ARTICLE

Type 2 Diabetes *TCF7L2* Risk Genotypes Alter Birth Weight: A Study of 24,053 Individuals

Rachel M. Freathy, Michael N. Weedon, Amanda Bennett, Elina Hyppönen, Caroline L. Relton, Beatrice Knight, Beverley Shields, Kirstie S. Parnell, Christopher J. Groves, Susan M. Ring, Marcus E. Pembrey, Yoav Ben-Shlomo, David P. Strachan, Chris Power, Marjo-Riitta Jarvelin, Mark I. McCarthy, George Davey Smith, Andrew T. Hattersley, and Timothy M. Frayling

The role of genes in normal birth-weight variation is poorly understood, and it has been suggested that the genetic component of fetal growth is small. Type 2 diabetes genes may influence birth weight through maternal genotype, by increasing maternal glycemia in pregnancy, or through fetal genotype, by altering fetal insulin secretion. We aimed to assess the role of the recently described type 2 diabetes gene *TCF7L2* in birth weight. We genotyped the polymorphism *rs7903146* in 15,709 individuals whose birth weight was available from six studies and in 8,344 mothers from three studies. Each fetal copy of the predisposing allele was associated with an 18-g (95% confidence interval [CI] 7–29 g) increase in birth weight ($P = .001$) and each maternal copy with a 30-g (95% CI 15–45 g) increase in offspring birth weight ($P = 2.8 \times 10^{-5}$). Stratification by fetal genotype suggested that the association was driven by maternal genotype (31-g [95% CI 9–48 g] increase per allele; corrected $P = .003$). Analysis of diabetes-related traits in 10,314 nondiabetic individuals suggested the most likely mechanism is that the risk allele reduces maternal insulin secretion (disposition index reduced by ~0.15 standard deviation; $P = 1 \times 10^{-4}$), which results in increased maternal glycemia in pregnancy and hence increased offspring birth weight. We combined information with the other common variant known to alter fetal growth, the −30G→A polymorphism of glucokinase (*rs1799884*). The 4% of offspring born to mothers carrying three or four risk alleles were 119 g (95% CI 62–172 g) heavier than were the 32% born to mothers with none (for overall trend, $P = 2 \times 10^{-7}$), comparable to the impact of maternal smoking during pregnancy. In conclusion, we have identified the first type 2 diabetes–susceptibility allele to be reproducibly associated with birth weight. Common gene variants can substantially influence normal birth-weight variation.

The role of genes in normal variation in birth weight is poorly understood, and it has been suggested that the genetic component of fetal growth in the general population is small.[1–4] In contrast, the maternal intrauterine environment, including maternal glucose tolerance, BMI, and smoking, has a large impact on birth weight.[5–8] However, the importance of these environmental factors does not negate the role of common maternal or fetal gene variants as determinants of normal fetal growth. Family, twin, and linkage studies suggest a role for common genetic variants,[1,3,4,9–11] but, to date, specific genetic loci remain largely unknown.

Diabetes-susceptibility genes or genes that alter fasting glucose are good candidates for genes that influence birth weight, since they may impact insulin secretion or insulin action in nondiabetic individuals. Altered fetal insulin secretion would alter fetal growth and hence birth weight, since insulin is a key intrauterine growth factor. A diabetes-risk allele in the mother may alter fetal growth indirectly, by altering maternal glycemia during pregnancy and thereby influencing fetal insulin secretion. Alternatively, a diabetes-risk allele in the fetus may act directly on fetal insulin secretion. Reduced birth weight is associated with an increased risk of type 2 diabetes (MIM 125853) later in life,[12,13] and it has been proposed under the fetal insulin hypothesis that this association could have a genetic explanation.[14] There is some evidence of this from population studies.[15–18] Direct evidence that birth weight is altered by fetal and maternal inheritance of diabetes-susceptibility genes is provided by several rare subtypes of diabetes. These include mutations in monogenic diabetes genes that reduce birth weight as a result of reduced fetal insulin secretion in utero[19–24] and mutations in other monogenic diabetes genes that increase birth weight as a result of increased fetal insulin secretion.[25]

The observations in monogenic diabetes and the general

From the Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, United Kingdom (R.M.F.; M.N.W.; B.K.; B.S.; K.S.P.; A.T.H.; T.M.F.); Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, United Kingdom (A.B.; C.J.G.; M.I.M.); Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health (E.H.; C.P.), Division of Community Health Sciences, St George's, University of London (D.P.S.), and Department of Epidemiology and Public Health, Imperial College London (M.-R.J.), London; Child Health, School of Clinical Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom (C.L.R.); Avon Longitudinal Study of Parents and Children, (S.M.R.; M.E.P.) and Department of Social Medicine (Y.B.-S.; G.D.S.), University of Bristol, Bristol, United Kingdom; and Department of Public Health Science and General Practice, University of Oulu, Oulu, Finland (M.-R.J.)

Received January 25, 2007; accepted for publication March 22, 2007; electronically published April 23, 2007.

Address for correspondence and reprints: Dr. T. M. Frayling, Institute of Biomedical and Clinical Science, Peninsula Medical School, Magdalen Road, Exeter EX1 2LU, United Kingdom. E-mail: tim.frayling@pms.ac.uk

Am. J. Hum. Genet. 2007;80:1150–1161. 0002-9297/2007/8006-0014$15.00
DOI: 10.1086/518517

Table 1. Clinical Characteristics of Subjects Included in the Analysis of the *TCF7L2 rs7903146* Genotype with Birth Weight

Characteristic	Characteristics by Study					
	BCG	EFSOCH	NCCGP	1958BC	NFBC1966	ALSPAC
N[a] (% male)	571 (52.2)	792 (52.9)	1,096 (49.9)	1,779 (49.5)	4,578 (47.7)	6,893 (51.2)
Mean (SD) birth weight, in g	3,387 (496)	3,504 (478)	3,441 (485)	3,351 (494)	3,536 (490)	3,489 (474)
Median (IQR) gestation, in wk	40.0 (39.0–41.0)	40.3 (39.3–41.1)	40.0 (39.0−41.0)	40.3 (39.4–41.3)	40.0 (38.0–42.0)	40.0 (39.0–41.0)
Median (IQR) maternal age, in years	NA	31.0 (27.0–34.0)	28.8 (24.0–32.8)	27.0 (23.0–31.0)	27.2 (17.4–36.9)	29.0 (26.0–32.0)
Median (IQR) maternal prepregnancy BMI[b]	NA	23.0 (21.1–25.7)	NA	22.1 (20.4–24.5)	22.7 (18.9–26.5)	22.2 (20.5–24.4)
Primiparous births (%)	NA	55.3	66.2	35.2	30.6	43.3
Maternal smoking during pregnancy (%)	NA	13.8	26.0	32.9	13.4	19.9

NOTE.—The clinical characteristics of offspring with maternal *rs7903146* genotype data available (917, 1,133, and 6,294 individuals from EFSOCH, NCCGP, and ALSPAC, respectively) were very similar (data not shown). In some cases, offspring birth weight and maternal genotype were available, whereas offspring genotype was unavailable. Therefore, for EFSOCH and NCCGP, the number of subjects in the maternal genotype analysis was larger than that included in the fetal genotype analysis. NA = not available; IQR = interquartile range.

[a] Includes white, singleton individuals, genotyped for *rs7903146,* with birth weight available, born at minimum gestation of 36 wk.

[b] Calculated as weight in kilograms divided by the square of height in meters.

population led to the hypothesis that inheritance of type 2 diabetes–susceptibility alleles by the fetus would reduce birth weight, whereas their inheritance by the mother would increase offspring birth weight. Some studies have suggested that inheritance by the fetus of polymorphisms associated with type 2 diabetes is associated with reduced birth weight,[26,27] but these results have not been replicated.[28–32] A maternal copy of the A allele of the common glucokinase (*GCK* [MIM 138079]) promoter polymorphism (*GCK-30*; *rs1799884*), which is associated with raised fasting glucose at all ages in the general population ($P = 1 \times 10^{-9}$), is also associated with a 32-g (95% CI 11–53 g) increase in offspring birth weight ($P = .002$). However, there is no evidence of an independent effect of fetal genotype.[33,34]

The gene encoding transcription factor 7–like 2 (*TCF7L2* [MIM 602228]) is the most important type 2 diabetes susceptibility gene found to date.[35] Since its discovery, the association has been replicated in subjects of U.K., Amish, Finnish, French, U.S., Polish, Scandinavian, Dutch, Indian, and West African origin.[36–45] In the U.K. population, the allelic odds ratio for *rs7903146* (risk-allele frequency ~30%) is 1.36 (95% CI 1.24–1.48; $P = 1.3 \times 10^{-11}$), and individuals carrying two risk (T) alleles are at nearly twice the risk of type 2 diabetes as are those with none.[36] Studies of nondiabetic subjects indicate that *TCF7L2* diabetes-risk genotypes alter insulin secretion.[37,42,46] The impact of this polymorphism on birth weight has not been studied.

In the present study, we hypothesized that fetal *TCF7L2* type 2 diabetes–predisposing genotypes at *rs7903146* and *rs12255372* would be associated with reduced birth weight and that maternal genotypes would be associated with increased offspring birth weight through elevated maternal glucose. We investigated this hypothesis in >24,000 individuals from six population-based studies. In addition, we explored the role of these variants in diabetes-related intermediate traits, including beta-cell function, in >10,000 young (median age ≤45 years), nondiabetic individuals from five studies.

Subjects and Methods

Subjects for Analysis of Fetal TCF7L2 *Genotype with Birth Weight*

To assess the association of fetal *TCF7L2* genotypes with birth weight, we used subjects from six studies (table 1). All subjects were born at 36 full wk gestation or later and were of white European origin, either from the United Kingdom (Barry Caerphilly Growth Study [BCG],[29] Exeter Family Study of Childhood Health [EFSOCH],[47] North Cumbria Community Genetics Project [NCCGP],[48] British 1958 Birth Cohort [1958BC],[36,49] and Avon Longitudinal Study of Parents and Children [ALSPAC][50]) or Finland (Northern Finland 1966 Birth Cohort [NFBC1966]).[51–53] These studies have been described in depth elsewhere, but brief details are as follows. BCG is a longitudinal study of individuals born between 1972 and 1974 whose growth was monitored from birth to age 5 years. Data for analysis of the association of *rs7903146* with birth weight were available for 571 subjects. EFSOCH is a prospective study of children, born between 2000 and 2004, and their parents from a geographically defined region of Exeter, United Kingdom. Data for 792 EFSOCH babies were available for the present analysis. Maternal fasting glucose, assessed at 26–28 wk gestation, was available for these subjects. Maternal smoking status was also assessed at that time. NCCGP is a community-based DNA-banking project. Data were available for 1,096 babies born between April 1999 and March 2002. Maternal smoking status was assessed during the first 12 wk of pregnancy. The 1958BC is a national cohort of U.K. subjects born during the same week in March 1958. The 1,779 subjects included in the present analysis are from the first group of subjects from whom DNA was extracted during 2003–2005. Information about smoking during pregnancy was reported by the mothers after the birth of the child and was coded as smoking continuing after the 4th mo of pregnancy ("yes/no"). ALSPAC is a prospective study, which recruited pregnant women from Bristol, United Kingdom, with expected delivery dates between April 1991 and December 1992. We were able to include 6,893 of the ALSPAC children in the present analysis. Maternal smoking status was assessed during the first 12 wk of gestation. NFBC1966 is a study of offspring born in the two northernmost provinces of Finland to mothers with expected dates of delivery in 1966. The 4,578 subjects included in the present analysis are from a subset of individuals who had anthropometric data taken and DNA extracted at age 31 years.

Maternal smoking status was assessed throughout the pregnancy and was classified as "yes/no" after the 8th gestational wk.

Only singleton pregnancies were included in the analyses. In all studies, birth weight was obtained from hospital records, and gestation was inferred from the last menstrual period or ultrasound scan. In most studies, data were available on parity (dichotomized as first child or subsequent), maternal prepregnancy BMI, and maternal smoking status (smoking or nonsmoking during pregnancy). All subjects involved gave their informed consent, and ethical approval was obtained from the local institutional review board for each study.

Subjects for Analysis of Maternal TCF7L2 *Genotype with Offspring Birth Weight*

To assess the association of maternal *TCF7L2* genotypes with offspring birth weight, we used a total of 8,344 genotyped mothers from the three studies in which maternal DNA was available (917 from EFSOCH, 1,133 from NCCGP, and 6,294 from ALSPAC). Altogether, there were 6,044 mother-offspring pairs with both mother's and child's *rs7903146* genotype and offspring birth weight available (754 from EFSOCH, 1,024 from NCCGP, and 4,266 from ALSPAC). Maternal DNA was not available for BCG, 1958BC, or NFBC1966 subjects.

Subjects for Analysis of TCF7L2 *Genotype with Diabetes-Related Intermediate Trait Data*

We investigated possible associations of *TCF7L2* genotypes with diabetes-related intermediate traits in the BCG, EFSOCH, 1958BC, NFBC1966, and ALSPAC studies. Since the BCG, 1958BC, and NFBC1966 studies are long-term follow-up cohorts, detailed quantitative trait data were available for the individuals as young adults, in addition to their birth and early-life data. In the case of ALSPAC, diabetes-related intermediate traits were measured in subsets of children at ages 7 and 8 years. For EFSOCH, we used data from the mothers and fathers. Again, analysis was restricted to individuals of white European origin. In addition, when information was available, subjects with diabetes, fasting plasma glucose >6 mmol/liter, or glycated hemoglobin (HbA_1c) >6% were excluded. The clinical characteristics of these subjects are shown in table 2.

Oral glucose-tolerance test data were available for 619 BCG adults and 697 ALSPAC children. These consisted of plasma glucose and insulin measures taken after fasting and then repeated 30 min after administration of an oral glucose load. We calculated early insulin response to glucose[54]

$$\frac{\text{Ins}_{30} - \text{fasting insulin}}{\text{Gluc}_{30}}$$

and insulin disposition index (insulinogenic index × homeostasis model assessment of insulin resistance [HOMA-IR]), where

$$\text{insulogenic index} = \frac{\text{Ins}_{30} - \text{fasting insulin}}{\text{Gluc}_{30} - \text{fasting glucose}}$$

and[42]

$$\text{HOMA-IR} = \frac{\text{fasting glucose} \times \text{fasting insulin}}{22.5}\ .$$

Whereas early insulin response is a measure of insulin secretion, disposition index is a corrected measure, accounting for an individual's insulin resistance. Fasting-glucose and insulin data were also available for 1,810 EFSOCH parents and 4,486 NFBC1966 adults. Using the online HOMA Calculator version 2.2 (available from the Diabetes Trials Unit Web site) we obtained model-derived estimates of HOMA-IR for these subjects. HbA_1c was available for 1,280 ALSPAC children, 1,442 EFSOCH parents, and 1,813 subjects from 1958BC. Finally, BMI was available for all subjects and for 5,817 ALSPAC mothers.

Genotyping and Quality Control

The *rs7903146* and *rs12255372* polymorphisms were genotyped in all cohorts. Genotyping was performed on the BCG, 1958BC, EFSOCH, NFBC1966, and ALSPAC samples by KBiosciences, with use of their own novel system of fluorescence-based competitive allele-specific PCR (KASPar). Details of assay design are available from the KBiosciences Web site. Genotyping of the NCCGP samples was performed in-house (Peninsula Medical School), with use of TaqMan SNP genotyping assay (Applied Biosystems) ac-

Table 2. Clinical Characteristics of Individuals Included in the Diabetes-Related Trait Analyses for the *TCF7L2 rs7903146* Genotype

	Characteristics by Study				
Characteristic	BCG	EFSOCH	1958BC	NFBC1966	ALSPAC
N[a] (% male)	619 (52.8)	1,810 (49.1)	1,813 (49.7)	4,486 (46.3)	697 (53.8)
Median (IQR[b]) age, in years	25.0 (24.5–25.6)	31.0 (28.0–35.0)	45.0	31.0	8.0
Median (IQR) BMI[c]	24.3 (22.0–27.3)	24.8 (22.2–27.7)[d]	26.5 (23.9–29.8)	24.0 (19.2–28.8)	16.2 (15.2–17.6)
Median (IQR) fasting plasma:					
Glucose, in mmol/liter	4.6 (4.3–4.8)	4.5 (4.2–4.8)	NA	5.0 (4.5–5.5)	4.9 (4.7–5.1)
Insulin, in pmol/liter	39.6 (29.4–55.8)	54.8 (40.4–79.0)	NA	52.1 (29.2–75.0)	33.3 (21.5–54.9)
HbA_1c (IQR) [%]	NA	5.1 (4.8–5.3)	5.2 (5.0–5.5)	NA	4.9[e] (4.7–5.1)

NOTE.—NA = not available; IQR = interquartile range.

[a] Includes white, unrelated individuals genotyped for *rs7901346*. When information was available, subjects with diabetes, HbA_1c >6%, or fasting plasma glucose >6 mmol/liter were excluded.

[b] IQR not applicable for 1958BC, NFBC1966, and ALSPAC, since participants were all studied at the same age.

[c] Calculated as weight in kilograms divided by the square of height in meters.

[d] Females were pregnant (28 wk gestation) at time of study, but BMI is the prepregnancy value.

[e] HbA_1c was measured in 1,280 subjects at age 7 years (50.6% male), whereas all other data in this column were gathered 1 year later. A total of 391 ALSPAC children were included at both times.

cording to the manufacturer's protocol. Additional EFSOCH samples (70 children and 104 parents) were genotyped in this way for *rs7903146*. These genotypes were added to the genotypes from KBiosciences, since the concordance for samples genotyped by both centers (n = 1,706) was 98.9%.

The percentage of successful genotype calls was >90% in all groups, apart from NCCGP children (88% *rs7903146*; 89% *rs12255372*). The percentage of duplicate samples included for genotyping in each study group was 1% (1958BC), 3% (ALSPAC children), 6% (ALSPAC mothers and NFBC1966), 10% (BCG and EFSOCH), and 21% (NCCGP). Concordance between duplicate samples was ⩾99% in all groups except NFBC1966 (98.1% *rs7903146*) and ALSPAC children (98.4% *rs7903146*). No deviation from Hardy-Weinberg equilibrium was observed (all $P > .05$). The minor-allele frequency (MAF) of *rs7903146* was 19.1% in NFBC1966 and had a range of 29.0%–30.4% in the U.K. samples. The MAF of *rs12255372* was 16.8% in NFBC1966 and had a range of 28.4%–29.8% in the U.K. samples. These figures are consistent with those observed elsewhere in Finnish[38] and U.K.[36] subjects, and the difference is therefore likely to reflect underlying differences in population allele frequencies than differences due to our sampling or genotyping. The SNPs were in linkage disequilibrium in all study groups (r^2 range 0.74–0.79 in U.K. samples; 0.69 in NFBC1966).

Statistical Analysis

We performed linear regression analysis within each study, with birth weight as the dependent and SNP genotype (coded as zero, one, or two risk alleles) as the independent variable and with sex and gestation as covariates. We performed additional analyses correcting also for parity, maternal smoking, maternal prepregnancy BMI, and maternal fasting plasma–glucose concentration, when these variables were available. All regression analyses were performed using Stata SE version 9 (StataCorp) or SPSS version 11.5. We looked for evidence of deviation from an additive genetic model in ALSPAC by comparing the full genotype model with the additive model, using the likelihood-ratio test. We found no evidence to reject the additive model ($P > .05$), so this was assumed for all regression analyses. Inverse variance meta-analysis (fixed effects) statistics and plots were generated using StatsDirect version 2.5.6. The I^2 (inconsistency) statistic was used to estimate the proportion of variance attributable to between-study heterogeneity.[55] By performing a meta-analysis of summary data from the separate studies, we were able to avoid the potential confounding effect of the difference between the NFBC1966 and U.K. allele frequencies. To investigate the relative contributions to birth weight of maternal and fetal genotypes, we generated standardized residuals from a regression of birth weight on sex and gestation within the EFSOCH, NCCGP, and ALSPAC studies. We combined these sex- and gestational age–corrected birth weight Z scores (mean = 0; SD = 1) into one data set. We then performed a regression analysis with birth-weight Z score as the dependent and both maternal and fetal genotypes as independent variables. Since there is a degree of colinearity between maternal and fetal genotype ($r \approx 0.5$), we performed stratified analyses to verify this result; within each of the three strata defined by maternal genotype, birth-weight Z score was regressed on fetal genotype, then the three regression coefficients were combined using a meta-analysis approach (fixed effects, inverse-variance method). The same analysis was performed for maternal genotype with data stratified by fetal genotype.

All diabetes-related trait variables, apart from fasting glucose, required log transformation to obtain a normal distribution before analysis. Linear regression analyses were performed for each trait against genotype (additive model). When appropriate, we included age, sex, and ln(BMI) as covariates.

To investigate the combined effects of maternal *TCF7L2 rs7903146* and *GCK rs1799884* genotype on offspring birth weight, we combined sex- and gestational age–corrected birth-weight Z scores from the 6,122 ALSPAC and 737 EFSOCH subjects for whom genotypes at both loci were available. We performed a regression analysis with Z score as the independent variable and "*TCF7L2-GCK* genotype" (values of zero, one, two, and three or four maternal risk alleles) as the dependent variable. We combined individuals with three or four risk alleles, because of small numbers in the final category. We looked for evidence of deviation from an additive genetic model in the separate analyses of birth-weight Z score versus *TCF7L2 rs7903146* or *GCK rs1799884* and, in the combined analysis, by comparing the full genotype model with the additive model with use of the likelihood-ratio test. We found no evidence to reject the additive model (all $P >$ .4), so this was assumed for the combined analysis. The mean birth-weight Z score of offspring born to mothers with no risk alleles was compared with those born to mothers carrying three or four risk alleles, with use of an independent-samples t test.

Power calculations were performed using Lenth's Java Applets for Power and Sample Size (available from the Russ Lenth's Power and Sample Size Web site), with the assumption of an MAF of 30%, birth-weight SD of 480 g, and a two-tailed $P < .05$. Our sample of 15,709 subjects with available birth weight gave us 80% power to detect differences of 39 g in birth weight between homozygotes. In assessment of the effect of maternal genotype, our sample of 8,344 genotyped mothers gave us 80% power to detect differences of 53 g between homozygotes.

Results

Association of Fetal TCF7L2 *Genotype with Birth Weight*

Since SNPs *rs7903146* and *rs12255372* are highly correlated ($r^2 \approx 0.75$), the results obtained for each were similar. In this work, we focus on *rs7903146,* since that SNP is associated more strongly with type 2 diabetes.[36,45] Detailed data for *rs12255372* are available from the authors.

Meta-analysis of the association between birth weight (corrected for sex and gestation) and fetal *rs7903146* genotype showed an 18-g (95% CI 7–29 g) increase in birth weight per T allele (P = .001) (fig. 1 and table 3). Heterogeneity between studies was low (I^2 = 21.0%). The association observed with *rs12255372* was similar (combined per-allele difference = 19 g [95% CI 8–30 g]; P = .0009; I^2 = 0%). The largest contribution to the overall effect size was made by ALSPAC, the largest individual study. In that study, fetal *rs7903146* genotype was associated with increased birth weight (per–T allele increase 23 g [95% CI 7–39 g]; P = .004). Correction for other covariates of birth weight made little difference to the results (data available from the authors).

Table 3. Analysis of Birth Weight by Fetal *TCF7L2 rs7903146* Genotype

Study	Total *N*	CC		CT		TT		Per–T Allele Birth-Weight[a] Difference (SE)[b]	*P*		
		Mean Birth Weight[a] (95% CI)	*n*	Mean Birth Weight[a] (95% CI)	*n*	Mean Birth Weight[a] (95% CI)	*n*		Uncorrected	Corrected for Sex and Gestation	Corrected for Additional Covariates[c]
BCG	571	3,355 (3,301–3,409)	281	3,411 (3,352–3,471)	233	3,444 (3,324–3,564)	57	49 (29)	.13	.10	NA
EFSOCH	792	3,475 (3,432–3,517)	399	3,523 (3,476–3,570)	316	3,583 (3,487–3,679)	77	52 (23)	.11	.03	.05
NCCGP	1,096	3,455 (3,419–3,491)	542	3,412 (3,374–3,451)	467	3,505 (3,416–3,596)	87	−4 (20)	.38	.83	NA
1958BC	1,605	3,359 (3,327–3,392)	764	3,358 (3,324–3,392)	700	3,365 (3,290–3,440)	141	1 (18)	.76	.96	.87
NFBC1966	4,578	3,534 (3,516–3,551)	2,978	3,541 (3,516–3,566)	1,444	3,540 (3,466–3,615)	156	9 (12)	.66	.47	.52
ALSPAC	6,893	3,474 (3,460–3,489)	3,372	3,499 (3,483–3,515)	2,876	3,517 (3,483–3,550)	645	23 (8)	.03	.004	.01

NOTE.—All weights are given in grams.

[a] Corrected for sex and gestation.

[b] Rounded to the nearest 1 g.

[c] Corrected for sex, gestation, parity (first child/subsequent), maternal smoking (yes/no), and maternal prepregnancy BMI. NA = not available.

Table 4. Analysis of Offspring Birth Weight by Maternal *TCF7L2 rs7903146* Genotype

Study	Total *N*	CC		CT		TT		Per–T Allele Birth-Weight[a] Difference (SE)[b]	*P*		
		Mean Birth Weight[a] (95% CI)	*n*	Mean Birth Weight[a] (95% CI)	*n*	Mean Birth Weight[a] (95% CI)	*n*		Uncorrected	Corrected for Sex and Gestation	Corrected for Additional Covariates[c]
EFSOCH	917	3,471 (3,432–3,509)	460	3,488 (3,446–3,530)	384	3,549 (3,452–3,646)	73	30 (22)	.25	.18	.34
NCCGP	1,133	3,439 (3,403–3,475)	563	3,448 (3,409–3,487)	470	3,434 (3,349–3,519)	100	2 (20)	.39	.91	NA[d]
ALSPAC	6,294	3,443 (3,426–3,460)	3,051	3,491 (3,473–3,509)	2,702	3,494 (3,453–3,534)	541	35 (9)	.0002	.00004	.00002

NOTE.—All weights are given in grams.

[a] Corrected for sex and gestation.

[b] Rounded to the nearest 1 g.

[c] Corrected for sex, gestation, parity (first child/subsequent), maternal smoking (yes/no), and maternal prepregnancy BMI.

[d] NA = not available.

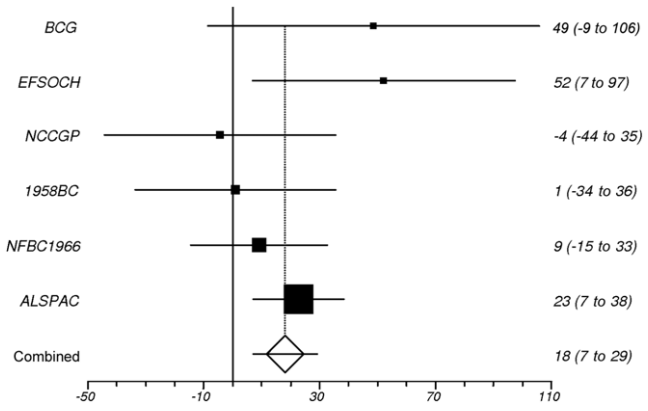

Figure 1. Meta-analysis of the association of birth weight (corrected for sex and gestation) with fetal *rs7903146* genotype across six studies, arranged in order of increasing size. The effect size (in grams) and 95% CI per risk allele is presented. Combined per-allele difference = 18 g (95% CI 7–29 g); $P = .001$; $I^2 = 21.0\%$.

Association of Maternal TCF7L2 *Genotype with Offspring Birth Weight*

Meta-analysis of the association between birth weight (corrected for sex and gestation) and maternal *rs7903146* genotype showed a 30-g (95% CI 15–45 g) increase in birth weight per T allele ($P = 2.8 \times 10^{-5}$) (fig. 2 and table 4). Again, heterogeneity between studies was low ($I^2 = 14.3\%$). The association observed with *rs12255372* was similar (combined per-allele difference = 29 g [95% CI 14–43 g]; $P = 4.5 \times 10^{-5}$; $I^2 = 0\%$). Again, the largest individual study, ALSPAC, made the largest contribution to the combined association. In ALSPAC, the maternal *rs7903146* genotype was associated with a per–T allele increase in offspring birth weight of 35 g (95% CI 17–53 g; $P = 4 \times 10^{-5}$). Correction for other covariates of birth weight again made little difference to the meta-analysis result (data available from the authors).

Data on maternal diabetes status were variable and incomplete across the cohorts, so no subject was excluded from the main analysis on that basis. Data on diabetes status were available for 96% of the ALSPAC mothers. These mothers had been asked whether they had ever had diabetes. Removal of the 64 mothers who had answered "yes" from the analysis of offspring birth weight versus maternal *rs7903146* genotype, sex, and gestation did not alter the results.

Adjustment of Fetal and Maternal Genotype Effects for One Another

Maternal and fetal genotypes are 50% correlated. To establish whether maternal or fetal genotypes were driving the association, we used 6,044 mother-offspring pairs for whom both maternal and fetal *rs7903146* genotypes were available. Regression of birth-weight *Z* score on fetal and maternal genotype revealed an association of maternal (per–T allele increase 0.07 [95% CI 0.02–0.11]; $P = .003$) but not fetal (per–T allele increase 0.02 [95% CI −0.02 to 0.07]; $P = .29$) genotype with birth weight. Stratified analyses confirmed this result. With use of the SD of birth weight (440 g), corrected for sex and gestation, in ALSPAC children with genotyped mothers, the effect size of 0.07 (95% CI 0.02–0.11) *Z* scores is equivalent to an increase of 31 g (9–48 g) per maternal T allele. The results of this analysis for *rs12255372* were similar: there was an association of maternal (per–T allele increase 0.05 [95% CI 0.01–0.10]; $P = .025$) but not fetal (per–T allele increase 0.03 [95% CI −0.01 to 0.08]; $P = .16$) genotype with birth weight.

Combined Analysis of Maternal TCF7L2 *and* GCK *Genotype with Offspring Birth Weight*

A previous study showed that maternal genotypes of the glucokinase (*GCK*) variant *rs1799884* (*GCK*-30) are reproducibly associated with offspring birth weight.[33,34] To assess the combined effect of maternal *TCF7L2 rs7903146* and *GCK rs1799884* variants on birth weight, we used the 737 EFSOCH and 6,122 ALSPAC subjects for whom maternal genotype was available at both loci. In this data set, maternal *GCK rs1799884* genotype was associated with a per–A allele increase in offspring birth-weight *Z* score of 0.06 (95% CI 0.02–0.10; $P = .006$; ~27 g [95% CI 8–46 g]). This is similar to the effect size observed in the present study for *TCF7L2 rs7903146*. We analyzed sex- and gestational age–corrected birth-weight *Z* score against the number of *TCF7L2 rs7903146* and *GCK rs1799884* maternal risk alleles (zero, one, two, and three or four) in 6,859 individuals (fig. 3). We confirmed that it was valid to combine data from the two polymorphisms in this way, because there was no deviation from an additive model ($P = .53$). The addition of each maternal allele (or alleles) was associated with an increase in *Z* score of 0.08 (95%

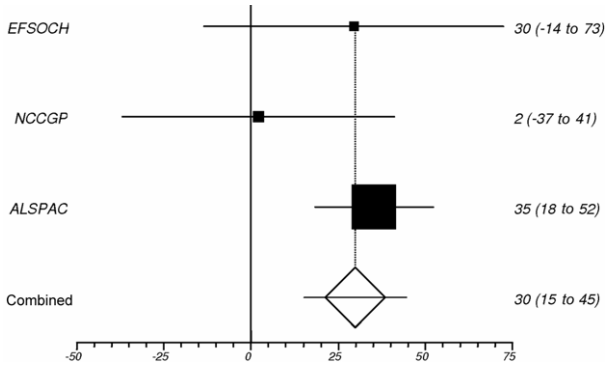

Figure 2. Meta-analysis of the association of birth weight (corrected for sex and gestation) with maternal *rs7903146* genotype across three studies, arranged in order of increasing size. The effect size (in grams) and 95% CI per risk allele is presented. Combined per-allele difference = 30 g (95% CI 15–45 g); $P = 2.8 \times 10^{-5}$. $I^2 = 14.3\%$.

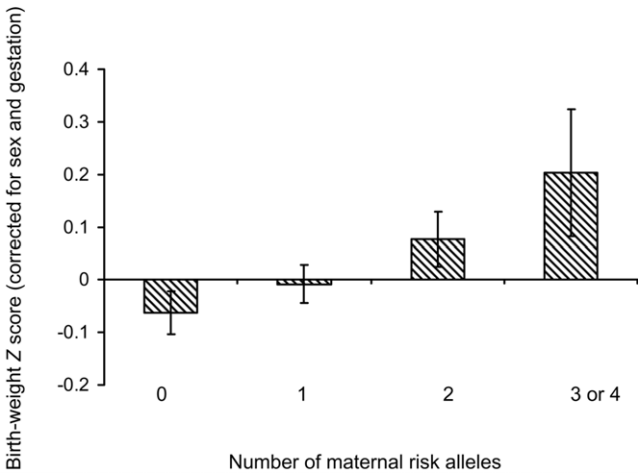

Figure 3. Bar graph showing sex- and gestational age–corrected birth-weight *Z* score plotted against the number of maternal risk alleles for *TCF7L2 rs7903146* and *GCK rs1799884*. Error bars show 95% CIs.

CI 0.05–0.10; $P = 2 \times 10^{-7}$). This equates to a per-allele increase of 35 g (95% CI 22–44 g). Offspring born to the 4% of mothers with three or four risk alleles had a 0.27 (95% CI 0.14–0.39) higher birth-weight *Z* score than those born to the 32% of mothers with no risk alleles ($P = 3 \times 10^{-5}$), which equates to a difference of 119 g (95% CI 62–172 g).

Association of TCF7L2 *Genotypes with Diabetes-Related Intermediate Traits*

To help establish the mechanism through which maternal *TCF7L2* genotype may be altering birth weight, we analyzed a number of type 2 diabetes–related continuous traits in 10,314 subjects. The results of the intermediate trait analysis for *rs7903146* are shown in table 5. The type 2 diabetes–risk allele (T) was associated with a reduction in insulin secretion in the BCG and ALSPAC studies, as measured by early-insulin response (combined per–T allele decrease [logged data] −0.067 [95% CI −0.118 to −0.016]; $P = .02$; $n = 1{,}235$) and insulin-disposition index (combined per–T allele decrease [logged data] −0.192 [95% CI −0.288 to −0.096]; $P = 1 \times 10^{-4}$; $n = 1{,}184$). An association was also seen with increased fasting plasma glucose across the BCG, EFSOCH, ALSPAC, and NFBC1966 studies (combined per–T allele increase 0.020 mmol/liter [95% CI 0.005–0.035 mmol/liter]; $P = .01$; $n = 7{,}612$; $I^2 = 9.2\%$). After a Bonferroni correction for the six studied traits was applied, the early-insulin response and fasting-glucose associations did not remain at $P < .05$. However, the correlation between these traits means that this is a conservative correction, and there is insufficient statistical power to rule out the associations observed. Whereas the *rs7903146* T allele was associated with reduced fasting insulin and HOMA-IR in the BCG cohort ($P < .05$), this association was not seen in the two larger cohorts ($P > .1$). No association was observed between the *TCF7L2* genotype and HbA_1c ($P > .05$). There was no evidence of association of *TCF7L2* genotype with BMI in any cohort (all $P > .05$) (data not shown). The results of the analyses for *rs12255372* were similar (data available from the authors).

Discussion

Using a total of 24,053 subjects from six studies, we have shown that *TCF7L2* is the first type 2 diabetes gene to be reproducibly associated with altered birth weight. Each maternal copy of the T allele at *rs7903146* increased offspring birth weight by 30 g (95% CI 15–45 g), and our data suggest that the most likely mechanism is through reduced maternal-insulin secretion resulting in maternal hyperglycemia and increased insulin-mediated fetal growth.

The understanding of the regulation of birth weight is important, not only because of direct effects of high or low birth weight on perinatal mortality and morbidity, but also because of the association of altered birth weight with altered adult phenotypes. It has been proposed under the fetal-insulin hypothesis that diabetes-susceptibility alleles might reduce birth weight by reducing fetal insulin secretion or action.[14] Our results do not support this hypothesis for *TCF7L2,* since we found an increase and not a reduction in birth weight with fetal inheritance of the type 2 diabetes risk allele. Our analysis of mother-child pairs suggests that the increase seen in the offspring is not a direct effect of the fetal risk allele but, rather, is a reflection of its presence in the mother.

There was clear evidence in support of the hypothesis that the presence of a maternal type 2 diabetes–susceptibility allele would result in increased offspring birth weight. Maternal *TCF7L2* risk genotypes increase birth weight by

Table 5. Type 2 Diabetes–Related Intermediate Trait Analyses for the *TCF7L2 rs7903146* Genotype

Trait (units) and Study	*N*	Uncorrected Mean (95% CI)			Per–T Allele Trait Difference (SE)	*P*	
		CC	CT	TT		Uncorrected	Corrected[a]
Fasting plasma glucose (mmol/liter):							
BCG	619	4.61 (4.57–4.65)	4.61 (4.56–4.66)	4.56 (4.47–4.66)	−.016 (.023)	.48	.72
ALSPAC	697	4.92 (4.88–4.96)	4.92 (4.90–4.95)	4.96 (4.88–5.03)	.012 (.019)	.52	.53
EFSOCH	1,810	4.49 (4.46–4.52)	4.55 (4.52–4.58)	4.52 (4.44–4.59)	.031 (.016)	.06	.08
NFBC1966	4,486	4.98 (4.96–4.99)	4.99 (4.97–4.99)	5.03 (4.97–5.09)	.025 (.011)	.03	.06
Combined	7,612				.020 (.008)	.01	.03
Fasting plasma insulin (pmol/liter):							
BCG	619	42.8 (40.4–45.4)	40.9 (38.3–43.5)	35.7 (31.5–40.4)	−.076 (.031)	.02	.002
ALSPAC	697	37.2 (34.5–40.0)	34.8 (33.5–37.6)	35.8 (31.0–41.4)	−.052 (.038)	.17	.26
EFSOCH	1,780	56.1 (54.2–58.1)	59.7 (57.5–62.0)	55.3 (50.8–60.2)	.023 (.020)	.25	.16
NFBC1966	4,453	53.6 (52.9–54.3)	53.7 (52.7–54.7)	55.7 (52.5–59.0)	.007 (.010)	.45	.99
Combined	7,549				.0009 (.008)	.92	.75
HOMA-IR:							
BCG	619	.89 (.84–1.06)	.85 (.80–.91)	.75 (.66–.84)	−.076 (.031)	.01	.002
EFSOCH	1,780	1.15 (1.11–1.19)	1.23 (1.18–1.23)	1.14 (1.05–1.24)	.025 (.019)	.21	.14
NFBC1966	4,452	1.00 (.99–1.01)	1.00 (.99–1.01)	1.02 (.99–1.04)	.004 (.004)	.41	.95
Combined	6,851				.004 (.004)	.35	1.00
Early insulin response (pmol/mmol/liter):							
BCG	604	42.8 (40.0–45.9)	41.6 (38.6–44.8)	34.8 (30.1–40.3)	−.078 (.036)	.03	.02
ALSPAC	631	33.0 (30.4–35.8)	30.4 (28.2–32..8)	31.6 (27.7–36.1)	−.055 (.039)	.16	.27
Combined	1,235				−.067 (.026)	.01	.02
Disposition index ($pmol^2/liter^2$):							
BCG	593	1,151.7 (1,011.3–1,310.3)	1,028.6 (894.3–1,182.0)	709.8 (541.9–929.8)	−.199 (.068)	.004	.001
ALSPAC	591	849.8 (737.3–979.5)	648.1 (561.7–747.7)	639.7 (477.7–856.6)	−.184 (.072)	.01	.03
Combined	1,184				−.192 (.049)	.0001	.0001
HbA_1c (%):							
ALSPAC	1,280	4.90 (4.87–4.92)	4.90 (4.87–4.92)	4.87 (4.81–4.92)	−.002 (.002)	.41	.14
EFSOCH	1,442	5.06 (5.03–5.08)	5.07 (5.04–5.10)	5.04 (4.97–5.10)	.0001 (.003)	.97	.77
1958BC	1,813	5.20 (5.18–5.22)	5.21 (5.19–5.24)	5.25 (5.19–5.31)	.004 (.003)	.10	.07
Combined	4,535				−.0001 (.001)	.95	.35

NOTE.—All variables apart from fasting glucose were log-transformed before analysis. The per–T allele trait difference is presented as the logged value, but means and 95% CIs were back-transformed. The combined values for each trait are from fixed-effects inverse-variance meta-analysis. There was a large amount of heterogeneity among studies in the fasting insulin and HOMA-IR analyses (I^2 = 68% and 75%, respectively).

[a] Corrected for age, sex, and ln(BMI), except in ALSPAC, 1958BC, and NFBC1966, which were corrected for sex and BMI only, since subjects do not differ in age.

30 g (95% CI 15–45 g) per risk allele. This result was seen in a meta-analysis of three U.K. studies that included 8,344 subjects and was independent of any fetal genotype effect.

Studies that have analyzed birth weight in relation to the established type 2 diabetes gene variants—E23K in *KCNJ11* (MIM 600937) and Pro12Ala in *PPARG* (MIM 601487)—have found no evidence of association,[56,57] although, in the first of these studies, maternal genotype data were not reported. These variants may genuinely have no effect on birth weight. However, it is possible that, with the smaller type 2 diabetes risk conferred by these variants (odds ratio ~1.2) compared with *TCF7L2* (odds ratio ~1.4), any effect on birth weight via alterations in insulin secretion and action would be concordantly smaller and would require a sample size of >10,000 individuals for detection. Other studies have investigated the role of common variation in genes that represent good biological candidates for diabetes and related traits in relation to birth weight.[58] Positive associations with both diabetes-related traits and birth weight have been shown for the insulin gene (*INS* [MIM 176730]) variable number tandem repeats (VNTR) locus,[59–61] a microsatellite polymorphism in the insulin-like growth factor 1 gene (*IGF1* [MIM 147440]),[27,62] and a variant in the *H19* gene (MIM 103280),[63] but the former two have not been consistently replicated,[26,28–32,64] and the latter is currently the only study of this gene in relation to common birth-weight variation. Taken together, these results highlight the need for large sample sizes and replication in the study of genetic associations between diabetes genes and fetal growth.

We previously studied the −30G→A (*rs1799884*) polymorphism in the glucokinase gene in two of the studies with maternal DNA available, including the largest individual study, ALSPAC.[33,34] Presence of the A allele confers a 0.06-mmol/liter increase in fasting glucose (95% CI 0.04–0.09 mmol/liter) across all ages in the normal population and, when carried by the mother, is associated with an increase in offspring birth weight of 32 g (95% CI 11–53 g).[34] Combining information from these two confirmed common variants, we have shown that the 4% of offspring born to mothers carrying three or four risk alleles were 119 g (95% CI 62–172 g) heavier than the 32% born to mothers with no risk alleles. This effect is similar to the effect of mothers smoking 4–5 cigarettes per day in the third trimester of pregnancy.[65,66] It confirms that common genetic variation can have a substantial effect on birth weight.

To understand the mechanism that leads to the increase in offspring birth weight, we studied >10,000 subjects, which represents the largest study to date of the effect of *TCF7L2* genotypes on continuous traits related to type 2 diabetes. We found that the T allele at *rs7903146* is associated with reduced insulin secretion when correcting for insulin resistance (as measured by disposition index). This supports the findings of previous studies of nondiabetic subjects.[37,40,42,46] We also found a weaker association with increased fasting glucose (per–T allele increase 0.020 [95% CI 0.005–0.035] mmol/liter). Results of analyses of glucose concentrations from previous studies show either an increase in fasting glucose or no change in glucose.[37,42,44,46] Our data suggest that the increase in birth weight is likely to result from the risk allele reducing maternal insulin secretion, which results in an increase in the pregnant maternal glycemia to which the fetus is exposed. However, further studies in very large cohorts of pregnant women will be needed to confirm this, since most of our data come from young adults, with only 921 females pregnant at the time of study.

To conclude, we have shown that maternal *TCF7L2* type 2 diabetes–risk genotypes at *rs7903146* are associated with increased offspring birth weight, probably through impaired maternal insulin secretion. Together with the common *GCK rs1799884* variant, *TFC7L2 rs7903146* is associated with differences in normal birth weight that are comparable to those conferred by smoking, suggesting an important role for genes in this complex trait.

Acknowledgments

R.M.F. holds a Diabetes UK research studentship. A.T.H. is a Wellcome Trust Research Leave Fellow, and M.N.W. is a Vandervell Foundation Research Fellow. The majority of the work was supported by Medical Research Council grant G0500070/73468. We are extremely grateful to all the families who took part in this study and to the midwives who helped recruit them. We are also grateful to the various study teams, which include interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, nurses, receptionists, and managers. The U.K. Medical Research Council, the Wellcome Trust, and the University of Bristol provide core support for ALSPAC. The NFBC1966 study is supported by Wellcome Trust grant GR069224MA and the Academy of Finland. We acknowledge use of DNA from the 1958BC collection, funded by Medical Research Council grant G0000934 and Wellcome Trust grant 068545/Z/02. E.H. is a Department of Health (United Kingdom) Public Health Career Scientist, and research at the Institute of Child Health and Great Ormond Street Hospital for Children National Health Service (NHS) Trust benefits from research and development funding received from the NHS executive.

Web Resources

The URLs for data presented herein are as follows:

Diabetes Trials Unit, http://www.dtu.ox.ac.uk/
KBiosciences, http://www.kbioscience.co.uk/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for type 2 diabetes, *GCK, TCF7L2, KCNJ11, PPARG, INS, IGF1,* and *H19*)
Russ Lenth's Power and Sample Size, http://www.stat.uiowa.edu/~rlenth/Power/

References

1. van Baal CG, Boomsma DI (1998) Etiology of individual differences in birth weight of twins as a function of maternal smoking during pregnancy. Twin Res 1:123–130
2. Baird J, Osmond C, MacGregor A, Snieder H, Hales CN, Phil-

lips DI (2001) Testing the fetal origins hypothesis in twins: the Birmingham twin study. Diabetologia 44:33–39
3. Clausson B, Lichtenstein P, Cnattingius S (2000) Genetic influence on birthweight and gestational length determined by studies in offspring of twins. BJOG 107:375–381
4. Magnus P, Gjessing HK, Skrondal A, Skjaerven R (2001) Paternal contribution to birth weight. J Epidemiol Community Health 55:873–877
5. Farmer G, Russell G, Hamilton-Nicol DR, Ogenbede HO, Ross IS, Pearson DW, Thom H, Kerridge DF, Sutherland HW (1988) The influence of maternal glucose metabolism on fetal growth, development and morbidity in 917 singleton pregnancies in nondiabetic women. Diabetologia 31:134–141
6. Breschi MC, Seghieri G, Bartolomei G, Gironi A, Baldi S, Ferrannini E (1993) Relation of birthweight to maternal plasma glucose and insulin concentrations during normal pregnancy. Diabetologia 36:1315–1321
7. Kieffer EC, Tabaei BP, Carman WJ, Nolan GH, Guzman JR, Herman WH (2006) The influence of maternal weight and glucose tolerance on infant birthweight in Latino mother-infant pairs. Am J Public Health 96:2201–2208
8. Lindley AA, Gray RH, Herman AA, Becker S (2000) Maternal cigarette smoking during pregnancy and infant ponderal index at birth in the Swedish Medical Birth Register, 1991–1992. Am J Public Health 90:420–423
9. Lindsay RS, Kobes S, Knowler WC, Hanson RL (2002) Genome-wide linkage analysis assessing parent-of-origin effects in the inheritance of birth weight. Hum Genet 110:503–509
10. Arya R, Demerath E, Jenkinson CP, Goring HH, Puppala S, Farook V, Fowler S, Schneider J, Granato R, Resendez RG, et al (2006) A quantitative trait locus (QTL) on chromosome 6q influences birth weight in two independent family studies. Hum Mol Genet 15:1569–1579
11. Fradin D, Heath S, Lepercq J, Lathrop M, Bougneres P (2006) Identification of distinct quantitative trait loci affecting length or weight variability at birth in humans. J Clin Endocrinol Metab 91:4164–4170
12. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD (1991) Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303:1019–1022
13. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM (1993) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62–67
14. Hattersley AT, Tooke JE (1999) The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 353:1789–1792
15. Lindsay RS, Dabelea D, Roumain J, Hanson RL, Bennett PH, Knowler WC (2000) 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
16. Hypponen E, Smith GD, Power C (2003) Parental diabetes and birth weight of offspring: intergenerational cohort study. BMJ 326:19–20
17. Davey Smith G, Sterne JA, Tynelius P, Rasmussen F (2004) Birth characteristics of offspring and parental diabetes: evidence for the fetal insulin hypothesis. J Epidemiol Community Health 58:126–128
18. Wannamethee SG, Lawlor DA, Whincup PH, Walker M, Ebrahim S, Davey-Smith G (2004) Birthweight of offspring and paternal insulin resistance and paternal diabetes in late adulthood: cross sectional survey. Diabetologia 47:12–18
19. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S (1998) Mutations in the glucokinase gene of the fetus result in reduced birth weight. Nat Genet 19:268–270
20. Edghill EL, Bingham C, Slingerland AS, Minton JA, Noordam C, Ellard S, Hattersley AT (2006) Hepatocyte nuclear factor-1 beta mutations cause neonatal diabetes and intrauterine growth retardation: support for a critical role of HNF-1β in human pancreatic development. Diabet Med 23:1301–1306
21. Wright NM, Metzger DL, Borowitz SM, Clarke WL (1993) Permanent neonatal diabetes mellitus and pancreatic exocrine insufficiency resulting from congenital pancreatic agenesis. Am J Dis Child 147:607–609
22. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF (1997) Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet 15:106–110
23. Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, Howard N, Srinivasan S, Silva JM, Molnes J, et al (2004) Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 350:1838–1849
24. Slingerland AS, Hattersley AT (2006) 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
25. Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, Ellard S, Ferrer J, Hattersley AT (2007) Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. PLOS Med 4:e118
26. Lindsay RS, Hanson RL, Wiedrich C, Knowler WC, Bennett PH, Baier LJ (2003) The insulin gene variable number tandem repeat class I/III polymorphism is in linkage disequilibrium with birth weight but not type 2 diabetes in the Pima population. Diabetes 52:187–193
27. Vaessen N, Janssen JA, Heutink P, Hofman A, Lamberts SW, Oostra BA, Pols HA, van Duijn CM (2002) Association between genetic variation in the gene for insulin-like growth factor-I and low birthweight. Lancet 359:1036–1037
28. Day IN, King TH, Chen XH, Voropanov AM, Ye S, Syddall HE, Sayer AA, Cooper C, Barker DJ, Phillips DI (2002) Insulin-like growth factor-I genotype and birthweight. Lancet 360: 945 (author reply 945–946)
29. Frayling TM, Hattersley AT, McCarthy A, Holly J, Mitchell SM, Gloyn AL, Owen K, Davies D, Davey Smith G, Ben-Shlomo Y (2002) A putative functional polymorphism in the IGF-I gene: association studies with type 2 diabetes, adult height, glucose tolerance, and fetal growth in UK populations. Diabetes 51:2313–2316
30. Mitchell SM, Hattersley AT, Knight B, Turner T, Metcalf BS, Voss LD, Davies D, McCarthy A, Wilkin TJ, Davey Smith G, et al (2004) Lack of support for a role of the insulin gene variable number of tandem repeats minisatellite (INS-VNTR) locus in fetal growth or type 2 diabetes-related intermediate traits in United Kingdom populations. J Clin Endocrinol Metab 89:310–317
31. Hansen SK, Gjesing AP, Rasmussen SK, Glumer C, Urhammer SA, Andersen G, Rose CS, Drivsholm T, Torekov SK, Jensen DP, et al (2004) Large-scale studies of the HphI insulin gene variable-number-of-tandem-repeats polymorphism in rela-

tion to type 2 diabetes mellitus and insulin release. Diabetologia 47:1079–1087
32. Bennett AJ, Sovio U, Ruokonen A, Martikainen H, Pouta A, Taponen S, Hartikainen AL, King VJ, Elliott P, Jarvelin MR, et al (2004) Variation at the insulin gene VNTR (variable number tandem repeat) polymorphism and early growth: studies in a large Finnish birth cohort. Diabetes 53:2126–2131
33. Weedon MN, Frayling TM, Shields B, Knight B, Turner T, Metcalf BS, Voss L, Wilkin TJ, McCarthy A, Ben-Shlomo Y, et al (2005) Genetic regulation of birth weight and fasting glucose by a common polymorphism in the islet cell promoter of the glucokinase gene. Diabetes 54:576–581
34. Weedon MN, Clark VJ, Qian Y, Ben-Shlomo Y, Timpson N, Ebrahim S, Lawlor DA, Pembrey ME, Ring S, Wilkin TJ, et al (2006) 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
35. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, et al (2006) Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. Nat Genet 38:320–323
36. Groves CJ, Zeggini E, Minton J, Frayling TM, Weedon MN, Rayner NW, Hitman GA, Walker M, Wiltshire S, Hattersley AT, et al (2006) Association analysis of 6,736 U.K. subjects provides replication and confirms *TCF7L2* as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. Diabetes 55:2640–2644
37. Damcott CM, Pollin TI, Reinhart LJ, Ott SH, Shen H, Silver KD, Mitchell BD, Shuldiner AR (2006) Polymorphisms in the transcription factor 7-like 2 (*TCF7L2*) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. Diabetes 55:2654–2659
38. Scott LJ, Bonnycastle LL, Willer CJ, Sprau AG, Jackson AU, Narisu N, Duren WL, Chines PS, Stringham HM, Erdos MR, et al (2006) Association of transcription factor 7-like 2 (*TCF7L2*) variants with type 2 diabetes in a Finnish sample. Diabetes 55:2649–2653
39. Cauchi S, Meyre D, Dina C, Choquet H, Samson C, Gallina S, Balkau B, Charpentier G, Pattou F, Stetsyuk V, et al (2006) Transcription factor *TCF7L2* genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. Diabetes 55: 2903–2908
40. Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D (2006) *TCF7L2* polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 355:241–250
41. Zhang C, Qi L, Hunter DJ, Meigs JB, Manson JE, van Dam RM, Hu FB (2006) Variant of transcription factor 7-like 2 (*TCF7L2*) gene and the risk of type 2 diabetes in large cohorts of U.S. women and men. Diabetes 55:2645–2648
42. Saxena R, Gianniny L, Burtt NP, Lyssenko V, Giuducci C, Sjogren M, Florez JC, Almgren P, Isomaa B, Orho-Melander M, et al (2006) Common single nucleotide polymorphisms in *TCF7L2* are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. Diabetes 55:2890–2895
43. van Vliet-Ostaptchouk JV, Shiri-Sverdlov R, Zhernakova A, Strengman E, van Haeften TW, Hofker MH, Wijmenga C (2007) Association of variants of transcription factor 7-like 2 (*TCF7L2*) with susceptibility to type 2 diabetes in the Dutch Breda cohort. Diabetologia 50:59–62
44. Chandak GR, Janipalli CS, Bhaskar S, Kulkarni SR, Mohankrishna P, Hattersley AT, Frayling TM, Yajnik CS (2007) Common variants in the *TCF7L2* gene are strongly associated with type 2 diabetes mellitus in the Indian population. Diabetologia 50:63–67
45. Helgason A, Palsson S, Thorleifsson G, Grant SF, Emilsson V, Gunnarsdottir S, Adeyemo A, Chen Y, Chen G, Reynisdottir I, et al (2007) Refining the impact of *TCF7L2* gene variants on type 2 diabetes and adaptive evolution. Nat Genet 39: 218–225
46. Munoz J, Lok KH, Gower BA, Fernandez JR, Hunter GR, Lara-Castro C, De Luca M, Garvey WT (2006) Polymorphism in the transcription factor 7-like 2 (*TCF7L2*) gene is associated with reduced insulin secretion in nondiabetic women. Diabetes 55:3630–3634
47. Knight B, Shields BM, Hattersley AT (2006) The Exeter Family Study of Childhood Health (EFSOCH): study protocol and methodology. Paediatr Perinat Epidemiol 20:172–179
48. Chase DS, Tawn EJ, Parker L, Jonas P, Parker CO, Burn J (1998) The North Cumbria Community Genetics Project. J Med Genet 35:413–416
49. Power C, Elliott J (2006) Cohort profile: 1958 British Birth Cohort (National Child Development Study). Int J Epidemiol 35:34–41
50. Golding J, Pembrey M, Jones R (2001) ALSPAC—the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol 15:74–87
51. Rantakallio P (1988) The longitudinal study of the northern Finland birth cohort of 1966. Paediatr Perinat Epidemiol 2: 59–88
52. Jarvelin MR, Sovio U, King V, Lauren L, Xu B, McCarthy MI, Hartikainen AL, Laitinen J, Zitting P, Rantakallio P, et al (2004) Early life factors and blood pressure at age 31 years in the 1966 Northern Finland Birth Cohort. Hypertension 44:838–846
53. Bennett A, Sovio U, Ruokonen A, Martikainen H, Pouta A, Taponen S, Hartikainen AL, Franks S, Peltonen L, Elliott P, et al (2005) No association between insulin gene variation and adult metabolic phenotypes in a large Finnish birth cohort. Diabetologia 48:886–891
54. Wareham NJ, Phillips DI, Byrne CD, Hales CN (1995) The 30 minute insulin incremental response in an oral glucose tolerance test as a measure of insulin secretion. Diabet Med 12: 931
55. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327:557–560
56. Weedon MN, Gloyn AL, Frayling TM, Hattersley AT, Davey Smith G, Ben-Shlomo Y (2003) Quantitative traits associated with the type 2 diabetes susceptibility allele in Kir6.2. Diabetologia 46:1021–1023
57. Pfab T, Poralla C, Richter CM, Godes M, Slowinski T, Priem F, Halle H, Hocher B (2006) Fetal and maternal peroxisome proliferator-activated receptor γ2 Pro12Ala does not influence birth weight. Obesity (Silver Spring) 14:1880–1885
58. Rasmussen SK, Urhammer SA, Hansen T, Almind K, Moller AM, Borch-Johnsen K, Pedersen O (2000) Variability of the insulin receptor substrate-1, hepatocyte nuclear factor-1α (HNF-1α), HNF-4α, and HNF-6 genes and size at birth in a

population-based sample of young Danish subjects. J Clin Endocrinol Metab 85:2951–2953
59. Dunger DB, Ong KK, Huxtable SJ, Sherriff A, Woods KA, Ahmed ML, Golding J, Pembrey ME, Ring S, Bennett ST, et al (1998) Association of the INS VNTR with size at birth: ALSPAC Study Team. Nat Genet 19:98–100
60. Ong KK, Phillips DI, Fall C, Poulton J, Bennett ST, Golding J, Todd JA, Dunger DB (1999) The insulin gene VNTR, type 2 diabetes and birth weight. Nat Genet 21:262–263
61. Le Stunff C, Fallin D, Schork NJ, Bougneres P (2000) The insulin gene VNTR is associated with fasting insulin levels and development of juvenile obesity. Nat Genet 26:444–446
62. Vaessen N, Heutink P, Janssen JA, Witteman JC, Testers L, Hofman A, Lamberts SW, Oostra BA, Pols HA, van Duijn CM (2001) A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. Diabetes 50:637–642
63. Petry CJ, Ong KK, Barratt BJ, Wingate D, Cordell HJ, Ring SM, Pembrey ME, Reik W, Todd JA, Dunger DB (2005) Common polymorphism in H19 associated with birthweight and cord blood IGF-II levels in humans. BMC Genet 6:22
64. Ong KK, Petry CJ, Barratt BJ, Ring S, Cordell HJ, Wingate DL, Pembrey ME, Todd JA, Dunger DB (2004) Maternal-fetal interactions and birth order influence insulin variable number of tandem repeats allele class associations with head size at birth and childhood weight gain. Diabetes 53:1128–1133
65. Bernstein IM, Mongeon JA, Badger GJ, Solomon L, Heil SH, Higgins ST (2005) Maternal smoking and its association with birth weight. Obstet Gynecol 106:986–991
66. Jarvelin MR, Elliott P, Kleinschmidt I, Martuzzi M, Grundy C, Hartikainen AL, Rantakallio P (1997) Ecological and individual predictors of birthweight in a northern Finland birth cohort 1986. Paediatr Perinat Epidemiol 11:298–312