

## Type 2 Diabetes Risk Alleles are Associated with Reduced Size at Birth

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## ABSTRACT

*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 7986 mothers and 19200 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: 21g [95%CI:11-31g],  $P=2\times 10^{-5}$  and 14g [4-23g],  $P=0.004$  lower birth weight per risk allele, respectively. The 4% of offspring carrying four risk alleles at these two loci were 80g [39-120g] 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.

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. Under 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 533g (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 in later 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 to 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 respectively to hyperglycemia and type 2 diabetes, were reproducibly associated with higher offspring birth weight (13; 14). However, neither of these risk alleles at *TCF7L2* and *GCK*, nor the type 2 diabetes risk alleles in the *PPARG* and *KCNJ11* genes, were associated with birth weight directly through the fetal genotype (13-15).

In this study we aimed to test further the relationship between known type 2 diabetes variants and size at birth. We selected variants at five loci recently identified through type 2 diabetes genome-wide association studies (16-21), which have not been investigated in relation to fetal growth: *CDKALI*, *CDKN2A/B*, *HHEX-IDE*, *IGF2BP2*, and *SLC30A8*. Each of these loci has been shown to predispose to diabetes by reducing insulin secretion (22-24). We used data from 19200 offspring and 7986 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.** 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, which recruited pregnant women from Bristol, UK, 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, UK. The Northern Finland Birth Cohort of 1966 (NFBC1966) (27) is a study of individuals born in the two northernmost 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 UK, 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 pre-pregnancy BMI, parity and maternal smoking. Subjects included in the analyses were of white European ancestry, singleton births and were born at a gestational age of 36 weeks or later. All subjects (or for children, their parents) gave informed consent and ethical 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 (*CDKALI*); 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**.

### **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 0, 1 or 2 risk alleles, and sex and gestational age as covariates. Consistent with previous studies confirming associations of the 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 (>4SD from the gender mean) were removed before analysis (details in the **Online Appendix**). We repeated the analysis with maternal pre-pregnancy 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, Texas, USA) (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 UK 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 (i) fetal and (ii) maternal genotype 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 (correlation coefficient,  $\approx 0.5$ ) 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 ( $n=5342$  to 5507). 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 standard errors using inverse-variance meta-analysis ( $n=18,438$ ). To gain estimates of the differences in birth weights between individuals with 0 risk alleles and individuals with either 1, 2, 3 or 4 risk alleles, we repeated the within-study analysis including the fetal risk allele score as indicator variables, and then meta-analysed 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: 21g [95%CI: 11-31g,  $P = 2 \times 10^{-5}$ ] and 14g [95%CI: 4-23g,  $P = 0.004$ ] lower birth weight per risk allele, respectively (**Table 2** and **Figure 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  $P > 0.1$ ). Adjustment 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 the 5 loci were not associated with offspring birth weight (all  $P > 0.05$ , except *HHEX-IDE*:

$P=0.045$  **Supplementary Table 1**). Using the mother-offspring pairs with both genotypes available ( $n = 5342$  to 5507), we assessed the association of fetal genotype with birth weight that was independent of maternal genotype (**Supplementary Table 2**). For *CDKAL1*, the per-risk allele effect size estimate of the association between fetal genotype and birth weight was -25g [95%CI: -43, -7g] ( $P=0.005$ ) before adjustment for maternal genotype and -36g [95%CI: -56, -16g] ( $P=0.0005$ ) 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 -25g [95%CI: -43, -9g] ( $P=0.003$ ) before adjustment for maternal genotype and -29g [95%CI: -48, -10g] ( $P=0.003$ ) after adjustment. The maternal risk allele at *HHEX-IDE* showed no association with birth weight after adjustment for fetal genotype ( $P=0.5$ ).

Using 18438 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 17g [95%CI: 10-24g] 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 80g [95%CI: 39-120g] lighter at birth than the 8% carrying none (**Figure 2**).

## **DISCUSSION**

Using a total of 19200 offspring and 7986 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 80g [95%CI: 39-120g] 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 *CDKALI* changed from -25g [95%CI: -43, -7g] to -36g [95%CI: -56, -16g]. 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 of maternal genotype was limited due to its availability in only two of the four studies (maximum  $n=7821$ ). While this gave us 80% power to detect changes in birth weight of 30g for the most common minor allele (frequency 40%;  $\alpha=0.005$ ), we estimate that we would have needed a maternal sample size ranging from  $n=10200$  to  $n=19250$  to detect effects of 20g 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. Thirdly, our statistical evidence for association of *CDKALI* ( $P=2\times 10^{-5}$ ) 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 beta-cell function (22-24; 32). Genetic variants at *CDKALI* and *HHEX-IDE* are associated with reduced beta-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 beta-cell defect. If a variant reduces fetal insulin secretion *in utero*, this could result in reduced birth weight (e.g. *CDKALI* 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 *CDKALI* 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 which first established the principle of a genetic link between low birth weight and diabetes (3-8), but which 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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## REFERENCES

1. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM: Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 36:62-67, 1993
2. Godfrey KM, Barker DJ: Fetal nutrition and adult disease. *Am J Clin Nutr* 71:1344S-1352S, 2000
3. Hattersley AT, Tooke JE: The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet* 353:1789-1792, 1999
4. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S: Mutations in the glucokinase gene of the fetus result in reduced birth weight. *Nat Genet* 19:268-270, 1998
5. Stoy J, Edghill EL, Flanagan SE, Ye H, Paz VP, Pluzhnikov A, Below JE, Hayes MG, Cox NJ, Lipkind GM, Lipton RB, Greeley SA, Patch AM, Ellard S, Steiner DF, Hattersley AT, Philipson LH, Bell GI: Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci U S A* 104:15040-15044, 2007
6. Slingerland AS, Hattersley AT: Activating mutations in the gene encoding Kir6.2 alter fetal and postnatal growth and also cause neonatal diabetes. *J Clin Endocrinol Metab* 91:2782-2788, 2006
7. Babenko AP, Polak M, Cave H, Busiah K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, Froguel P: Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. *N Engl J Med* 355:456-466, 2006
8. Edghill EL, Bingham C, Slingerland AS, Minton JA, Noordam C, Ellard S, Hattersley AT: Hepatocyte nuclear factor-1 beta mutations cause neonatal diabetes and intrauterine growth retardation: support for a critical role of HNF-1beta in human pancreatic development. *Diabet Med* 23:1301-1306, 2006
9. Davey Smith G, Sterne JA, Tynelius P, Rasmussen F: Birth characteristics of offspring and parental diabetes: evidence for the fetal insulin hypothesis. *J Epidemiol Community Health* 58:126-128, 2004
10. Hypponen E, Davey Smith G, Power C: Parental diabetes and birth weight of offspring: intergenerational cohort study. *Bmj* 326:19-20, 2003
11. Wannamethee SG, Lawlor DA, Whincup PH, Walker M, Ebrahim S, Davey-Smith G: Birthweight of offspring and paternal insulin resistance and paternal diabetes in late adulthood: cross sectional survey. *Diabetologia* 47:12-18, 2004
12. Lindsay RS, Dabelea D, Roumain J, Hanson RL, Bennett PH, Knowler WC: Type 2 diabetes and low birth weight: the role of paternal inheritance in the association of low birth weight and diabetes. *Diabetes* 49:445-449, 2000
13. Weedon MN, Clark VJ, Qian Y, Ben-Shlomo Y, Timpson N, Ebrahim S, Lawlor DA, Pembrey ME, Ring S, Wilkin TJ, Voss LD, Jeffery AN, Metcalf B, Ferrucci L, Corsi AM, Murray A, Melzer D, Knight B, Shields B, Smith GD, Hattersley AT, Di Rienzo A, Frayling TM: A common haplotype of the glucokinase gene alters fasting glucose and birth weight: association in six studies and population-genetics analyses. *Am J Hum Genet* 79:991-1001, 2006
14. Freathy RM, Weedon MN, Bennett A, Hypponen E, Relton CL, Knight B, Shields B, Parnell KS, Groves CJ, Ring SM, Pembrey ME, Ben-Shlomo Y, Strachan DP, Power C, Jarvelin MR, McCarthy MI, Davey Smith G, Hattersley AT, Frayling TM: Type 2 diabetes TCF7L2 risk genotypes alter birth weight: a study of 24,053 individuals. *Am J Hum Genet* 80:1150-1161, 2007

15. Bennett AJ, Sovio U, Ruukonen A, Martikainen H, Pouta A, Hartikainen AL, Franks S, Elliott P, Jarvelin MR, McCarthy MI: No evidence that established type 2 diabetes susceptibility variants in the PPAR $\gamma$  and KCNJ11 genes have pleiotropic effects on early growth. *Diabetologia* 51:82-85, 2008
16. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS, McCarthy MI, Hattersley AT: Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316:1336-1341, 2007
17. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, Styrkarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorraddottir S, Bjarnason H, Ng MC, Hansen T, Bagger Y, Wilensky RL, Reilly MP, Adeyemo A, Chen Y, Zhou J, Gudnason V, Chen G, Huang H, Lashley K, Doumatey A, So WY, Ma RC, Andersen G, Borch-Johnsen K, Jorgensen T, van Vliet-Ostaptchouk JV, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Rotimi C, Gurney M, Chan JC, Pedersen O, Sigurdsson G, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K: A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet* 39:770-775, 2007
18. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341-1345, 2007
19. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Rieke D, Purcell S: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331-1336, 2007
20. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshzhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P: A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881-885, 2007
21. Wellcome Trust Case Control Consortium: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447:661-678, 2007
22. Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, Weedon MN, Mari A, Hattersley AT, McCarthy MI, Frayling TM, Walker M: Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. *Diabetes* 56:3101-3104, 2007
23. Grarup N, Rose CS, Andersson EA, Andersen G, Nielsen AL, Albrechtsen A, Clausen JO, Rasmussen SS, Jorgensen T, Sandbaek A, Lauritzen T, Schmitz O, Hansen T, Pedersen O:

- Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. *Diabetes* 56:3105-3111, 2007
24. Palmer ND, Goodarzi MO, Langefeld CD, Ziegler J, Norris JM, Haffner SM, Bryer-Ash M, Bergman RN, Wagenknecht LE, Taylor KD, Rotter JI, Bowden DW: Quantitative trait analysis of type 2 diabetes susceptibility loci identified from whole genome association studies in the Insulin Resistance Atherosclerosis Family Study. *Diabetes* 57:1093-1100, 2008
25. Golding J, Pembrey M, Jones R: ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatr Perinat Epidemiol* 15:74-87, 2001
26. Knight B, Shields BM, Hattersley AT: The Exeter Family Study of Childhood Health (EFSOCH): study protocol and methodology. *Paediatr Perinat Epidemiol* 20:172-179, 2006
27. Rantakallio P: The longitudinal study of the northern Finland birth cohort of 1966. *Paediatr Perinat Epidemiol* 2:59-88, 1988
28. Power C, Elliott J: Cohort profile: 1958 British birth cohort (National Child Development Study). *Int J Epidemiol* 35:34-41, 2006
29. Harris R, Bradburn M, Deeks J, Altman D, Harbord R, Steichen T, Sterne J: METAN: Stata module for fixed and random effects meta-analysis. *Statistical Software Components S456798*, Boston College Department of Economics, revised 19 Feb 2007. <http://ideas.repec.org/c/boc/bocode/s456798.html> 2006
30. Higgins JP, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses. *Bmj* 327:557-560, 2003
31. Bernstein IM, Mongeon JA, Badger GJ, Solomon L, Heil SH, Higgins ST: Maternal smoking and its association with birth weight. *Obstet Gynecol* 106:986-991, 2005
32. Frayling TM: Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev Genet* 8:657-662, 2007
33. Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, Ellard S, Ferrer J, Hattersley AT: Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med* 4:e118, 2007
34. Poulsen P, Vaag AA, Kyvik KO, Moller Jensen D, Beck-Nielsen H: Low birth weight is associated with NIDDM in discordant monozygotic and dizygotic twin pairs. *Diabetologia* 40:439-446, 1997
35. Grunnet L, Vielwerth S, Vaag A, Poulsen P: Birth weight is nongenetically associated with glucose intolerance in elderly twins, independent of adult obesity. *J Intern Med* 262:96-103, 2007
36. Gluckman PD, Hanson MA, Cooper C, Thornburg KL: Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 359:61-73, 2008

**Table 1. Clinical characteristics of subjects**

Characteristic	Study			
	ALSPAC children <sup>†</sup>	EFSOCH children <sup>†</sup>	NFBC1966	1958BC
Year(s) of birth	1991-3	2000-4	1965-7	1958
Total N (% male)*	7687 (52.0)	763 (53.1)	4838 (48.0)	5912 (50.4)
Mean (SD) birth weight in g	3482 (480)	3507 (475)	3534 (491)	3345 (489)
Median (IQR) gestation in wks	40 (39, 41)	40 (39, 41)	40 (39, 41)	40 (39, 41)
Median (IQR) maternal age in yrs	28 (25, 32)	31 (27, 34)	27 (23, 34)	27 (23, 31)
Median (IQR) maternal pre-pregnancy BMI in kg/m <sup>2</sup>	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

\*Includes individuals of white European ancestry, singleton pregnancy, with birth weight available, born at a minimum gestational age of 36 wk and genotyped for at least one of the five SNPs.

<sup>†</sup>Maternal genotype available: ALSPAC  $n = 7176$ ; 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).

SD, standard deviation; IQR, inter-quartile range; NA, not available.

**Table 2. Meta-analysis of the association of birth weight with fetal genotype**

<b>Locus (SNP)</b>	<b>Total N in meta-analysis</b>	<b>Per-allele effect size, in g (95% CI)</b>	<b>P value</b>
<i>CDKALI</i> (rs10946398)	18679	-21 (-31, -11)	$2 \times 10^{-5}$
<i>CDKN2A-2B</i> (rs10811661)	18751	11 (-1, 24)	0.07
<i>HHEX-IDE</i> (rs1111875)	18958	-14 (-23, -4)	0.004
<i>IGF2BP2</i> (rs4402960)	18187	4 (-6, 14)	0.43
<i>SLC30A8</i> (rs13266634)	18702	12 (2, 21)	0.02

Analyses are adjusted for sex and gestational age.

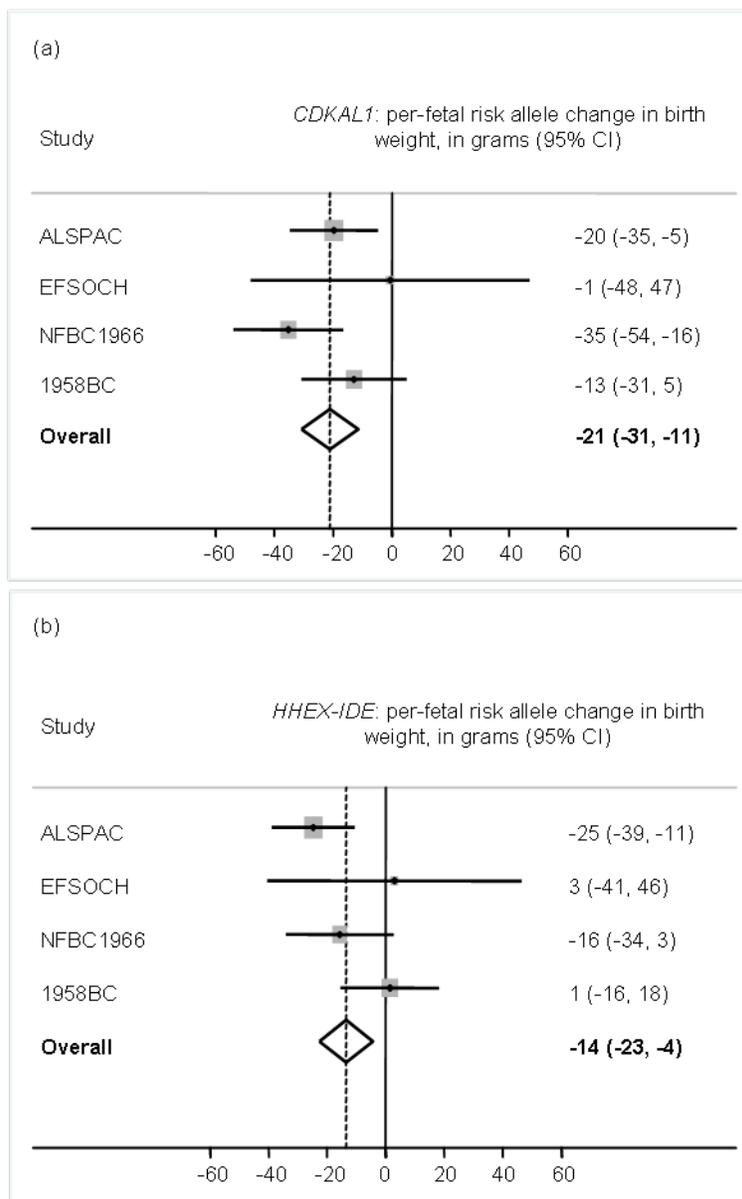
**Table 3. Analysis of fetal genotype and birth weight within four studies**

SNP (locus)	Study	Genotype (number of type 2 diabetes risk alleles)						Total N	Per-risk allele effect size in g (SE)*	P value*
		0		1		2				
		Mean birth weight in g (95% CI)	N	Mean birth weight in g (95% CI)	N	Mean birth weight in g (95% CI)	N			
rs10946398 ( <i>CDKALI</i> )	ALSPAC	3496 (3481, 3510)	3494	3471 (3456, 3486)	3210	3462 (3431, 3494)	758	7462	-20 (8)	0.010
	EFSOCH	3512 (3466, 3557)	350	3504 (3457, 3551)	329	3520 (3420, 3620)	72	751	-1 (24)	0.983
	NFBC1966	3568 (3546, 3589)	1795	3516 (3497, 3534)	2295	3506 (3474, 3539)	742	4832	-35 (10)	0.0002
	1958BC	3380 (3362, 3397)	2675	3367 (3348, 3386)	2354	3354 (3319, 3390)	605	5634	-13 (9)	0.1618
rs10811661 ( <i>CDKN2A/B</i> )	ALSPAC	3451 (3395, 3507)	236	3482 (3463, 3500)	2105	3483 (3471, 3495)	5176	7517	7 (9)	0.465
	EFSOCH	3396 (3196, 3597)	18	3506 (3443, 3569)	182	3509 (3473, 3545)	554	754	20 (31)	0.515
	NFBC1966	3543 (3463, 3624)	125	3523 (3498, 3549)	1249	3537 (3522, 3552)	3448	4822	8 (13)	0.539
	1958BC	3349 (3283, 3414)	162	3358 (3335, 3380)	1618	3380 (3365, 3394)	3878	5658	20 (11)	0.084
rs1111875 ( <i>HHEX/IDE</i> )	ALSPAC	3529 (3505, 3553)	1289	3475 (3461, 3489)	3652	3471 (3454, 3488)	2573	7514	-25 (7)	0.0007
	EFSOCH	3495 (3421, 3569)	132	3509 (3464, 3555)	350	3505 (3453, 3556)	275	757	3 (22)	0.894
	NFBC1966	3547 (3520, 3574)	1100	3536 (3518, 3554)	2466	3516 (3490, 3541)	1266	4832	-16 (9)	0.095
	1958BC	3373 (3345, 3400)	1020	3372 (3355, 3389)	2836	3375 (3355, 3395)	1999	5855	1 (9)	0.8721
rs4402960 ( <i>IGF2BP2</i> )	ALSPAC	3480 (3466, 3494)	3718	3481 (3466, 3497)	3128	3500 (3468, 3533)	692	7538	7 (8)	0.385
	EFSOCH	3469 (3423, 3514)	345	3554 (3508, 3599)	346	3443 (3336, 3550)	63	754	29 (25)	0.247
	NFBC1966	3536 (3518, 3555)	2386	3528 (3509, 3555)	1972	3540 (3498, 3583)	446	4804	-2 (10)	0.829
	1958BC	3375 (3356, 3393)	2413	3366 (3348, 3385)	2172	3391 (3351, 3430)	506	5091	2 (10)	0.851
rs13266634 ( <i>SLC30A8</i> )	ALSPAC	3471 (3438, 3504)	666	3480 (3465, 3495)	3178	3485 (3471, 3499)	3619	7463	6 (8)	0.422
	EFSOCH	3421 (3315, 3528)	63	3548 (3501, 3596)	315	3479 (3435, 3523)	368	746	-13 (25)	0.613
	NFBC1966	3504 (3469, 3540)	631	3536 (3517, 3555)	2274	3512 (3521, 3562)	1933	4838	15 (10)	0.124
	1958BC	3321 (3284, 3358)	558	3372 (3353, 3390)	2383	3377 (3359, 3394)	2714	5655	19 (9)	0.037

\*Linear regression of birth weight against fetal genotype (coded 0, 1, 2 risk alleles), with sex and gestation as covariates. Mean birth weights (and 95% confidence intervals) are adjusted for sex and gestational age.

Type 2 diabetes genes and birth weight

**Figure 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  $N = 18679$ ; 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  $N = 18958$ ; heterogeneity statistics:  $I^2=49.7\%$ ,  $P=0.11$ ). Analyses are adjusted for sex and gestational age.



**Figure 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 ( $n = 18438$ ). Estimates of the difference in birth weight are adjusted for sex and gestational age. Error bars show 95% confidence intervals.

