Type 2 Diabetes Risk Alleles Are Associated With Reduced Size at Birth

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OBJECTIVE—Low birth weight is associated with an increased risk of type 2 diabetes. The mechanisms underlying this association are unknown and may represent intrauterine programming or two phenotypes of one genotype. The fetal insulin hypothesis proposes that common genetic variants that reduce insulin secretion or action may predispose to type 2 diabetes and also reduce birth weight, since insulin is a key fetal growth factor. We tested whether common genetic variants that predispose to type 2 diabetes also reduce birth weight.

RESEARCH DESIGN AND METHODS—We genotyped single-nucleotide polymorphisms (SNPs) at five recently identified type 2 diabetes loci (*CDKAL1*, *CDKN2A/B*, *HHEX-IDE*, *IGF2BP2*, and *SLC30A8*) in 7,986 mothers and 19,200 offspring from four studies of white Europeans. We tested the association between maternal or fetal genotype at each locus and birth weight of the offspring.

RESULTS—We found that type 2 diabetes risk alleles at the *CDKAL1* and *HHEX-IDE* loci were associated with reduced birth weight when inherited by the fetus (21 g [95% CI 11–31], $P = 2 \times 10^{-5}$, and 14 g [4–23], $P = 0.004$, lower birth weight per risk allele, respectively). The 4% of offspring carrying four risk alleles at these two loci were 80 g (95% CI 39–120) lighter at birth than the 8% carrying none ($P_{trend} = 5 \times 10^{-7}$). There were no associations between birth weight and fetal genotypes at the three other loci or maternal genotypes at any locus.

CONCLUSIONS—Our results are in keeping with the fetal insulin hypothesis and provide robust evidence that common disease-associated variants can alter size at birth directly through the fetal genotype. ***Diabetes*** **58:1428–1433, 2009**

Reduced birth weight is associated with late-onset diseases including type 2 diabetes, hypertension, and heart disease (1). The cause of this association is not known. It is often proposed to reflect fetal programming in utero in response to maternal malnutrition in pregnancy (2). An alternative explanation is that genetic variants that increase disease risk could also reduce fetal growth. In accordance with the fetal insulin hypothesis (3), we proposed that genetic variants that reduce insulin secretion or insulin sensitivity might reduce birth weight as well as predisposing to type 2 diabetes in adulthood, since fetal insulin is a key fetal growth factor.

The fetal insulin hypothesis was initially based on observations of subjects with glucokinase (*GCK*) mutations, whose birth weight is reduced by 533 g (4) and who have mild hyperglycemia postnatally. Markedly reduced birth weights in patients with monogenic diabetes due to mutations in the *INS*, *INSR*, *IPF1*, *KCNJ11*, *ABCC8*, and *HNF1B* genes (3,5–8) have further established the principle that gene variants can cause both low birth weight and diabetes. However, mutations causing monogenic diabetes are too rare to explain the association between reduced birth weight and type 2 diabetes observed in population studies.

There is epidemiological support for the fetal insulin hypothesis. Offspring of fathers who go on to develop type 2 diabetes later in life have lower birth weights than those born to fathers who do not develop diabetes (9–12). This is consistent with the fetus inheriting, on average, 50% of the father's genetic predisposition to diabetes and this genetic predisposition reducing fetal growth.

Maternal genotypes may have opposing effects on offspring birth weight compared with fetal genotypes (4). Type 2 diabetes risk alleles, which are present in the mother and which raise maternal glycemia in pregnancy, will increase fetal growth by increasing fetal insulin secretion. Maternal inheritance of common risk alleles in the *GCK* and *TCF7L2* genes, which predispose to hyperglycemia and type 2 diabetes, respectively, were reproducibly associated with higher offspring birth weight (13,14). However, neither of these risk alleles at *TCF7L2* and *GCK* or the type 2 diabetes risk alleles in the *PPARG* and *KCNJ11* genes was associated with birth weight directly through the fetal genotype (13–15).

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TABLE 1
Clinical characteristics of subjects

	Study			
	ALSPAC children*	EFSOCH children*	NFBC1966	1958BC
Year(s) of birth	1991–1993	2000–2004	1965–1967	1958
Total n (% male)†	7,687 (52.0)	763 (53.1)	4,838 (48.0)	5,912 (50.4)
Birth weight (g)	3,482 ± 480	3,507 ± 475	3,534 ± 491	3,345 ± 489
Gestation (weeks)	40 (39–41)	40 (39–41)	40 (39–41)	40 (39–41)
Maternal age (years)	28 (25–32)	31 (27–34)	27 (23–34)	27 (23–31)
Maternal prepregnancy BMI (kg/m^2)	22.14 (20.47–24.38)	23.03 (21.14–25.63)	22.68 (20.96–24.80)	22.53 (20.55–24.51)
Primiparous births	43.5	45.0	31.1	37.3
Maternal smoking during pregnancy	21.3	14.1	13.3	32.3

Data are means ± SD, median (interquartile range), or percentages. *Maternal genotype available: ALSPAC (n = 7,176) and EFSOCH (n = 810) (includes total number of mothers genotyped for at least one SNP, with offspring birth weight available, regardless of whether fetal genotype was also available). †Includes individuals of white European ancestry, from a singleton pregnancy, with birth weight available, born at a minimum gestational age of 36 weeks, and genotyped for at least one of five SNPs.

In this study, we aimed to further test the relationship between known type 2 diabetes variants and size at birth. We selected variants at five loci (*CDKAL1*, *CDKN2A/B*, *HHEX-IDE*, *IGF2BP2*, and *SLC30A8*), recently identified through type 2 diabetes genome-wide association studies (16–21), that have not been investigated in relation to fetal growth. Each of these loci has been shown to predispose to diabetes by reducing insulin secretion (22–24). We used data from 19,200 offspring and 7,986 mothers from four studies of white Europeans to test the hypothesis that these variants are associated with birth weight, either through the fetal or maternal genotype.

RESEARCH DESIGN AND METHODS

Subjects included in our analyses were selected from four studies (Table 1). The Avon Longitudinal Study of Parents and Children (ALSPAC) (25) is a prospective study that recruited pregnant women from Bristol, U.K., with expected delivery dates between April 1991 and December 1992. The Exeter Family Study of Childhood Health (EFSOCH) (26) is a prospective study of children born between 2000 and 2004 and their parents from a geographically defined region of Exeter, U.K. The Northern Finland Birth Cohort of 1966 (NFBC1966) (27) is a study of individuals born in the two northern-most provinces of Finland to women with expected dates of delivery in 1966. The 1958 British Birth Cohort (1958BC) (28) is a national cohort of subjects from the U.K. born during the same week in March 1958. Fetal DNA was available from all studies, and maternal DNA was available in the ALSPAC and EFSOCH studies. In all studies, birth weight and gestational age were obtained from hospital records. Important covariates were recorded, including maternal prepregnancy BMI, parity, and maternal smoking. Subjects included in the analyses were of white European ancestry, were singleton births, and were born at gestational age ≥36 weeks. All subjects (or for children, their parents) gave informed consent, and ethics approval was obtained from the local review committee for each study.

Genotyping. One single nucleotide polymorphism (SNP) was chosen to represent the type 2 diabetes association signal at each of the five loci (rs10946398 [*CDKAL1*], rs10811661 [*CDKN2A/B*], rs1111875 [*HHEX-IDE*], rs4402960 [*IGF2BP2*], and rs13266634 [*SLC30A8*]). Genotyping was performed using standard methods with robust quality-control criteria, details of which are presented in the online appendix (available at http://diabetes.diabetesjournals.org/cgi/content/full/db08-1739/DC1).

Statistical analysis

Analysis of fetal genotype and birth weight. Within each of the four studies, we examined the association between birth weight and fetal genotype for each SNP using linear regression, with genotype coded as zero, one, or two risk alleles and sex and gestational age as covariates. Consistent with previous studies confirming associations of five SNPs with type 2 diabetes (16–20), we used an additive genetic model, assuming a constant change in birth weight per additional risk allele. The distribution of birth weight was approximately normal, so it was not transformed for analysis. Subjects with extreme birth weight values (>4 SD from the sex mean) were removed before analysis (see the online appendix). We repeated the analysis, with maternal prepregnancy BMI; smoking; parity; and, in the EFSOCH study, maternal fasting glucose included as additional covariates.

We produced meta-analysis statistics and plots using the inverse-variance method (fixed effects), implemented in the METAN module developed for Stata (StataCorp, College Station, TX) (29). Summary data were pooled from the linear regression analyses performed in the individual studies. We used the I^2 statistic to estimate the percentage of total variation in study estimates that is due to between-study heterogeneity (30). In addition, we used Cochran's Q test to evaluate the evidence for between-study heterogeneity. By performing meta-analyses of summary data from individual studies, we avoided any potential confounding effect of allele frequency differences between the Finnish and U.K. studies.

Analysis of maternal genotype and offspring birth weight. Within each of the two studies with maternal genotype available (ALSPAC and EFSOCH), we examined the association between birth weight and maternal genotype for each SNP using linear regression under the same model as was used for fetal genotype, with sex and gestational age as covariates. We combined data from the two studies using inverse-variance meta-analysis. Since we tested the associations with birth weight of *1*) fetal and *2*) maternal genotypes for all five SNPs, we used $\alpha = 0.05/10$ to make study-wide adjustments of P values.

Adjustment of maternal and fetal genotype effects for one another. Maternal and fetal genotypes are not independent ($r = \sim 0.5$) and may have opposing effects on birth weight (4). To examine the effects of maternal and fetal genotypes that were independent of one another, we used the mother-offspring pairs from the ALSPAC and EFSOCH cohorts with both maternal and fetal genotype available (n = 5,342–5,507). Within each study, we performed a linear regression analysis of birth weight against maternal genotype, fetal genotype, sex, and gestation. We performed two meta-analyses for each SNP, combining regression coefficients from the two studies for fetal, and then maternal, genotype.

Analysis of the combined effects of *CDKAL1* and *HHEX-IDE* on birth weight. To assess the combined effect of the fetal risk alleles at *CDKAL1* and *HHEX-IDE* on birth weight, we generated a risk allele score (from 0 to 4) for individuals genotyped at both loci. We then performed a linear regression analysis, within each of the four studies, of birth weight against the fetal risk allele score (additive model), sex, and gestation. We combined the per–risk allele effect sizes and SEs using inverse-variance meta-analysis (n = 18,438). To gain estimates of the differences in birth weights between individuals with no risk alleles and individuals with either one, two, three, or four risk alleles, we repeated the within-study analysis including the fetal risk allele score as indicator variables and then meta-analyzed the effect size estimates for each comparison.

RESULTS

The fetal risk alleles of SNPs rs10946398 (*CDKAL1*) and rs1111875 (*HHEX-IDE*) were associated with reduced birth weight in the meta-analysis (21 g [95% CI 11–31], $P = 2 \times 10^{-5}$, and 14 g [4–23], $P = 0.004$, lower birth weight per risk allele, respectively) (Table 2 and Fig. 1) (see Table 3 for individual study results). Fetal genotypes at the other three loci were not associated with birth weight (all $P > 0.01$). The variability of effect size estimates among studies was consistent with random statistical fluctuations, suggesting no underlying heterogeneity (all $P > 0.1$). Adjust-

TABLE 2
Meta-analysis of the association of birth weight with fetal genotype

Locus (SNP)	Total n in meta-analysis	Per–risk allele effect size [g (95% CI)]	P
CDKAL1 (rs10946398)	18,679	−21 (−31 to −11)	2×10^{-5}
CDKN2A-2B (rs10811661)	18,751	11 (−1 to 24)	0.07
HHEX-IDE (rs1111875)	18,958	−14 (−23 to −4)	0.004
IGF2BP2 (rs4402960)	18,187	4 (−6 to 14)	0.43
SLC30A8 (rs13266634)	18,702	12 (2–21)	0.02

Analyses are adjusted for sex and gestational age.

ment for additional covariates of birth weight made little difference to the results (data not shown).

In the two studies with maternal DNA available, maternal genotypes at five loci were not associated with offspring birth weight (all $P > 0.05$; except *HHEX-IDE*, $P = 0.045$) (online appendix Table 1). Using the mother-offspring pairs with both genotypes available (n = 5,342–5,507), we assessed the association of fetal genotype with birth weight that was independent of maternal genotype (online appendix Table 2). For *CDKAL1*, the per–risk allele effect size estimate of the association between fetal genotype and birth weight was −25 g (95% CI −43 to −7) ($P = 0.005$) before adjustment for maternal genotype and −36 g (−56 to −16) ($P = 0.0005$) after adjustment. In accordance with this, the maternal risk allele at *CDKAL1* showed a nominal association with increased birth weight after adjustment for fetal genotype ($P = 0.04$). For *HHEX-IDE*, the per–risk allele effect size estimate of the association between fetal genotype and birth weight was −25 g (−43 to −9) ($P = 0.003$) before adjustment for maternal genotype and −29 g (−48 to −10) ($P = 0.003$) after adjustment. The maternal risk allele at *HHEX-IDE* showed no association with birth weight after adjustment for fetal genotype ($P = 0.5$).

Using 18,438 individuals from all four studies, we combined information from the *CDKAL1* and *HHEX-IDE* loci into a fetal risk allele score and tested the association with birth weight. We observed a 17-g (95% CI 10–24) reduction in birth weight per additional risk allele ($P = 5 \times 10^{-7}$). The 4% of offspring who carried four type 2 diabetes risk alleles were 80 g (39–120) lighter at birth than the 8% carrying none (Fig. 2).

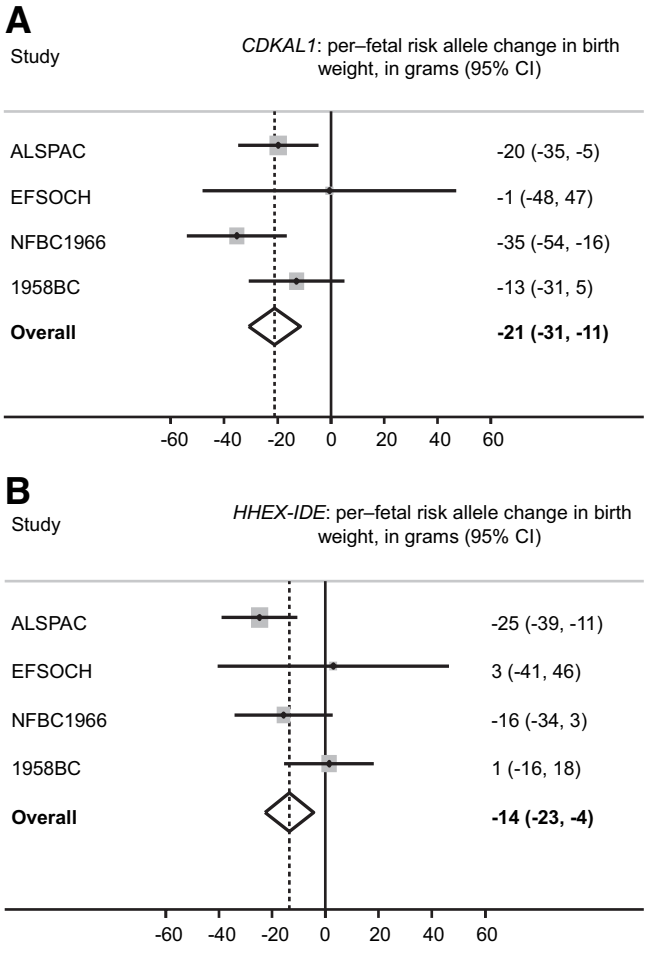

FIG. 1. *A*: Meta-analysis plot showing the association of fetal *CDKAL1* genotype with birth weight across all four studies (overall $P = 2 \times 10^{-5}$; total n = 18,679; heterogeneity statistics: $I^2 = 19.9\%$, $P = 0.29$). *B*: Meta-analysis plot showing association of fetal *HHEX-IDE* genotype with birth weight across all four studies (overall $P = 0.004$; total n = 18,958; heterogeneity statistics: $I^2 = 49.7\%$, $P = 0.11$). Analyses are adjusted for sex and gestational age.

DISCUSSION

Using a total of 19,200 offspring and 7,986 mothers from four studies of white Europeans, we have shown that fetal inheritance of the type 2 diabetes risk alleles at *CDKAL1* and *HHEX-IDE* is associated with reduced birth weight. This is consistent with the fetal insulin hypothesis (3) and provides the first robust evidence that common disease-associated genetic variants can directly influence size at birth. While the individual effect sizes were small, our combined analysis showed a difference in birth weight of 80 g (95% CI 39–120) between offspring carrying four risk alleles and those carrying none. This is similar to the effect on birth weight of a mother smoking three cigarettes per day in the third trimester of pregnancy (31).

We did not observe an association between maternal genotype and offspring birth weight. However, maternal and fetal genotypes are 50% correlated and may confound each other. When we assessed the effects of maternal and fetal genotype that were independent of one another using mother-offspring pairs, the effect size of the association between fetal genotype and birth weight at *CDKAL1* changed from −25 g (95% CI −43 to −7) to −36 g (−56 to −16). This suggests that maternal and fetal genotypes at this locus may have opposing effects on birth weight, as has been observed in mother-offspring pairs with heterozygous mutations in the *GCK* gene (4). However, this result requires confirmation in further large studies of mothers and offspring.

We acknowledge some limitations to our study. First, although we have studied the largest cohorts available for genetic studies of birth weight, our power to detect effects

TABLE 3
Analysis of fetal genotype and birth weight within four studies

	Genotype (number of type 2 diabetes risk alleles)								
	0		1		2				
	Mean birth weight (g)	*n*	Mean birth weight (g)	*n*	Mean birth weight (g)	*n*	Total *n*	Per–risk allele effect size (g)*	*P**
rs10946398 (*CDKAL1*)									
ALSPAC	3,496 (3,481–3,510)	3,494	3,471 (3,456–3,486)	3,210	3,462 (3,431–3,494)	758	7,462	−20 ± 8	0.010
EFSOCH	3,512 (3,466–3,557)	350	3,504 (3,457–3,551)	329	3,520 (3,420–3,620)	72	751	−1 ± 24	0.983
NFBC1966	3,568 (3,546–3,589)	1,795	3,516 (3,497–3,534)	2,295	3,506 (3,474–3,539)	742	4,832	−35 ± 10	0.0002
1958BC	3,380 (3,362–3,397)	2,675	3,367 (3,348–3,386)	2,354	3,354 (3,319–3,390)	605	5,634	−13 ± 9	0.162
rs10811661 (*CDKN2A/B*)									
ALSPAC	3,451 (3,395–3,507)	236	3,482 (3,463–3,500)	2,105	3,483 (3,471–3,495)	5,176	7,517	7 ± 9	0.465
EFSOCH	3,396 (3,196–3,597)	18	3,506 (3,443–3,569)	182	3,509 (3,473–3,545)	554	754	20 ± 31	0.515
NFBC1966	3,543 (3,463–3,624)	125	3,523 (3,498–3,549)	1,249	3,537 (3,522–3,552)	3,448	4,822	8 ± 13	0.539
1958BC	3,349 (3,283–3,414)	162	3,358 (3,335–3,380)	1,618	3,380 (3,365–3,394)	3,878	5,658	20 ± 11	0.084
rs1111875 (*HHEX/IDE*)									
ALSPAC	3,529 (3,505–3,553)	1,289	3,475 (3,461–3,489)	3,652	3,471 (3,454–3,488)	2,573	7,514	−25 ± 7	0.0007
EFSOCH	3,495 (3,421–3,569)	132	3,509 (3,464–3,555)	350	3,505 (3,453–3,556)	275	757	3 ± 22	0.894
NFBC1966	3,547 (3,520–3,574)	1,100	3,536 (3,518–3,554)	2,466	3,516 (3,490–3,541)	1,266	4,832	−16 ± 9	0.095
1958BC	3,373 (3,345–3,400)	1,020	3,372 (3,355–3,389)	2,836	3,375 (3,355–3,395)	1,999	5,855	1 ± 9	0.872
rs4402960 (*IGF2BP2*)									
ALSPAC	3,480 (3,466–3,494)	3,718	3,481 (3,466–3,497)	3,128	3,500 (3,468–3,533)	692	7,538	7 ± 8	0.385
EFSOCH	3,469 (3,423–3,514)	345	3,554 (3,508–3,599)	346	3,443 (3,336–3,550)	63	754	29 ± 25	0.247
NFBC1966	3,536 (3,518–3,555)	2,386	3,528 (3,509–3,555)	1,972	3,540 (3,498–3,583)	446	4,804	−2 ± 10	0.829
1958BC	3,375 (3,356–3,393)	2,413	3,366 (3,348–3,385)	2,172	3,391 (3,351–3,430)	506	5,091	2 ± 10	0.851
rs13266634 (*SLC30A8*)									
ALSPAC	3,471 (3,438–3,504)	666	3,480 (3,465–3,495)	3,178	3,485 (3,471–3,499)	3,619	7,463	6 ± 8	0.422
EFSOCH	3,421 (3,315–3,528)	63	3,548 (3,501–3,596)	315	3,479 (3,435–3,523)	368	746	−13 ± 25	0.613
NFBC1966	3,504 (3,469–3,540)	631	3,536 (3,517–3,555)	2,274	3,512 (3,521–3,562)	1,933	4,838	15 ± 10	0.124
1958BC	3,321 (3,284–3,358)	558	3,372 (3,353–3,390)	2,383	3,377 (3,359–3,394)	2,714	5,655	19 ± 9	0.037

Data are means (95% CI) or means ± SE, unless otherwise indicated. *Linear regression of birth weight against fetal genotype (coded zero, one, or two risk alleles), with sex and gestation as covariates. Mean birth weights (95% CI) are adjusted for sex and gestational age.

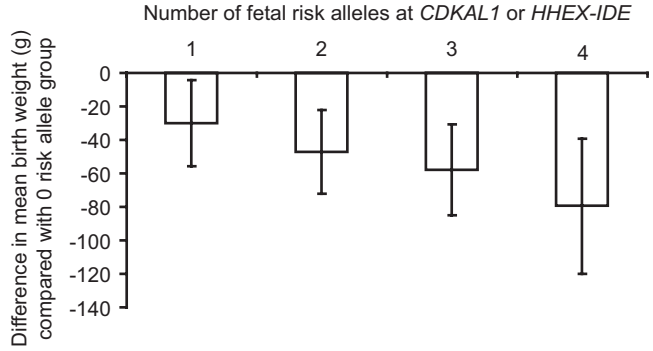

FIG. 2. Bar graph showing the association between birth weight and the number of fetal type 2 diabetes risk alleles at *CDKAL1* (rs10946398) and *HHEX-IDE* (rs1111875) across all four studies (n = 18,438). Estimates of the difference in birth weight are adjusted for sex and gestational age. Error bars show 95% CIs.

of maternal genotype was limited due to its availability in only two of four studies (maximum n = 7,821). While this gave us 80% power to detect changes in birth weight of 30 g for the most common minor allele (frequency 40%; α = 0.005), we estimate that we would have needed a maternal sample size ranging from n = 10,200 to 19,250 to detect effects of 20 g per risk allele, given the allele frequency variation of the SNPs tested. The second limitation is that we have only studied individuals of European origin. Further studies are needed in large cohorts of other ethnic groups. Third, our statistical evidence for association of *CDKAL1* ($P = 2 \times 10^{-5}$) and *HHEX-IDE* (P = 0.004) with birth weight does not meet the generally accepted criterion for genome-wide adjustment. However, the robust prior evidence for association of all five loci with type 2 diabetes ($P < 5 \times 10^{-8}$) (32) and the association of each with insulin secretion (22–24) indicate that such an adjustment would be too stringent. In addition, the associations survive study-wide adjustment ($P <$ 0.005), suggesting that they are unlikely to be false-positives. Finally, it is also possible that population substructure has influenced our results, but the use of meta-analysis across studies that individually consist of white Europeans from relatively homogenous regions means that this is unlikely.

The majority of type 2 diabetes genetic variants increase diabetes risk by reducing β-cell function (22–24,32). Genetic variants at *CDKAL1* and *HHEX-IDE* are associated with reduced β-cell function in adults, and we hypothesize that the associations with birth weight are mediated via reduced fetal insulin secretion. As cord insulin was not measured in our fetal samples, we cannot test this hypothesis directly. Our results and previous studies (14,15) support heterogeneity in the impact of common type 2 diabetes variants on fetal growth, and this could suggest differences in the timing of the β-cell defect. If a variant reduces fetal insulin secretion in utero, this could result in reduced birth weight (e.g., *CDKAL1* and *HHEX-IDE*). If insulin secretion is reduced at child-bearing age, this could result in maternal hyperglycemia and hence increased offspring birth weight (e.g., *TCF7L2* [14]). Finally, if insulin secretion is not reduced until old age, then birth weight will not be altered. The heterogeneity of fetal effects on birth weight is consistent with rare autosomal dominant forms of young-onset diabetes, in which different genetic aetiologies have contrasting impacts on fetal growth (4,8,33).

The associations of the *CDKAL1* and *HHEX-IDE* variants with reduced birth weight provide the first direct evidence that common genetic variation may account, in part, for the epidemiological association between reduced birth weight and type 2 diabetes. This is a crucial addition to the observations of patients with rare mutations that first established the principle of a genetic link between low birth weight and diabetes (3–8) but that are too rare to explain the epidemiological data. It is important to appreciate that genetic associations cannot explain all of the epidemiological data, in particular the associations seen in identical twins (34,35), and the associations we have seen explain <0.2% of the variation in birth weight. However, the association between type 2 diabetes and birth weight is not strong and it is possible, with the identification of additional type 2 diabetes gene variants, that genetic factors will explain a substantial fraction of the correlation between low birth weight and type 2 diabetes. An alternative mechanism, which has gained support from experimental animal models, is that low birth weight results from maternal malnutrition, and subsequent programming in utero results in a predisposition to diabetes (2). The roles for genetic variation and programming are not mutually exclusive. However, further work to define the relative contributions of these two potential mechanisms is important because if a large component of the association is genetic, then this would argue against targeting preventative interventions to pregnant women to influence the health outcomes of their offspring (36).

In conclusion, our study provides the first robust evidence that common type 2 diabetes susceptibility variants can alter size at birth directly through the fetal genotype. Risk alleles at *CDKAL1* and *HHEX-IDE* are both associated with reduced birth weight. This is consistent with the fetal insulin hypothesis, which proposed that predisposition to both type 2 diabetes and low birth weight are two phenotypes of a single genotype and explains, at least in part, the association of low birth weight with type 2 diabetes.

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