and

TABLE 1
Basic characteristics of study participants

	Belfast, U.K.	Manchester, U.K.	Brisbane, Australia	Newcastle, Australia	Bangkok, Thailand
Pregnant women (*n*)*	1,284	1,085	959	483	1,706
Gestational age at which OGTT was performed (weeks)	29.0 ± 1.2	28.4 ± 1.0	28.1 ± 1.2	28.1 ± 1.5	28.1 ± 1.7
Age at OGTT (years)	29.8 ± 5.5	30.8 ± 5.6	29.2 ± 5.3	29.5 ± 5.5	27.9 ± 5.5
BMI at OGTT (kg/m^2)	28.4 ± 4.9	29.0 ± 5.5	29.0 ± 5.7	29.7 ± 6.0	25.6 ± 3.6
Mean arterial pressure at OGTT (mmHg)	83.6 ± 7.9	83.5 ± 8.0	83.8 ± 7.7	82.9 ± 8.1	80.1 ± 7.8
FPG (mmol/l)	4.63 ± 0.37	4.61 ± 0.40	4.43 ± 0.35	4.54 ± 0.41	4.44 ± 0.37
1-h plasma glucose (mmol/l)	7.50 ± 1.70	7.46 ± 1.80	7.40 ± 1.51	7.33 ± 1.66	8.27 ± 1.75
2-h plasma glucose (mmol/l)	6.08 ± 1.23	5.99 ± 1.35	6.25 ± 1.20	6.21 ± 1.33	6.66 ± 1.40
Offspring birth weight in g†	3,526 ± 489	3,511 ± 498	3,552 ± 466	3,585 ± 466	3,142 ± 399
Gestational age at delivery (weeks)†	40.1 ± 1.1	40.0 ± 1.3	40.0 ± 1.2	40.1 ± 1.2	39.4 ± 1.2
Male offspring (%)†	50.6	52.1	51.1	51.2	48.7
Maternal smoking during pregnancy (%)†	23.7	19.2	13.2	15.5	0.6
Primiparous births (%)†	50.2	47.8	55.8	46.1	53.3

Data are means ± SD unless otherwise indicated. *Number of women with fasting glucose, *GCK* rs1779984 genotype, age, BMI, and mean arterial pressure available. The numbers with *TCF7L2* rs7903146 genotype were very similar. †Excluding births before 37 completed weeks of gestation and pregnancies in which caregivers were not blinded to maternal glucose levels. FPG, fasting plasma glucose.

gestational age at delivery as covariates. For the meta-analysis, we used the METAN module developed for Stata (42). Heterogeneity between studies was estimated using Cochran's *Q* test and the I^2 statistic (43). We also used Cochran's *Q* test to assess evidence of heterogeneity between the analyses of maternal glucose outcomes in the European and Thai samples.

Associations between fetal genotype and birth weight. We analyzed the association between birth weight and fetal genotype using linear regression (additive model), adjusting for field center (European ancestry only), sex, and gestational age and again excluding preterm births and unblinded pregnancies. Since maternal and fetal genotypes are correlated, we then repeated the analysis stratifying by maternal genotype and tested for evidence of a fetal genotype–birth weight association within each stratum. To address whether fetal genotype alters the impact of maternal glucose levels on offspring birth weight, fat mass, or skinfold thickness, we additionally tested for evidence of interaction between fetal genotype and maternal glucose using a likelihood ratio test.

Power calculations. Our sample of 3,811 Europeans gave us 80% power to detect per-allele differences in an outcome of 0.09 SD and 0.07 SD for the *GCK* and *TCF7L2* SNPs, respectively, at $P < 0.05$. Due to the lower sample size and allele frequencies in the Thai sample, power to detect these associations was reduced (35 and 14%, respectively). Power calculations were performed using Quanto, version 1.2 (44).

Comparing the discriminatory impact of maternal genotype with measured glucose on birth weight and neonatal adiposity. To address whether maternal *GCK* and *TCF7L2* genotypes improve prediction of birth weight or neonatal adiposity in the presence of different combinations of other known variables, we used logistic regression to model the odds of birth weight, skinfold sum, and percent body fat >90th percentile against various explanatory variables, including both maternal *GCK* and *TCF7L2* genotypes (included as indicator variables). We constructed receiver operator characteristic (ROC) curves and calculated the area under the curve (AUC) to estimate the discriminatory power of the model. The AUCs were compared for various models using a χ^2 test. For this analysis, we combined all of the study subjects and adjusted for study center.

RESULTS

Associations between maternal genotype and maternal glycemia. Basic characteristics of the study participants are presented in Table 1. The associations between continuous measures of maternal glucose, as determined during an OGTT, and the *GCK* and *TCF7L2* variants are presented in Table 2 and Table 3, respectively. Summary data from each of the field centers with European ancestry women are presented in supplementary Tables 1 and 2, available in an online appendix (http://diabetes.diabetesjournals.org/cgi/content/full/db10-0177/DC1).

We observed associations between the *GCK* rs1799884 variant and fasting glucose in both the European (0.03 mmol/l higher per T-allele [95% CI 0.01–0.05]; $P = 0.001$)

TABLE 2
Association between maternal *GCK* rs1799884 genotype and maternal glucose levels in pregnancy

		Mean ± SE plasma glucose level by *GCK* rs1799884 genotype				
	Total *N*	CC	CT	TT	Effect size ± SE per T-allele (mmol/l)*†	*P*†
FPG (mmol/l)						
European	3,811	4.55 ± 0.01	4.59 ± 0.01	4.63 ± 0.03	0.03 ± 0.01	0.001
Thai	1,706	4.42 ± 0.01	4.51 ± 0.02	4.52 ± 0.08	0.08 ± 0.02	<0.0001
1-h plasma glucose (mmol/l)						
European	3,811	7.39 ± 0.03	7.54 ± 0.05	7.69 ± 0.15	0.15 ± 0.05	0.001
Thai	1,706	8.25 ± 0.05	8.35 ± 0.09	8.54 ± 0.37	0.11 ± 0.09	0.24
2-h plasma glucose (mmol/l)						
European	3,811	6.11 ± 0.02	6.12 ± 0.04	6.18 ± 0.11	0.02 ± 0.04	0.56
Thai	1,706	6.61 ± 0.04	6.85 ± 0.08	6.81 ± 0.30	0.21 ± 0.07	0.005

The European plasma glucose levels by genotype are the mean values across all four field centers. These are presented separately in supplementary Table 1. *The T-allele of *GCK* rs1799884 is associated with raised fasting glucose in nondiabetic, nonpregnant populations. The T-allele frequency ranged from 17.1 to 18.7% in the European ancestry samples and was 10.3% in the Thai samples. †The effect sizes and *P* values are from linear regression of maternal glucose level against genotype (coded 0, 1, or 2 T-alleles), with field center (European ancestry only), age, BMI, and mean arterial pressure as covariates. Age, BMI, and mean arterial pressure were measured at a median of 28–29 weeks' gestation, depending on the field center. Mean ± SE values are adjusted for these three covariates. FPG, fasting plasma glucose.

TABLE 3
Association between maternal *TCF7L2* rs7903146 genotype and maternal glucose levels in pregnancy

	Total *N*	Mean ± SE plasma glucose level by *TCF7L2* rs7903146 genotype			Effect size ± SE per T-allele (mmol/l)*†	*P*†
		CC	CT	TT		
FPG (mmol/l)						
European	3,811	4.56 ± 0.01	4.55 ± 0.01	4.63 ± 0.02	0.02 ± 0.01	0.03
Thai	1,706	4.44 ± 0.01	4.45 ± 0.03	4.44 ± 0.21	0.01 ± 0.03	0.66
1-h plasma glucose (mmol/l)						
European	3,811	7.34 ± 0.04	7.51 ± 0.04	7.65 ± 0.08	0.16 ± 0.04	<0.0001
Thai	1,706	8.25 ± 0.04	8.53 ± 0.13	7.39 ± 0.96	0.23 ± 0.14	0.09
2-h plasma glucose (mmol/l)						
European	3,811	6.04 ± 0.03	6.18 ± 0.03	6.27 ± 0.06	0.13 ± 0.03	<0.0001
Thai	1,706	6.64 ± 0.03	6.86 ± 0.11	5.81 ± 0.77	0.17 ± 0.11	0.11

The European plasma glucose levels by genotype are the mean values across all four field centers. These are presented separately in supplementary Table 2. *The T-allele of *TCF7L2* rs7903146 is associated with an increased risk of type 2 diabetes. The T-allele frequency ranged from 29.2 to 30.6% in the European ancestry samples and was 4.7% in the Thai samples. †The effect sizes and *P* values are from linear regression of maternal glucose level against genotype (coded 0, 1, or 2 T-alleles), with field center (European ancestry only), age, BMI, and mean arterial pressure as covariates. Age, BMI, and mean arterial pressure were measured at a median of 28–29 weeks' gestation, depending on the field center. Mean ± SE values by genotype are adjusted for these three covariates. FPG, fasting plasma glucose.

and Thai samples (0.08 mmol/l higher per T-allele [0.04–0.12]; $P < 0.0001$). We also observed an association with 1-h glucose in the Europeans ($P = 0.001$) but not in the Thais ($P = 0.24$). However, the estimated differences were similar (per T-allele increase 0.15 mmol/l [0.06–0.25] in the Europeans and 0.11 mmol/l [−0.07 to 0.29] in the Thais). There was evidence of association with 2-h glucose in the Thai sample (0.21 mmol/l higher per T-allele [95% CI 0.06–0.35]; $P = 0.005$), but not in the Europeans (0.02 mmol/l per T-allele [−0.05–0.09]; $P = 0.56$). There was evidence of heterogeneity between the European and Thai samples for the fasting ($P = 0.03$) and 2-h ($P = 0.02$) glucose measures but not the 1-h measure ($P = 0.70$). When we repeated these analyses including additional covariates, we observed similar results (supplementary Table 3).

In the European samples, we observed associations between the *TCF7L2* rs7903146 variant and both 1-h (0.16 mmol/l higher per T-allele [95% CI 0.08–0.24]; $P < 0.0001$) and 2-h (0.13 mmol/l higher per T-allele [0.07–0.19]; $P < 0.0001$) glucose levels. There was also some evidence for association with fasting glucose (0.02 mmol/l higher per T-allele [0.002–0.03]; $P = 0.03$). We observed no evidence for association at $P < 0.05$ between the *TCF7L2* variant and the maternal glucose measures in the Thai samples, reflecting either no association in this population or reduced power due to the lower minor allele frequency. The estimated differences were similar to those in the Europeans (all heterogeneity *P* values >0.6). When we repeated these analyses including additional covariates, we observed similar results (supplementary Table 4). There were also no associations between any of the maternal glucose outcomes and the *TCF7L2* rs290487 or rs11196218 SNPs in the Thai sample ($P > 0.05$; data not shown).

Consistent with the results for continuous glucose measures, we observed associations between both the *GCK* and *TCF7L2* variant and the odds of high glucose, defined by the IADPSG (28) as having one or more of the following: fasting plasma glucose ≥5.1 mmol/l, 1-h glucose ≥10.0 mmol/l, and 2-h glucose ≥8.5 mmol/l (Table 4). In the Europeans, 26% of women with the *GCK* rs1799884 TT genotype had high glucose, compared with 15% with the CC genotype, and the per-allele OR was 1.29 (95% CI 1.09–1.50). The corresponding OR in the Thai sample was 1.42 (1.06–1.77). For *TCF7L2* rs7903146, 20% of women of European ancestry with the TT genotype had high glucose, compared with 16% with the CC genotype. The per-allele ORs were 1.15 (1.00–1.31) in the Europeans and 1.39 (0.88–1.90) in the Thais. Analysis of the complete dataset, combining information from both *GCK* and *TCF7L2*, showed strong evidence for a trend to increased odds of high glucose with an increasing number of T-alleles (OR 1.25 [95% CI 1.14–1.37]; $P < 0.00001$) (supplementary Fig.

TABLE 4
Association between *GCK* or *TCF7L2* genotype and high* maternal glucose levels

Ancestry and genotype	No. of women with high/normal glucose (% high)	Per T-allele OR (95% CI) for high glucose†	*P*†
GCK rs1799884			
European		1.29 (1.09–1.50)	0.001
CC	388/2,575 (15.1)		
CT	194/1,114 (17.4)		
TT	32/122 (26.2)		
Thai		1.42 (1.06–1.77)	0.007
CC	288/1,375 (20.9)		
CT	91/311 (29.3)		
TT	5/20 (25.0)		
TCF7L2 rs7903146			
European		1.15 (1.00–1.31)	0.04
CC	293/1,884 (15.6)		
CT	246/1,557 (15.8)		
TT	75/370 (20.3)		
Thai		1.39 (0.88–1.90)	0.08
CC	338/1,549 (21.8)		
CT/TT‡	46/157 (29.3)		

*Defined as fasting glucose ≥5.1 mmol/l or 1-h glucose ≥10.0 mmol/l or 2-h glucose ≥8.5 mmol/l. †ORs and *P* values are from logistic regression of high glucose status (1 or 0) against genotype (coded 0, 1, or 2 T-alleles), with field center (European ancestry only), age, BMI, and mean arterial pressure as covariates. Age, BMI, and mean arterial pressure were measured at a median of 28–29 weeks' gestation, depending on the field center. ‡Categories are combined because of the small number of TT homozygotes ($n = 3$).

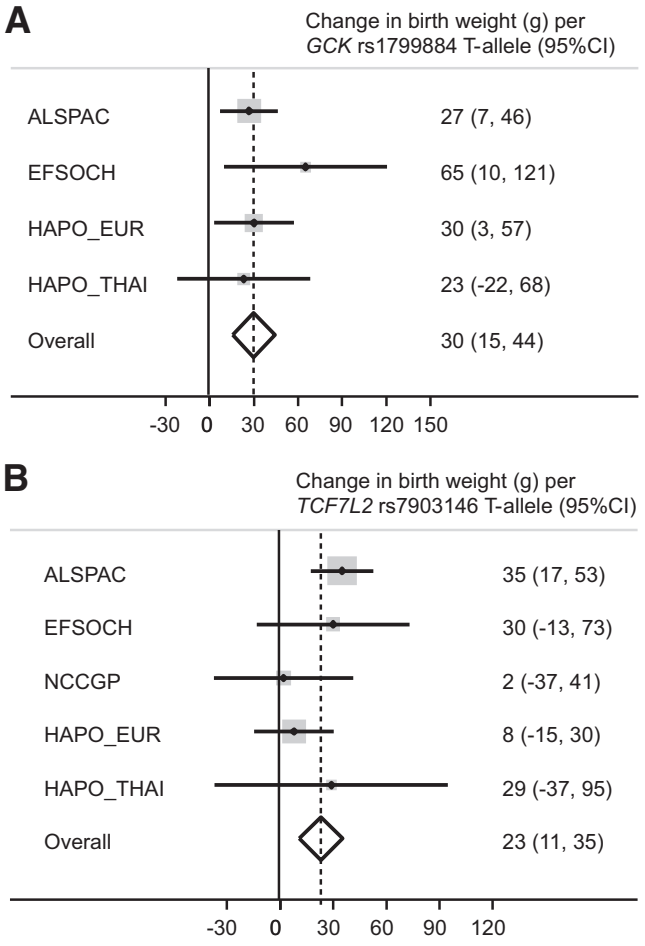

FIG. 1. Meta-analysis of the association between offspring birth weight and maternal *GCK* rs1799884 genotype (overall $P < 0.0001$) (*A*) or maternal *TCF7L2* rs7903146 genotype (overall $P < 0.001$) (*B*) in the HAPO study and previously published studies (4,20,22). Analyses were adjusted for sex and gestational age. ALSPAC, Avon Longitudinal Study of Parents and Children; EFSOCH, Exeter Family Study of Childhood Health; NCCGP, North Cumbria Community Genetics Project.

1). There was no evidence of deviation from a multiplicative model ($P = 0.28$).

Inclusion of the first two ancestry principal components as covariates did not change the associations between the *GCK* and *TCF7L2* variant and maternal glucose outcomes (data not shown). A sensitivity analysis including only participants whose caregivers remained blinded to glucose levels gave very similar results for all associations.

Associations between maternal genotype and neonatal anthropometric traits. Meta-analysis of the associations between the maternal *GCK* and *TCF7L2* variants and offspring birth weight, using both Thai and European study samples, showed that those in the HAPO study were consistent in size and direction with previously published studies of Europeans (heterogeneity P values >0.05; $I^2 < 20\%$) (Fig. 1). The overall P values for association with birth weight were $P < 0.0001$ for maternal *GCK* rs1799884 ($n = 12{,}643$) and $P < 0.001$ for maternal *TCF7L2* rs7903146 ($n = 13{,}406$).

We analyzed the associations between the maternal *GCK* and *TCF7L2* variants and various neonatal traits (supplementary Tables 5 and 6). We did not observe an association with cord C-peptide at either locus ($P > 0.05$). After Bonferroni adjustment for 10 tests, there was no association at $P < 0.05$ with neonatal anthropometric traits at either locus. However, in the Europeans, the *GCK* rs1799884 effect size estimates for all traits were in the direction expected, given the association with maternal glucose, and the unadjusted P values for association with birth weight, fat mass, percent body fat, and skinfold thickness were all <0.05.

Associations between fetal genotype and birth weight. We observed no overall association between birth weight and the fetal genotype at either locus ($P > 0.05$; data not shown). To address the correlation between maternal and fetal genotype, we stratified by maternal genotype, but there was no evidence of association ($P > 0.05$) after multiple testing correction (supplementary Fig. 2). We observed no evidence of interaction between fetal genotype and maternal glucose levels on offspring birth weight, fat mass, or skinfold thickness ($P > 0.05$; data not shown).

Using ROC curves to evaluate the impact of maternal genotype versus maternal glucose on birth weight and neonatal adiposity. We next addressed *1*) whether maternal *GCK* and *TCF7L2* genotypes improve prediction of birth weight or neonatal adiposity when maternal fasting glucose is not known and *2*) whether knowledge of maternal genotypes improves prediction of birth weight or neonatal adiposity when maternal fasting glucose is known but maternal 1- and 2-h glucose values are not known. When maternal fasting glucose was added to a model containing various covariates, the observed area under the ROC curves for birth weight, skinfold sum, and percent body fat >90th percentile increased ($P < 0.05$). In contrast, the addition of maternal genotype at *GCK* and *TCF7L2* did not result in increased discriminatory ability ($P > 0.05$). Similarly, genotype did not improve the AUCs when considered alongside fasting glucose, whereas measured 1- and 2-h glucose values did result in improvement ($P<0.05$; supplementary Table 7).

DISCUSSION

In our study of 5,517 women of European and Thai ancestry, we have shown for the first time associations between variants at the *GCK* and *TCF7L2* loci and continuous OGTT measures of maternal glucose in pregnant women without overt diabetes. An additional new finding in this study is association of these variants with gestational diabetes mellitus, as defined by the new IADPSG consensus recommendation (28), itself associated with higher risk of adverse birth outcomes.

The *GCK* rs1799884 variant was associated with fasting glucose in both of our study populations. The association in the Thai sample was similar to associations previously observed in nonpregnant East Asian subjects (37), while the estimated fasting glucose difference per allele in the Europeans was smaller than previously observed ($P = 0.02$) (7). We observed some evidence of heterogeneity, with the Thai sample showing a tendency to larger per-allele differences than the European ancestry sample ($P = 0.03$). This is consistent with previous observations from East Asian, but not South Asian, versus European analyses (36,37). Changes in glucokinase activity or content in pancreatic β-cells are known to impact primarily on fasting glucose levels (45). However, the rate of β-cell glucose metabolism—and, hence, insulin secretion—is controlled by glucokinase across the full range of glucose levels (46,47), and mutations of the glucokinase gene

result in a raising of glucose values throughout the glucose tolerance test compared with non–mutation-carrying family members (48). In keeping with this, *GCK* rs1799884 was associated with higher 1-h glucose levels in the European population in our study and higher 2-h glucose levels in the Thais. Association of *GCK* rs1799884 with 2-h glucose was demonstrated previously in a European study of nonpregnant individuals (49).

The *TCF7L2* rs7903146 variant showed associations at $P < 0.05$ with all measures of maternal glucose, but these were largest for 1- and 2-h glucose—in keeping with the known association of this locus with glucose-stimulated insulin secretion and incretin signaling in the islet (13). Despite the lower minor allele frequency in the Thai sample, the glucose differences per allele for all three measures were similar to those observed in the Europeans.

Previous studies of both *GCK* and *TCF7L2* have shown associations with gestational diabetes mellitus (23,24,26,27). In keeping with these, we have shown associations with the new consensus definition of gestational diabetes mellitus (28) in both the European and Thai samples. We observed strong evidence for a trend to higher odds of high glucose with increasing numbers of T-alleles at *GCK* and *TCF7L2* ($P < 0.00001$) but found no evidence for statistical interaction between these loci ($P > 0.2$). It is important that we now assess the impact of all confirmed fasting glucose and type 2 diabetes susceptibility loci on maternal glycemia because we are likely to identify extreme groups within the population who are at greatly increased genetic risk of high glucose levels in pregnancy. Due to the adverse impact of glucose levels per se on offspring phenotype, genetic variants that are only associated with steady-state glucose regulation may be associated with potentially harmful outcomes in pregnancy even if they are not associated with disease risk in nonpregnant adults.

It is important to understand the contribution of these variants to nenonatal outcomes. The associations that we observed between the maternal risk allele at both loci and higher offspring birth weight were similar to those previously published (4,20). Our finding that the *GCK* variant showed adiposity associations consistent with the continuous relationship between glucose and neonatal adiposity (31) is a novel observation that extends the previously demonstrated association of this variant with birth weight. Larger studies or meta-analyses will be necessary to provide enough statistical power with which to investigate thoroughly the associations between maternal genotype and neonatal anthropometrics. Consistent with previous studies (4,20,22), we observed no association between birth weight and fetal genotype, suggesting that, unlike rare fetal mutations in *GCK* (50), the common variants at *GCK* and *TCF7L2* do not influence fetal growth directly.

Measurement of maternal glucose can help identify women without overt diabetes who have a higher risk of neonatal adiposity >90th percentile (31). We hypothesized that maternal *GCK* and *TCF7L2* genotypes might add useful information for predicting neonatal birth weight, skinfold sum, and percent body fat >90th percentile when maternal glucose is not known. Using ROC curves, we found that maternal genotypes did not improve the discriminatory ability of the models ($P > 0.05$). This may reflect that genotypes at these variants explain only a small proportion (<1%) of variance in maternal glucose levels. However, a total of 16 genetic variants are now known to explain 3–4% of the variation in fasting glucose in Europeans (10). It will therefore be important to repeat these analyses with all known variants.

To conclude, variants at the *GCK* and *TCF7L2* loci, which predispose to higher fasting glucose and type 2 diabetes in the general population, are associated with *1*) higher glucose levels from OGTTs in pregnant women who do not have overt diabetes and *2*) gestational diabetes mellitus under the new consensus definition (28). Further well-powered studies will be important to assess fully the contribution of known genetic variants to maternal glycemia in pregnancy, pregnancy outcomes, and neonatal phenotypes.

ACKNOWLEDGMENTS

This study was funded by National Institute of Child Health and Human Development and National Institute of Diabetes and Digestive and Kidney Diseases grants R01-HD34242, R01-HD34243, and R01-DK067459 and by the American Diabetes Association. R.M.F. is funded by a Sir Henry Wellcome Postdoctoral Fellowship (Wellcome Trust grant 085541/Z/08/Z).

No potential conflicts of interest relevant to this article were reported.

R.M.F. carried out analyses, wrote the manuscript, reviewed and edited the manuscript, and contributed to discussion. M.G.H. researched data, wrote the manuscript, reviewed and edited the manuscript, and contributed to discussion. M.U. researched data, reviewed and edited the manuscript, and contributed to discussion. L.P.L. researched data, reviewed and edited the manuscript, and contributed to discussion. H.L. carried out analyses and contributed to discussion. C.A. researched data and contributed to discussion. T.M.F. reviewed and edited the manuscript and contributed to discussion. N.J.C. researched data and contributed to discussion. D.B.D. researched data and contributed to discussion. A.R.D. researched data, reviewed and edited the manuscript, and contributed to discussion. A.T.H. researched data and contributed to discussion. B.E.M. researched data, reviewed and edited the manuscript, and contributed to discussion. W.L.L. researched data, wrote the manuscript, reviewed and edited the manuscript, and contributed to discussion.

Parts of this study were presented orally at the American Diabetes Association 69th Scientific Sessions, New Orleans, Louisiana, 5–9 June 2009, and in poster form at the Wellcome Trust/Nature Genetics "Genomics of Common Diseases" meeting, Hinxton, U.K., 23–26 September 2009.

We acknowledge the role of the field centers and investigators who participated in this genetics study: Belfast, U.K. (D. McCance); Manchester, U.K. (K. Cruickshank); Brisbane, Australia (D. McIntyre); and Newcastle, Australia (J. Lowe).

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