Identification of HKDC1 and BACE2 as Genes Influencing Glycemic Traits During Pregnancy Through Genome-Wide Association Studies

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Maternal metabolism during pregnancy impacts the developing fetus, affecting offspring birth weight and adiposity. This has important implications for metabolic health later in life (e.g., offspring of mothers with pre-existing or gestational diabetes mellitus have an increased risk of metabolic disorders in childhood). To identify genetic loci associated with measures of maternal metabolism obtained during an oral glucose tolerance test at ~28 weeks’ gestation, we performed a genome-wide association study of 4,437 pregnant mothers of European (n = 1,367), Thai (n = 1,178), Afro-Caribbean (n = 1,075), and Hispanic (n = 817) ancestry, along with replication of top signals in three additional European ancestry cohorts. In addition to identifying associations with genes previously implicated with measures of glucose metabolism in nonpregnant populations, we identified two novel genome-wide significant associations: 2-h plasma glucose and HKDC1, and fasting C-peptide and BACE2. These results suggest that the genetic architecture underlying glucose metabolism may differ, in part, in pregnancy. Diabetes 62:3282–3291, 2013

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he intrauterine milieu of the developing fetus, as determined largely by maternal metabolism, impacts both fetal and later health outcomes. Offspring of mothers with pre-existing or gestational diabetes mellitus (GDM) have an increased risk of metabolic disorders in childhood, including obesity, impaired glucose tolerance, and higher lipid levels (1–3). Maternal glucose levels less than those diagnostic of GDM are also associated with greater offspring birth weight and adiposity and may impose similar risks later in childhood and adulthood (4–6). The mechanisms underlying these risks are not known, but maternal metabolism is important given the impact of the mother’s metabolic profile on the intrauterine milieu of the developing fetus.

Maternal glucose metabolism during pregnancy differs from the nongravid state because the mother must meet both her own and the growing fetus’s energy needs (7). Fasting glucose decreases progressively throughout gestation, but insulin resistance increases from the end of the first through the third trimester. As insulin resistance increases, basal and stimulated insulin secretion, postprandial glucose levels, and hepatic glucose production increase compared with the nongravid state.

Maternal metabolism is determined by genetic and environmental factors. Given the unique aspects of glucose metabolism in pregnancy, we examined whether genetic variation associated with glycemic traits during pregnancy differs from that known to be important in the nongravid state. This was accomplished using DNA and phenotype data collected by the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study, a multicenter, international study that collected high-quality phenotypic data related to fetal growth and maternal glucose metabolism from ~25,000 pregnant women of varied geographic, ethnic, and sociodemographic backgrounds. Standardized protocols that were uniform across centers were used to test for associations of maternal glycemia less severe than overt diabetes with risks of adverse pregnancy outcomes (6,8). Genetic loci important for maternal metabolism during pregnancy were identified by genome-wide mapping and replication of single nucleotide polymorphisms (SNPs) demonstrating association.

RESEARCH DESIGN AND METHODS

Samples and DNA source

HAPO cohort. All pregnant women at less than 32 weeks of gestation were eligible for enrollment in HAPO unless they met one of several exclusion

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criteria. All participants gave written informed consent, and an external data monitoring committee provided oversight. Study phenotype collection methods and inclusion and exclusion criteria have been published elsewhere (6,8).

Participants underwent a 75-g oral glucose tolerance test (OGTT) at ~28 weeks’ gestation. DNA was removed from the collected blood tube at 2 h during the OGTT, when phenotypes of interest were measured, including glucose, blood pressure, weight, and height. Glucose and C-peptide were measured in a central laboratory (6,8), and DNA was prepared using the automated Autopure LS from Gentra Systems.

Submitted for genotyping were 9,814 mother and offspring HAPO samples (2,581 Afro-Caribbean [AC], 3,152 European ancestry [EU], 1,615 Hispanic [HI], and 2,466 Thai [TH]), after DNA from mothers was removed from the Illumina 610 Quad v1 B SNP array. We used samples from 10,989 individuals from the four HAPO Phase 1 study populations, including 2,278 AC, 2,797 EU, 1,408 HI, and 2,435 TH survived quality control (QC). Demographic and phenotypic descriptions of the mothers whose samples survived QC are summarized in Supplementary Table 1, and the sampling locations of each cohort are listed in Supplementary Table 2.

**Sherbrooke cohort.** Women planning to deliver at the Centre Hospitalier Universitaire de Sherbrooke (CHUS) were recruited between 6 and 13 weeks of pregnancy. Exclusion criteria were age <18 or >40 years, multiple pregnancy, pregestational diabetes (type 1 or 2) or diabetes discovered at the first trimester (defined as glycosuria >10.3 mmol/L at 1 h after 50-g glucose ingestion), drugs and/or alcohol abuse, uncontrolled endocrine disease, renal failure, or other major medical conditions that would affect glucose regulation. The project was approved by the CHUS Ethical Review Board, and written informed consent was obtained before their inclusion in the study.

Demographics and baseline characteristics collected at the first trimester included maternal age, gestational weeks, medications, and personal and family medical history. Height and weight were measured using standardized procedures, and BMI was calculated. Systolic and diastolic blood pressures were measured in the sitting position after 5 min of rest; the average of 3 measurements was used for analyses.

During the second trimester (between 24 and 28 weeks of gestation), medical history and weight were again ascertained. Each participant had a 75-g OGTT in the fasting state (>8 h). Blood samples were maintained at 4°C and centrifuged; plasma was collected, aliquoted, and stored at −80°C until measurements. Plasma glucose was measured by glucose hexokinase (Roche Diagnostics, Indianapolis, IN). C-peptide levels were measured by ELISA (Luminex technology; Millipore Corp., Billerica, MA). C-peptide levels were measured using the commercially available ELISA kit (ALPCO Diagnostics, Salem, NH).

**Chicoutimi cohort (ECOGENE-21).** Women with a singleton pregnancy were recruited at their first trimester of pregnancy from a founder population of French-Canadian origin (Saguenay area, Canada). Women older than 40 years, those with pregestational diabetes or other disorders known to affect glucose metabolism, and those with a positive history of alcohol and/or drug abuse during the current pregnancy were excluded. The Chicoutimi Hospital Ethics Committee approved the project. All women provided written informed consent before their inclusion in the study.

BMI was measured using standardized procedures. Glucose tolerance was assessed using a 75-g OGTT performed at 24–28 weeks’ gestation after a 12-h fast. Blood glucose and C-peptide measurements were made on fresh serum samples. Glucose was evaluated using a Beckman analyzer (model CX7; Fullerton, CA). C-peptide levels were measured using a commercially available ELISA kit (ALPCO Diagnostics, Salem, NH).

**Genotyping.** AC DNA samples were genotyped using the Illumina Human1M-Duo v3 B SNP array, EU DNA samples were genotyped using the Illumina Human 610 Quad v1 B SNP array, and the HI DNA samples were genotyped using the Illumina Human1M-Duo v3 B SNP array at the Broad Institute. TH DNA samples were genotyped using the Illumina HumanOmni1-Quad v1-B SNP array at the Center for Inherited Disease Research, following agreed upon protocols of the Gene-Environment Association Studies (GENEVA) consortium (9).

**Genome-wide association study QC.** Genotype data that passed initial QC at the genotyping centers were released to the GENEVA Coordinating Center (CC), National Center for Biotechnology Information database of Genotypes and Phenotypes (dbGaP), and HAPLO study teams, who collectively performed QC using population-specific QC procedures previously described by the GENEVA consortium (9). Poorly performing samples or SNPs were removed based on misspecified sex, chromosomal anomalies, unDatasets removed with duplicates, sample relatedness, low call rate, high number of Mendelian errors, departures from Hardy-Weinberg equilibrium, duplicate discordance, sex differences in heterozygosity, and low minor allele frequencies, as detailed in Supplementary Tables 3 and 4. Complete QC reports are available through dbGaP, http://www.ncbi.nlm.nih.gov/htbin-study?db=studies&dbfrom=000674&pm=514.

**Ancestry.** Population structure was determined using principal components analysis (PCA), essentially as described by Price et al. (10). All unduplicated HAPO study samples were analyzed separately in each of the four HAPO populations, along with HapMap (Utah residents with ancestry from northern and western Europe; Han Chinese in Beijing, China; Japanese in Tokyo, Japan; and Yoruba in Ibadan, Nigeria) samples genotyped with the Haplo study subjects. From the autosomal SNPs with missing call rate <5% and minor allele frequency >5%, we selected a subset through two rounds of linkage disequilibrium (LD) pruning (short- and long-range), as described previously (9). Outliers (those ±5 SDs from the mean first and second principal component values) whose SNPs were removed. The PCA analysis was performed again when the PCA analysis was performed without HapMap samples. The first two eigenvectors from the results in these analyses were used as covariates in the association tests to adjust for possible population structure among the mothers (Supplementary Figs. 1–4).

**Imputation.** Imputation was performed separately in each of the four QC cleaned and filtered genotyping sets using BEAGLE (11) and a HapMap 2 (12). HapMap (phase 1) was used to impute genotypes for individuals from multiple HapMap Phase 3 populations for imputation, based on the PCA analysis described above (Supplementary Table 5). We first used the strand-checking utility of BEAGLE to ensure consistent strand assignments between the reference dataset and the QC cleaned and filtered datasets, and we subsequently corrected strand and/or removed SNPs where strandedness could not be resolved. Next, we conducted imputation runs in the mothers and offspring separately within each of the four HAPO cohorts. We used a conservative allele $r^2$ threshold of 0.9 to remove questionable imputed SNPs.

**Association tests.** The genotype call probabilities from the filtered BEAGLE output were used in a linear regression model between each of the phenotypes and the genotypes probabilities under an additive model adjusting for the set of maternal covariates. Trait values were adjusted as follows: fasting C-peptide (FPC) and plasma glucose (FPG): log10 (trait), and 1- and 2-h plasma glucose (1HPG and 2HPG): square root (trait). We used the frequentist approach in SNPTES v2.2.0 (13) to estimate the betas and SEs for each regression model and assess significance of the association between the SNP and the phenotype of interest.

We adjusted for confounders in two successive models: model 1 included mother’s age, gestational age at OGTT, parity, field center, and ancestry; and model 2 added maternal BMI, height, and mean arterial pressure measured at the OGTT, and maternal smoking and drinking status (yes/no). For the association tests between the maternal genotype and baby phenotypes (birth weight, fat mass, and sum of skinfolds), we adjusted for confounders in three successive models: model 1 included field center, ancestry using PCA, newborn sex, gestational age at delivery, parity, and maternal age at OGTT; model 2 included additional covariates (birth weight, fat mass, and sum of skinfolds), and maternal smoking and drinking status (yes/no); and model 3 included the covariates from model 2 plus maternal FPG and FCP during the OGTT.

**Meta-analysis.** The betas and SEs were combined across the four cohorts using meta-analysis under a fixed-effects model weighting each strata by sample size. METAL (14) calculates a $z$ statistic that summarizes the magnitude and direction of effect for the association of a reference allele selected at each marker. After aligning the SNPTES output from each of the four cohorts to the same reference allele, a weighted sum of individual cohort results was used to calculate an overall $z$ statistic and $P$ value. The square root of the cohort-specific sample size was used as the proportional weight, and these squared weights sum to 1.

Meta-analysis was initiated after analysis of the HI, AC, and EU genome-wide association study (GWAS) populations before availability of the TH GWAS data. Top associations (those with $P < 1 	imes 10^{-5}$ in the HI-AC-EU meta-analysis or EU cohort itself, and trimmed for LD $r^2 < 0.5$) were replicated in a second set of 2,192 EU HAPO mothers using a custom Illumina 384 SNP bead array consisting of 127 SNPs selected for replication of the traits described herein, 157 SNPs selected for replication of surface markers signals from related projects, and 100 ancestry informative markers, which were the total 50 SNPs associated with the first two principle components in the EU group in the GWAS discovery phase. Genotyping was performed at the Broad Institute.

For the external Sherbrooke and Chicoutimi replication cohorts, DNA was purified from whole-blood samples with Gentra Puregene Cell Kit (Qiagen, Valencia, CA). Selected gene polymorphisms (top 30 associations after replication phase 1) were genotyped using a quantitative RT-PCR assay (model 7500 Fast, Applied Biosystems) with Applied Biosystems TaqMan probes and primers (sequences can be obtained upon request), following the manufacturer’s recommendations (Life Technologies Inc., Burlington, ON, Canada).

**RESULTS**

We performed a discovery GWAS in a large subset of HAPO mothers from four different ancestry populations using the Illumina 610, 1M, and Omni1 platforms, with 4,528 participants (Supplementary Table 1) surviving genotyping QC control. Cohort-specific and meta-analyses of genome-wide SNP data were conducted to identify
common genetic variants associated with maternal FPG, 1HPG, and 2HPG levels as well as FCP levels measured at -28 weeks of gestation. Associations were assessed with linear regressions under an additive genetic model adjusting for confounders in two successive models using genotyped and imputed SNPs (see RESEARCH DESIGN AND METHODS for a description of the models). Results from cohorts were combined through meta-analyses weighting each stratum by sample size. Associations meeting a significance of $P < 1 \times 10^{-5}$ were replicated in a second cohort of EU HAPO mothers, and the top 30 signals were replicated in two independent cohorts of pregnant women of EU.

Several genes/SNPs associated with glycemic traits in nongravid populations (15,16) demonstrated genome-wide significant association ($P < 5 \times 10^{-8}$) in pregnant women (Table 1; Supplementary Table 6; Supplementary Figs. 5–8). Specifically, we found associations with FPG and SNPs in glucokinase regulator (GCKR), glucose-6-phosphatase 2 (G6PC2), propionate convertase subtilisin/kexin type 1 (PCSK1), protein phosphatase 1, regulatory subunit 3B (PPP1R3B), and melanotan receptor 1B (MTNR1B); 1HPG and SNPs in MTNR1B; and FCP and SNPs in PPP1R3B and GCKR. The top association in the vicinity of GCKR was rs1260326, which reached $P = 6.08 \times 10^{-13}$ with FPG and $P = 5.73 \times 10^{-11}$ with FCP in the seven-meta-group meta-analysis, with betas ranging from $-0.30$ to $-0.015 \log_{10}(\mu g/L)$ and from $-0.066$ to $0.0008 \sqrt{(mmol/L)}$ per T allele for FCP and FPG, respectively, in the four GWAS ancestry groups. Similarly, the top G6PC2 association was rs560887 with FPG ($P = 2.08 \times 10^{-16}$), with betas ranging from $-0.0082$ to $-0.0026 \sqrt{(mmol/L)}$ per T allele. The SNP rs6235 in PCSK1 was the SNP most strongly associated with FPG ($P = 4.96 \times 10^{-15}$), with betas ranging from $-0.0069$ to $-0.00054 \sqrt{(mmol/L)}$ per G allele. The top PPP1R3B SNP was rs4841132 at $P = 4.55 \times 10^{-14}$ for FCP (betas ranging from $-0.061$ to $-0.017 \log_{10}(\mu g/L)$ per G allele) and $P = 2.88 \times 10^{-13}$ for FPG (betas ranging from $-0.014$ to $-0.0038 \sqrt{(mmol/L)}$ per G allele). The SNP rs7936247 was the SNP in MTNR1B most strongly associated with FPG ($P = 2.11 \times 10^{-13}$, betas ranging from $0.000055$ to $0.0073 \sqrt{(mmol/L)}$ per T allele) and IHPG ($P = 3.44 \times 10^{-13}$, betas ranging from $0.013$ to $0.29 \sqrt{(mmol/L)}$ per T allele). Although the strength of association varied across cohorts, these data demonstrated evidence of association when combined through meta-analysis.

We also found strong (but not genome-wide significant) associations between SNPs previously found to be associated with glucose or insulin levels or type 2 diabetes in large meta-analyses of nongravid populations (Supplementary Table 7). Genes with SNPs reaching $P < 0.001$, which are not discussed above, include INS1A (rs9157197 with $2 \text{ HPG}$, $P_{\text{meta}} = 5.44 \times 10^{-5}$), CDKAL1 (rs9908322 with $1 \text{ HPG}$, $P_{\text{meta}} = 1.01 \times 10^{-8}$), YPS26A (rs1802295 with $2 \text{ HPG}$, $P_{\text{meta}} = 6.42 \times 10^{-10}$), and ARAP1 (rs11603394 with $2 \text{ HPG}$, $P_{\text{meta}} = 8.63 \times 10^{-4}$).

Several of these most strongly associated SNPs were not the SNPs previously reported to be associated with the phenotype of interest in nongravid populations. A query of those SNPs (Table 1) also showed strong evidence for association at or near genome-wide significance and, importantly, all in the expected direction based on nongravid populations.

The locus with strongest association in the GWAS was 10q22.1 with 2HPG. This locus, which showed a relatively narrow region of association, is found in a segment of high

| Table 1 | Genome-wide significant associations of glucose metabolism in gravid populations that overlap with those identified in nongravid populations |
|---------|---------------------------------------------------------------------------------------------------------------------------------|
| **SNP** | **Chr** | **Abbr** | **Freq.** | **Effect** | **Beta** | **P** | **Meta** |
| FPG rs1260326 | 2 | T/C | 0.330 | 2.94E-11 | 2.74E-11 | 8.1E-01 | 0.37 |
| FPG rs4841132 | 4 | G/C | 0.229 | 1.09E-05 | 7.09E-05 | 6.3E-06 | 0.36 |
| FPG rs13179048 | 5 | G/C | 0.213 | 2.21E-03 | 2.06E-03 | 4.02E-02 | 0.32 |
| FPG rs6235 | 5 | G/C | 0.213 | 2.21E-03 | 2.06E-03 | 4.02E-02 | 0.32 |
| FPG rs1387153 | 11 | T/C | 0.241 | 7.28E-03 | 4.25E-04 | 2.34E-02 | 0.35 |
| FPG rs17085593 | 5 | G/C | 0.229 | 5.60E-03 | 5.60E-03 | 3.37E-06 | 0.34 |
| FPG rs6235 | 5 | G/C | 0.213 | 2.21E-03 | 2.06E-03 | 4.02E-02 | 0.32 |
| FPG rs2764072 | 11 | T/C | 0.241 | 4.59E-03 | 2.34E-04 | 7.93E-02 | 0.35 |
| 1HPG rs7936247 | 11 | T/G | 0.278 | 1.37E-01 | 1.30E-02 | 7.93E-01 | 0.35 |
| 1HPG rs7936247 | 11 | G/C | 0.240 | 2.42E-01 | 1.30E-02 | 7.93E-01 | 0.35 |

*Effect allele/other allele. Results presented are for Model 2. Boldfaced SNPs are those showing evidence for association in previous studies (see refs. 15,16). †Bonferroni-corrected P-values indicate the most significantly associated SNP at the three meta-analytic stages. N/A, SNP not genotyped, did not pass genotyping QC, or was not relatively imputed.
FIG. 1. A: LocusZoom plot of association results and LD boundaries around HKDC1. The top panel reflects the meta-analysis results of the four GWAS cohorts. Each of the four middle panels contains the population-specific (AC, EU, HI, TH) association results and estimates of LD ($D'$) from the SNP with the strongest evidence for association in the meta-analysis. The LD estimates are color coded as a heat map from purple ($D' < 0.3$) to red ($D' > 0.9$). These coincide with the recombination hotspots indicated by the blue lines (recombination rate in genetic distance between markers [cM]/physical distance [Mb] from HapMap (12)). The bottom panel shows the genes and their directions in this region of chromosome 10. B: HKDC1 mRNA in human tissues as determined by RT-PCR: (1) adipose tissue, (2) bladder, (3) brain,
LD upstream from the first intron of *HKDC1* (hexokinase domain containing 1; no MIM number), a recently identified member of the hexokinase family (17). The LD structure in each of the four ancestry populations shows that this association locus spans a 400-kb region with $D^\prime > 0.5$ from the most strongly associated SNP and includes the following genes in addition to *HKDC1*: *SUPV3L1*, *SRGN*, *VPS26A*, and *HK1* (Fig. 1A). In the GWAS, the best SNPs at this locus demonstrated evidence for association with 2HPG in three of the four ancestry groups with $P$ values ranging from $1.52 \times 10^{-3}$ to $7.03 \times 10^{-2}$ in Northern EU mothers (Table 2). The SNP with strongest association in the GWAS, rs4746822, reached genome-wide significance in a meta-analysis that combined the four ancestry groups ($P = 8.26 \times 10^{-13}$; $b$ range $0.167$–$0.229$ $\mu$mol/L per T allele). The proportion of phenotypic variation explained by this SNP ranged from 1.2% in EU to 2.7% in HI. This association was replicated in a cohort of 2,192 additional EU HAPO mothers and two smaller ($n = 228$ and 606) independent EU cohorts from Quebec, Canada, yielding a $P$ value of $1.02 \times 10^{-22}$ in a meta-analysis that combined the seven GWAS and replication cohorts.

*HKDC1* mRNA was present in multiple human tissues, with highest levels in colon, small intestine, trachea, thymus, kidney, and endocrine tissues (Fig. 1B). Hepatic expression was also evident. Examination of the association locus using ENCODE and other databases demonstrated 2HPG-associated variants proximal to *HKDC1* in regions of open chromatin (OC) and histones H3K27ac and H3K27me, all of which are indicative of active regulatory elements (18). For example, rs4746822 overlaps OC, H3K27ac, and H3K27me in the first intron of *HKDC1* in HepG2 liver carcinoma cells and liver stellate cells (Fig. 1C), whereas rs5030937 is proximal to OC in liver stellate cells. The variants may therefore affect the function of these regulatory elements and alter liver HKDC1 levels.

A second novel finding was association of the rs6517656 G allele in *BACE2* (b-site amyloid polypeptide cleaving enzyme 2; MIM 605668) with higher FCP. This locus showed moderate association in each of the four GWAS cohorts ($P = 1.26 \times 10^{-2}$ to $1.74 \times 10^{-3}$) and approached genome-wide significance when combined across all four groups through meta-analysis ($P = 3.06 \times 10^{-7}$). The proportion of FCP phenotypic variation explained by this SNP ranged from 0.2% in Thais to 1.0% in AC. Strong association was present in the HAPO EU replication cohort ($P = 3.89 \times 10^{-11}$) and a meta-analysis combining the four discovery GWAS and three replication cohorts ($0.018$–$0.054$ log$_{10}$[mg/L] per G allele; $P = 6.30 \times 10^{-16}$; Table 2). The LD structure in the four ancestry populations shows this association locus spans a 200-kb region and includes, in addition to *BACE2*, *PLAC4*, *C21orf130*, and *FAM3B* (Fig. 2A).

OC near the *BACE2* transcription start site is common to many tissue types (18), suggesting that the gene is poised for expression (Fig. 2B). Several regions of islet-specific OC are located in the first intron of *BACE2*, and these regions may be important for *BACE2* expression in islets. The FCP-associated tag and imputed variants are

(4) cervix, (5) colon, (6) esophagus, (7) heart, (8) kidney, (9) liver, (10) lung, (11) ovary, (12) placenta, (13) prostate, (14) skeletal muscle, (15) small intestine, (16) spleen, (17) testes, (18) thymus, (19) thyroid, and (20) trachea. C: Aligned genes, SNPs, active enhancer marks, OC regions, and gene expression profiles of the 2HPG-associated *HKDC1* region on chromosome 10. SNPs upstream and within *HKDC1* align with peaks representing regions enriched for active histone marks and OC regions in cell types representing 16 different tissues. *HKDC1* is highly expressed in colon, lung, liver, and cervical carcinomas.

**FIG. 1.** Continued.
The above findings suggest some similarities between the genetic architecture of glucose metabolism in pregnant women and non-pregnant adults, with a possible role in regulating maternal metabolism. For any of the associations attaining genome-wide significance between the maternal alleles and glucose metabolism (Tables 1 and 2), we tested for associations between these maternal alleles and neonatal anthropometric outcomes such as birth weight, sum of skinfolds, and fat mass (Supplementary Table 8). Although we observed evidence of nominally significant associations at some of these loci, none remained significant after correcting for multiple testing.

### Table 2

| SNP        | Beta (95% CI) | P-value | FPG | EP3G | GCKR | MTNR1B | PPP1R3B | TCF7L2 | PCSK1 | G6PC2 | HKDC1 | BACE2 | 2HPG | 3HPG |
|------------|---------------|---------|-----|------|------|--------|---------|--------|-------|------|-------|------|------|------|
| rs6517656  | -0.0539       | 6.34E-01| 0.83 | 0.0075| 6.30E-16| 1.43E-15| 3.06E-07|        |       |      |      |      |      |      |
| rs5030937  | 0.209         | 1.67E-02| 0.834| 1.84E-02| 1.26E-02| 0.946    | 3.23E-02|        |       |      |      |      |      |      |
| rs4746822  | 0.675         | 1.67E-02| 0.834| 1.84E-02| 1.26E-02| 0.946    | 3.23E-02|        |       |      |      |      |      |      |
and nonpregnant populations. The absence of association between other previously identified glucose genes and glucose levels during pregnancy may have been due to partial differences in the genetic architecture of glucose metabolism in pregnant and nonpregnant populations and/or reduced power in the current study; determining this awaits the availability of additional large cohorts of pregnant women.
We also found evidence for genome-wide significant association of loci with maternal metabolic traits that have not been previously reported in nongravid populations. **HKDC1** has not been associated with metabolic traits in GWAS performed in nongravid populations, although a recent large meta-analysis using a gene-based approach reported modest association of **HKDC1** \((P = 1.24 \times 10^{-3})\) with 2HPG in 42,854 nongravid EU individuals (19). The lead SNP in that study, rs9645500, showed much stronger evidence for association with 2HPG in our GWAS of only 1,351 pregnant HAPO EU mothers \((P = 7.52 \times 10^{-10})\;\text{Supplementary Table 6}.\) Our top **HKDC1** SNP, rs4746822, which is located in the 5’-flanking region of **HKDC1**, is in high LD with rs9645500 and demonstrated strong association in an additional 2,192 pregnant EU HAPO mothers during replication \((P = 9.68 \times 10^{-8})\), as well as in the two smaller external EU replication cohorts of 228 \((P = 0.011)\) and 606 women \((P = 9.25 \times 10^{-5})\) for a combined \(P_{\text{meta-All}} = 1.022 \times 10^{-22}\). These data demonstrate association of **HKDC1** in pregnant women from multiple ancestry groups and suggest that **HKDC1** may play a more important role in glucose metabolism during pregnancy than in nongravid states. **HKDC1**, a recently identified member of the hexokinase family, is adjacent to hexokinase 1 on chromosome 10 in a head-to-tail arrangement, suggesting that **HKDC1** and **HK1** are products of a gene duplication event (17). **HKDC1** is conserved across multiple species, including mammals, birds, fish, and amphibians, and has both a glucose-binding domain and ATP-binding site in its COOH-terminal domain, suggesting that it has hexokinase activity (17). The biological role of **HKDC1** is unknown, but as shown in the current study, **HKDC1** mRNA is present in a wide distribution of human tissues. This top **HKDC1** variant (rs4746822) showed a nominally significant association \((P = 0.01)\) in the five HAPO population meta-analysis) with sum of skinfolds in the neonates. Although this does not remain significant after correcting for multiple testing, these data, which need confirmation in larger meta-analysis, suggest that maternal **HKDC1** variants impact neonatal outcomes modulated through maternal glucose metabolism.

A second locus, **BACE2**, which was associated with FCP in the current study, has not been previously associated with metabolic traits in nongravid populations. A recent large meta-analysis of nongravid cohorts consisting of 108,557 individuals did not report association of SNPs within the **BACE2** locus with fasting insulin levels or other metabolic traits (Supplementary Table 9) (39). Moreover, results from 38,238 individuals of EU ancestry in the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) demonstrated no evidence for association of the lead SNP in the **BACE2** locus, rs6517656, with fasting insulin levels (Supplementary Table 9). **BACE2** is capable of processing amyloid precursor protein (40) and is expressed in multiple tissues (41). In islets, **BACE2** expression is limited to β-cells, where it is located in endocytic vesicles (42,43). It is not thought to contribute to amyloid deposition in pancreatic islets, but it has been shown to both augment and inhibit insulin secretion and/or production in human islets (42,43). Thus, **BACE2** either represents a second locus that is uniquely associated in pregnancy or is a newly identified locus specifically associated with C-peptide as opposed to insulin levels, although a role for **BACE2** in proinsulin processing has not been reported.

Prior studies of the genetics of maternal metabolism during pregnancy have been largely candidate gene studies focused on GDM (44). This includes studies of candidate genes based on biological plausibility or, more recently, type 2 diabetes susceptibility genes identified through GWAS (44–46). The latter studies in European and, to a large degree, Asian populations have demonstrated association of a number of type 2 diabetes susceptibility genes with GDM, including **TCF7L2**, **MTNR1B**, **IGF2BP2**, **KCNJ11**, **CDKAL1**, **KCNQ1**, **CDKN2A-CDKN2B**, **SLC30A8**, **HHEX**, and **GCK**. More recently, a GWAS for GDM performed in a Korean cohort demonstrated genome-wide significant association of **CDKAL1** and **MTNR1B** and marginal association of **IGF2BP2** with GDM (47). With the exception of **MTNR1B** and **CDKAL1**, none of the loci identified in the current study were reported as demonstrating marginal evidence for association with GDM in that GWAS. Studies in nonpregnant populations have demonstrated both similarities and differences between the genetic architecture of metabolic traits and type 2 diabetes (19,48,49). We have previously demonstrated association of SNPs in

**FIG. 2.** Continued.
TCF7L2 and GCK with fasting, 1-h and/or 2-h glucose levels in women of EU or TH ancestry (50). Thus, the results of the current study, together with the results of the previous studies described above, suggest that the genetic architecture of GDM and maternal metabolism, similar to the non-gravid state, exhibit both similarities and differences.

This is the first GWAS of glycemic traits during pregnancy and is strengthened further by the inclusion of non-EU populations. We demonstrated that genes important to the genetic architecture of glycemic traits in largely EU non-pregnant populations are also important in pregnancy, suggesting similarities in the underlying genetic architecture of glycemic traits in gravid and nongravid populations that extend across ancestry groups. However, our data also suggest differences between the gravid and nongravid states. Two loci with the strongest evidence for association demonstrated either no or weak association with glycemic traits in nonpregnant populations. Together with the results of earlier studies, our findings suggest that the roles of HKDC1 in glucose metabolism and BACE2 in insulin secretion are more important during pregnancy than in the nongravid state. Defining the underlying genetic architecture of maternal glycemia during pregnancy may assist in future efforts to identify women at risk for hyperglycemia during pregnancy.

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M.G.H., M.U., M.F.H., T.E.R., N.J.C., and W.L.L conceived and designed the study. M.G.H., M.F.H., L.L.A., J.M., C.G., D.A.S., A.P., D.M.L., C.P.M., C.M.A., B.T.L., D.M., K.F.D., M.V.L., and R.N.L.-H. performed experiments and statistical analyses. L.P.L., L.B., D.B., A.R.D., and B.E.M. recruited study subjects and measured or analyzed phenotypic data. M.G.H., M.F.H., T.E.R., and W.L.L. wrote the manuscript. All authors critically reviewed and approved the manuscript. M.G.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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