nature genetics

Variants in *ADCY5* and near *CCNL1* are associated with fetal growth and birth weight

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To identify genetic variants associated with birth weight, we meta-analyzed six genome-wide association (GWA) studies (n = 10,623 Europeans from pregnancy/birth cohorts) and followed up two lead signals in 13 replication studies (n = 27,591). rs900400 near *LEKR1* and *CCNL1* ($P = 2 \times 10^{-35}$) and rs9883204 in *ADCY5* ($P = 7 \times 10^{-15}$) were robustly associated with birth weight. Correlated SNPs in *ADCY5* were recently implicated in regulation of glucose levels and susceptibility to type 2 diabetes[1], providing evidence that the well-described association between lower birth weight and subsequent type 2 diabetes[2,3] has a genetic component, distinct from the proposed role of programming by maternal nutrition. Using data from both SNPs, we found that the 9% of Europeans carrying four birth weight–lowering alleles were, on average, 113 g (95% CI 89–137 g) lighter at birth than the 24% with zero or one alleles ($P_{trend} = 7 \times 10^{-30}$). The impact on birth weight is similar to that of a mother smoking 4–5 cigarettes per day in the third trimester of pregnancy[4].

The extremes of birth weight are associated with high risks of perinatal morbidity and mortality[5,6]. In addition, there are well-documented observational associations between lower birth weight and later-life chronic disease, including type 2 diabetes, cardiovascular disease and higher blood pressure[2,3]. The mechanisms underlying these associations are poorly understood. Birth weight is a complex multifactorial trait[7,8]. The importance of genetic factors acting independently of the intrauterine environment is illustrated by correlations between paternal height or weight and offspring birth weight[7,9,10], and genetic variants that are associated both with low birth weight and with increased risk of type 2 diabetes may account for some of the observed correlation between these phenotypes[11–13]. However, the genetic loci that influence birth weight are largely unknown.

Birth weight may be influenced directly by fetal genotype and also indirectly by maternal genotype operating through the intrauterine environment. This is clearly illustrated by observations of mothers and offspring with rare, heterozygous mutations in *GCK*, the gene encoding glucokinase. By reducing insulin secretion, these mutations

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Received 21 October 2009; accepted 17 March 2010; published online 6 April 2010; doi:10.1038/ng.567

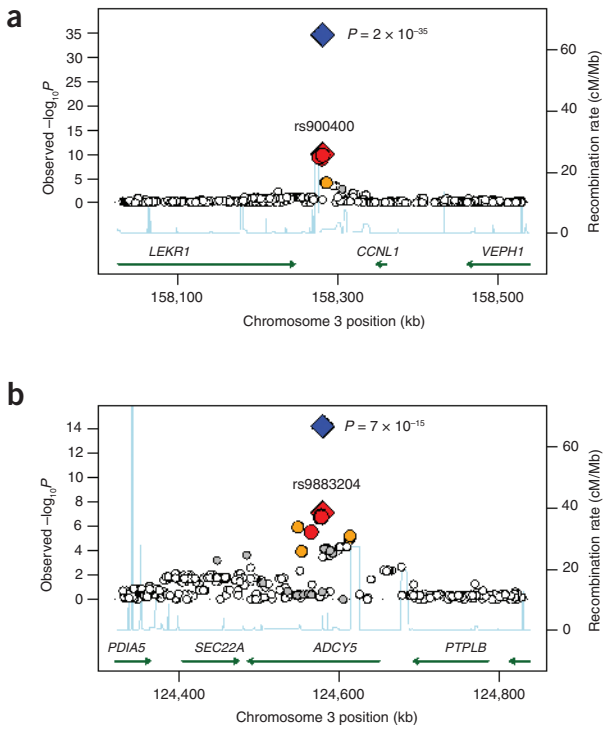

Figure 1 Regional plots of two previously unknown associations with birth weight. (**a**,**b**) For each of the two regions, 3q25 (**a**) and 3q21 (**b**), directly genotyped and imputed SNPs are plotted using filled circles with their meta-analysis *P* values (as $-\log_{10}$ values) as a function of genomic position (NCBI Build 35). In each plot, the discovery-stage SNP taken forward to replication stage is represented by a blue diamond (defining a global meta-analysis *P* value), with its discovery meta-analysis *P* value denoted by a red diamond. Local LD structure is reflected by the plotted estimated recombination rates (taken from HapMap) in the region around the associated SNPs and their correlated proxies. Each analyzed SNP is represented by a circle. The color scheme of the circles respects LD patterns (HapMap CEU pairwise r^2 correlation coefficients) between top discovery SNP and surrounding variants: white, $r^2 < 0.2$; gray, $0.5 > r^2 \geq 0.2$; orange, $0.8 > r^2 \geq 0.5$; red, $r^2 \geq 0.8$. Gene annotations were taken from the University of California Santa Cruz genome browser.

increase offspring birth weight by 600 g when carried by the mother and reduce birth weight by 530 g when inherited by the fetus[14].

To search for common genetic variants associated with birth weight, we performed a meta-analysis of GWA studies. We reasoned that finding such variants, even those with modest effects, would lead to enhanced understanding of pathways important for fetal growth and those underlying the associations between fetal growth and adult disease.

We meta-analyzed association statistics from 2,427,548 directly genotyped and imputed SNPs in singletons of European descent from six discovery GWA studies (n = 10,623; **Supplementary Table 1**). Birth weight was standardized to *z* scores within each study and adjusted for sex and gestational age. We observed SNPs at two independent loci on chromosome 3 that were associated with birth weight at, or close to, genome-wide significance ($P < 5 \times 10^{-8}$; **Supplementary Fig. 1**). The first locus was at 3q25, between *CCNL1* and *LEKR1*, and the second at 3q21 in *ADCY5* (**Fig. 1**). To replicate these associations, we genotyped the most strongly associated SNP from each locus (rs900400 from 3q25; rs9883204 from 3q21), or a closely correlated proxy (HapMap r^2 = 0.93–0.96), in 13 further samples of individuals of European descent (n = 27,591; **Supplementary Table 2**). Robust evidence of association was seen for both signals in these replication samples (**Fig. 2**; $P = 3 \times 10^{-26}$ and 3×10^{-9}, respectively). Combining all discovery and replication samples, each additional C allele of SNP rs900400 (frequency 32–47%) was associated with a 0.086-s.d. lower birth weight (95% CI 0.073–0.100; $P = 2 \times 10^{-35}$), whereas each C allele of SNP rs9883204 (frequency 71–83%) was associated with a 0.063-s.d. lower birth weight (95% CI 0.047–0.079; $P = 7 \times 10^{-15}$; **Table 1**). These effect sizes equate to approximate differences of 40 g and 30 g per allele, respectively (median study s.d. = 484 g). Analysis conditional on the index SNPs, rs900400 and rs9883204 did not produce any evidence for additional independent signals at either locus.

We found no evidence of heterogeneity between the studies examined ($P > 0.5$; $I^2 = 0\%$)[15], despite differences in the distribution of birth weight over time and between populations (**Table 2**), and the associations with birth weight were similar in males and females ($P > 0.05$ for difference in effect sizes). Gestational age was not available as a covariate in three of our replication studies (combined n = 6,235; **Supplementary Table 2**), but these studies did not introduce detectable heterogeneity, and their removal from the meta-analysis changed the results very little (**Fig. 2** and **Table 1** footnote). We also assessed the effects of the two SNPs on birth weight in a limited number of non-European or admixed samples from two studies (n = 1,415 Filipino subjects from the Cebu Longitudinal Health and Nutrition Survey, and n = 298–448 African-descended, Moroccan and Turkish subjects from Generation R; **Supplementary Tables 2** and **3**). There was no difference in the effect sizes observed relative to the European studies ($P > 0.5$), but the power to detect association was limited. Further well-powered studies will be needed to investigate these associations in non-Europeans.

Table 1 Associations between newly identified birth-weight loci and anthropometric traits at birth

Phenotype	Locus 3q25[a]				Locus 3q21[b]			
	n	Effect	95% CI	*P*[c]	*n*	Effect	95% CI	*P*[c]
Birth-weight *z* score[d]	37,745	–0.086	–0.100 to –0.073	2×10^{-35}	38,214	–0.063	–0.079 to –0.047	7×10^{-15}
Birth-length *z* score	21,512	–0.028	–0.046 to –0.010	0.002	21,782	–0.044	–0.066 to –0.022	4×10^{-5}
Birth head-circumference *z* score	17,349	–0.024	–0.044 to –0.004	0.017	17,693	–0.025	–0.048 to –0.004	0.030
Ponderal index[e] *z* score	21,515	–0.094	–0.113 to –0.074	5×10^{-21}	21,785	–0.032	–0.055 to –0.009	0.006

Results are from inverse variance, fixed-effects meta-analysis of all 19 study samples of European ancestry. The effect allele for each SNP is labeled on the positive strand according to HapMap. The effect is the change in phenotype *z* score per allele from linear regression, adjusted for sex and gestational age, assuming an additive genetic model. If the index SNP was unavailable, this was substituted with a closely correlated (HapMap $r^2 > 0.9$) proxy (rs1482853 or rs900399 for rs900400; rs2877716 or rs6798189 for rs9883204). There was no evidence of between-study heterogeneity of effect-size estimates (all $P > 0.18$; $I^2 < 26\%$).

[a]Index SNP rs900400, effect allele C (40% frequency in HapMap CEU; range 32–47% in our European study samples); nearest genes to the 3q25 signal are *CCNL1* and *LEKR1*. [b]Index SNP rs9883204, effect allele C (73% frequency in HapMap CEU; range 71–83% in our European study samples); nearest gene to the 3q21 signal is *ADCY5*. [c]The *P* value for the birth-weight meta-analysis includes the double–genomic control correction of the discovery meta-analysis. [d]Excluding the three studies that were unable to adjust for gestational age, the β (s.e.m.), *n* and *P* values in the birth-weight analysis were –0.089 (0.008), n = 31,510, $P = 7 \times 10^{-32}$ (3q25); –0.068 (0.009), n = 31,901, $P = 8 \times 10^{-15}$ (3q21). [e]Ponderal index = birth weight/length^3.

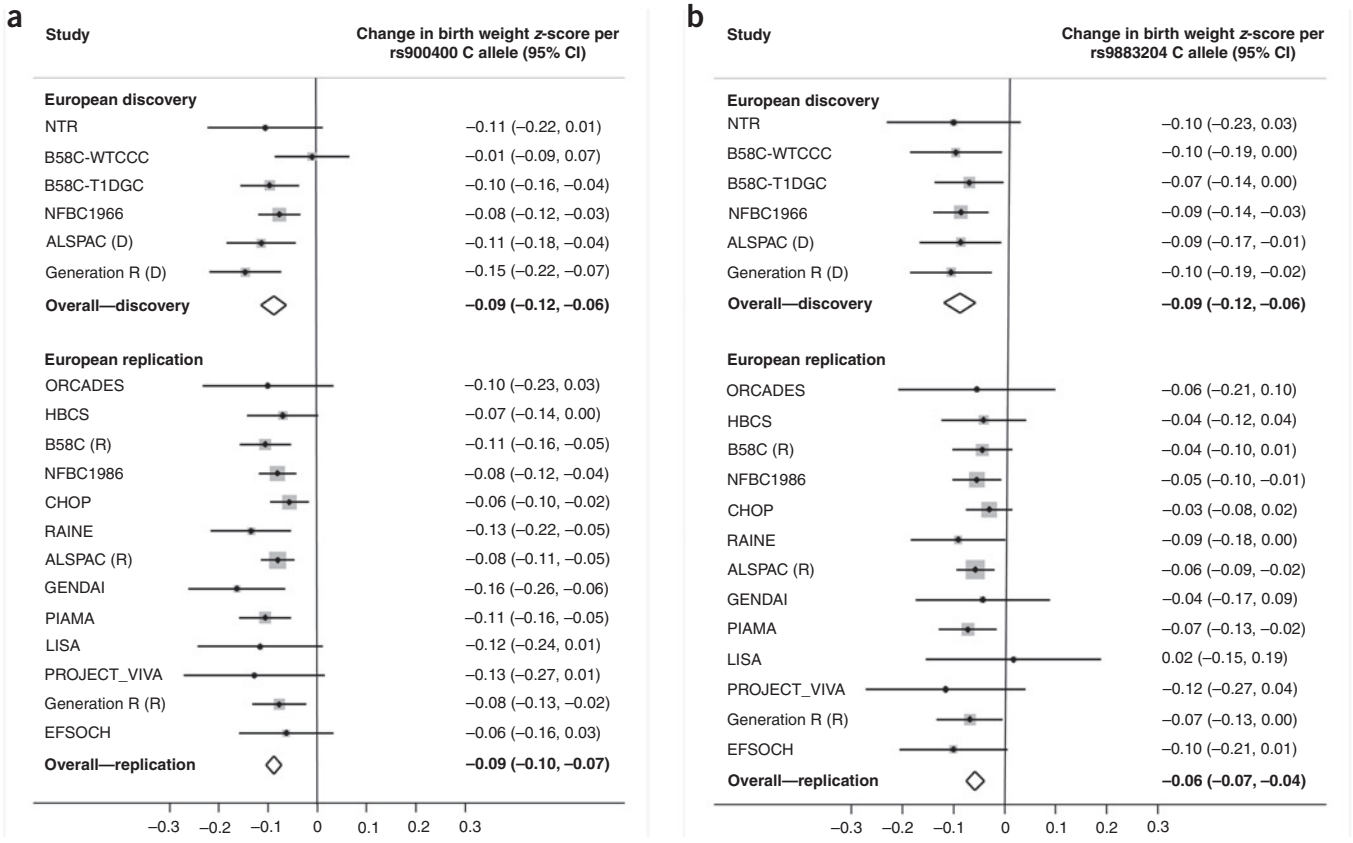

Figure 2 Forest plots of the association between birth weight and genotype at each locus. (**a**,**b**) Index SNP rs900400 at 3q25 (**a**) and index SNP rs9883204 at 3q21 (**b**). If the index SNP was unavailable, a closely correlated proxy (HapMap $r^2 > 0.9$) was used.

Maternal and fetal genotypes are correlated as a result of segregation. In a previous study, an observed association between fetal *TCF7L2* genotype and birth weight was driven by the effects of maternal *TCF7L2* variation on the intrauterine environment rather than by a direct effect on fetal growth[16]. To distinguish between these two mechanisms, we tested whether the newly identified birth-weight associations were independent of maternal genotype. We genotyped both SNPs in all available maternal DNAs ($n = 9{,}127$; 5 study samples). Meta-analysis of associations between birth weight and fetal genotype, conditional on maternal genotype, yielded results that were very similar to the original associations at both loci (**Supplementary Table 4**), showing that these are direct fetal effects. As expected, there was no association between fetal genotype and various covariates of birth weight that were not included in our main analysis (maternal smoking, body mass index (BMI), parity, education, age at delivery; all $P > 0.05$; data not shown).

Birth weight may be influenced by skeletal growth or fat mass. In available samples, we analyzed the association between each locus and birth length, birth head circumference and ponderal index (**Table 1** and **Supplementary Figs. 2–4**). The association with ponderal index, relative to those with birth length and head circumference, was particularly strong for the rs900400 SNP (0.094 s.d. (95% CI 0.074–0.113) per C allele; $P = 5 \times 10^{-21}$), suggesting that there is a greater association with fat mass than with skeletal growth. For the rs9883204 SNP, the measures showed more proportionate changes (**Table 1**). We investigated associations with adult height and BMI using published GWA meta-analyses from the GIANT consortium[17,18]. Only the rs900400 signal was captured in the published height data at $r^2 > 0.8$ (because that study included direct genotypes from the Affymetrix Genechip 500k only), and we found no association ($P = 0.64$; $n = 9{,}818$). There was no association with adult BMI for either locus ($n \approx 32{,}500$, $P > 0.1$). This is consistent with the weak observational association between birth weight and adult BMI[19], indicating that they are largely governed by different processes.

Although birth weight is a continuous trait, standard clinical cutoffs are used to identify neonates who are small for gestational age and who may require further observation. We therefore assessed whether each SNP increased the odds of gestational age-adjusted birth weight $<10^{th}$ percentile. Both loci were associated with smallness for gestational age: odds ratios (OR) 1.16 (95% CI 1.10–1.23) ($n = 30{,}370$; $P = 1 \times 10^{-7}$) and 1.09 (1.02–1.16) ($n = 30{,}778$; $P = 0.009$) per C allele of rs900400 and rs9883204, respectively (**Supplementary Fig. 5**).

The birth-weight signal marked by rs900400 maps approximately 35 kb 3′ to the *LEKR1* locus (encoding leucine, glutamate and lysine rich 1) and 67 kb 3′ to *CCNL1* (encoding cyclin L1). Neither gene has previously been implicated in fetal growth. The CCNL1 protein may be involved in pre-mRNA splicing and RNA processing and associates with cyclin-dependent kinases[20]. A noncoding RNA of unknown function, 682 bp from rs900400 (AK311218, Human March 2006 Assembly 18), overlaps with the signal. We found no evidence for association at a genome-wide level ($P > 5 \times 10^{-8}$) between our 3q25 birth-weight signal and mRNA expression in lymphocytes, using the publicly available 'mRNA by SNP Browser 1.0'[21], and there was no association between rs900400 or rs900399 and type 2 diabetes or related adult glycemic traits in the recent GWA meta-analysis from the MAGIC consortium ($P > 0.1$)[1]. A range of approaches (including re-sequencing and functional studies) will be required to establish which gene—*CCNL1*, *LEKR1* or another transcript—mediates the effect on fetal growth.

Table 2 Mean birth weight (SD) by genotype and individual association results by study

Study type	Study	Year(s) of birth	Total n^a	% male	Locus 3q25[b] TT Mean BW[c] (s.d.)	Locus 3q25[b] CT Mean BW[c] (s.d.)	Locus 3q25[b] CC Mean BW[c] (s.d.)	Locus 3q25[b] *P* value[d]	Locus 3q21[b] TT Mean BW[c] (s.d.)	Locus 3q21[b] CT Mean BW[c] (s.d.)	Locus 3q21[b] CC Mean BW[c] (s.d.)	Locus 3q21[b] *P* value[d]
Discovery	NTR	1923–86	414	37.9	3,470 (652)	3,401 (615)	3,329 (646)	0.08	3,500 (720)	3,402 (604)	3,359 (633)	0.09
	B58C-WTCCC	1958	1,227	50.4	3,367 (444)	3,337 (455)	3,364 (454)	0.77	3,459 (457)	3,357 (456)	3,336 (455)	0.05
	B58C-T1DGC	1958	2,037	49.2	3,399 (468)	3,339 (464)	3,308 (461)	1×10^{-3}	3,396 (463)	3,375 (484)	3,341 (463)	0.07
	NFBC1966	1966	4,333	48.1	3,567 (458)	3,519 (458)	3,503 (458)	5×10^{-4}	3,630 (459)	3,559 (459)	3,529 (459)	4×10^{-3}
	ALSPAC (D)	1991–2	1,418	48.8	3,486 (481)	3,419 (482)	3,374 (467)	2×10^{-3}	3,451 (458)	3,462 (465)	3,405 (514)	0.03
	Generation R (D)	2002–6	1,194	53.1	3,633 (435)	3,562 (447)	3,492 (448)	1×10^{-4}	3,655 (449)	3,593 (444)	3,549 (456)	0.01
Replication	ORCADES	1920–88	328	43.3	3,635 (599)	3,615 (594)	3,487 (602)	0.12	3,542 (612)	3,670 (605)	3,566 (595)	0.74
	HBCS	1934–44	1,566	42.7	3,462 (436)	3,434 (438)	3,403 (430)	0.06	3,391 (426)	3,479 (434)	3,431 (418)	0.33
	B58C (R)	1958	2,550	51.6	3,407 (454)	3,341 (451)	3,308 (456)	7×10^{-5}	3,338 (457)	3,387 (448)	3,340 (477)	0.14
	NFBC1986	1985-6	5,008	49.1	3,656 (440)	3,607 (440)	3,591 (440)	4×10^{-5}	3,674 (441)	3,646 (441)	3,620 (441)	0.03
	CHOP	1987–2009	5,149	53.3	3,384 (634)	3,333 (646)	3,318 (628)	5×10^{-3}	3,389 (641)	3,357 (647)	3,341 (609)	0.19
	RAINE	1989–92	988	52.4	3,507 (428)	3,432 (417)	3,384 (429)	1×10^{-3}	3,472 (426)	3,489 (431)	3,427 (425)	0.06
	ALSPAC (R)	1991–2	5,695	54.6	3,303 (547)	3,259 (568)	3,229 (493)	3×10^{-6}	3,305 (464)	3,288 (580)	3,257 (626)	3×10^{-3}
	GENDAI	1994–6	758	45.5	3,401 (530)	3,215 (528)	3,235 (529)	1×10^{-3}	3,291 (539)	3,286 (539)	3,260 (539)	0.53
	PIAMA	1996–7	1,789	51.3	3,629 (438)	3,575 (443)	3,512 (427)	9×10^{-5}	3,619 (441)	3,607 (425)	3,554 (430)	0.01
	LISA	1998–9	387	56.9	3,476 (366)	3,454 (363)	3,368 (363)	0.07	3,532 (365)	3,429 (366)	3,443 (367)	0.84
	PROJECT VIVA	1999–2003	300	50.0	3,711 (406)	3,646 (411)	3,594 (407)	0.08	3,698 (412)	3,703 (402)	3,625 (408)	0.15
	Generation R (R)	2002–6	1,885	50.3	3,558 (435)	3,527 (423)	3,481 (413)	6×10^{-3}	3,615 (433)	3,534 (435)	3,518 (430)	0.04
	EFSOCH	2003–4	719	53.1	3,556 (427)	3,509 (432)	3,504 (431)	0.20	3,660 (433)	3,513 (435)	3,503 (432)	0.07

BW, birth weight. All birth-weight values are adjusted for sex and, where available, gestational age.

[a]Study *n* in the birth-weight association analysis for rs900400 genotype. Total numbers of European discovery and replication samples, respectively, were $n = 10{,}623$ and $n = 27{,}122$ for rs900400; $n = 10{,}623$ and $n = 27{,}591$ for rs9883204. [b]If the index SNP was unavailable, this was substituted with a closely correlated (HapMap $r^2 > 0.9$) proxy (rs1482853 or rs900399 for rs900400 at 3q25; rs2877716 or rs6798189 for rs9883204 at 3q21); nearest genes to the 3q25 signal are *CCNL1* and *LEKR*; nearest gene to the 3q21 signal is *ADCY5*. [c]Mean BW in grams. [d]*P* value is from linear regression of birth-weight *z* score against SNP (additive model), with sex and gestational age, where available, as covariates. Gestational age was not available for the ORCADES, CHOP and GENDAI studies. All study samples comprised individuals of European descent. See **Supplementary Note** for description of study cohorts

The second birth-weight locus at 3q21 (index SNP rs9883204) maps within *ADCY5*, encoding adenylyl cyclase 5. ADCY5 belongs to the family of enzymes responsible for the synthesis of cyclic adenosine monophosphate (cAMP)[22–24]. Allele A of rs11708067, which is in linkage disequilibrium (LD) with the birth weight–lowering C allele of rs9883204 ($r^2 = 0.75$), is associated with a higher risk of type 2 diabetes (OR 1.12 (95% CI 1.04–1.15); $P = 9.9 \times 10^{-21}$; 40,655 cases/87,022 controls), higher fasting glucose (0.027 mmol/l (95% CI 0.021–0.033); $P = 7.1 \times 10^{-22}$; $n = 118{,}475$) and reduced values of the Homeostatic Model Assessment (HOMA)-derived indices of beta-cell function (HOMA-B; $P = 7.1 \times 10^{-12}$; $n = 94{,}212$)[1], suggesting that it may influence insulin secretion. Fetal insulin is a key fetal growth factor, and these metabolic associations suggest that one mechanism explaining the *ADCY5* association with birth weight might be a direct effect of the fetal risk allele on fetal growth via reduced insulin secretion, consistent with the fetal insulin hypothesis[11].

However, our previous studies suggest that an association between fetal genotype and birth weight is not characteristic of all type 2 diabetes–associated loci. For example, susceptibility variants at *CDKN2A–CDKN2B*, *IGF2BP2* and *SLC30A8* and at *TCF7L2* were not associated with birth weight in previous studies of $n > 15{,}000$, after adjusting for maternal genotype[12,16]. To test this more comprehensively, we examined the associations between birth weight and all published type 2 diabetes–associated ($n = 24$) and fasting glucose–associated ($n = 16$) loci in our discovery GWA meta-analysis ($n = 10{,}623$)[1,25,26]. Only *ADCY5* and *CDKAL1* variants (the latter in line with previous reports[12,13]) showed evidence of association at $P < 0.01$ (**Supplementary Table 5**). One explanation for the variable effects of different type 2 diabetes susceptibility loci on birth weight is that they may influence beta-cell function at different points of the life course, with *ADCY5* having a more marked effect *in utero* than the other loci. However, other mechanisms could be partially or wholly responsible for the association of *ADCY5* with birth weight, including the regulation of placental glucose transporter expression[27], vitamin B_2 uptake in the placenta[28], and the architecture and permeability of the materno-fetal placental barrier[29].

The associations at 3q25 and 3q21 explained 0.3% and 0.1% of the variance in birth weight, respectively. Given that estimates of the fetal genetic contribution to birth weight from twin and family studies are generally between 10% and 40%[30,31], the proportion of heritability explained may be up to ten times greater. The variance explained by the first locus is comparable to that of maternal age (0.5%). We used a weighted risk allele score to estimate the differences in birth weight attributable to combinations of birth weight–lowering alleles at both loci. The 9% of Europeans with four birth weight–lowering alleles were, on average, 113 g (95% CI 89–137 g) lighter at birth than the 24% with zero or one allele (P for trend $= 7 \times 10^{-30}$). For comparison, this effect on birth weight is similar to the impact of a mother smoking 4–5 cigarettes per day[4] and is approximately one-third of the impact of the severe malnutrition of the Dutch Famine of 1944–1945, during which pregnant women consumed, on average, <1,000 calories per day[32].

To conclude, we have identified previously unknown, robust associations between fetal genotype and birth weight at loci near *CCNL1* and at *ADCY5*. The causal mechanisms are not yet known, but the *ADCY5* locus has pleiotropic effects on glucose regulation and type 2 diabetes in adulthood. This is robust evidence that the widely described association between lower birth weight and subsequent type 2 diabetes has a genetic component, distinct from the proposed role of programming

by maternal nutrition. Further understanding of these associations will illuminate the biological pathways important for fetal growth and its relationship with adult diseases.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

See also **Supplementary Note** for detailed acknowledgments by study.

The authors of this manuscript would like to acknowledge the particular role of Leena Peltonen-Palotie in the research described in this manuscript, and to express our sadness at her untimely loss. Leena made a unique contribution to the field of human genetics research, and enriched the professional and social lives of all who worked with her. She will be sorely missed.

Major funding for the research in this paper is as follows: Academy of Finland (project grants 104781, 120315, 209072, 129255 and Center of Excellence in Complex Disease Genetics); Biocentrum Helsinki; Biocenter, University of Oulu, Finland; British Heart Foundation; Canadian Institutes of Health Research (grant MOP 82893); Center for Medical Systems Biology; Centre for Neurogenomics and Cognitive Research (CNCR-VU) (grant EU/QLRT-2001-01254); The Chief Scientist Office of the Scottish Government; The Children's Hospital of Philadelphia (Institute Development Award); Coca-Cola Hellas; Cotswold Foundation (Research Development Award); Darlington Trust; Department of Health's National Institute of Health Research UK; Diabetes UK (grant RD08/0003704); Dutch Asthma Foundation; Dutch Ministry of the Environment; Erasmus Medical Center Rotterdam; Erasmus University Rotterdam; European Commission (EURO-BLCS, Framework 5 award QLG1-CT-2000-01643); The European Community's Seventh Framework Programme (FP7/2007-2013), ENGAGE project, grant agreement HEALTH-F4-2007- 201413; The European Union Framework Program 6 EUROSPAN Project (LSHG-CT-2006-018947); Exeter National Health Service Research and Development; Friedrich-Schiller University Jena; Genetic Association Information Network; Healthway Western Australia; Helmholtz Zentrum Muenchen–German Research Center for Environment and Health; Institute of Epidemiology Neuherberg; Institut für Umweltmedizinische Forschung (IUF) Düsseldorf; Juvenile Diabetes Research Foundation International; Kompetenznetz Adipositas (Competence Network Obesity) funded by the German Federal Ministry of Education and Research (FKZ: 01GI0826); Marien-Hospital Wesel; MRC UK (grants G0601261, G0600705, studentship grant G0500539, G0000934, G0601653); Munich Center of Health Sciences (MCHEALTH); Municipal Health Service Rotterdam; National Health and Medical Research Council of Australia (grant 572613); National Human Genome Research Institute (US); National Institute of Allergy and Infectious Diseases (US); National Institute of Child Health and Human Development (US) (HD056465, HD034568, HD05450); National Institute of Diabetes and Digestive and Kidney Diseases (US) (DK075787, DK078150, DK56350); National Institute for Environmental Health Sciences (US) (ES10126); National Institute of Mental Health (US) (MH083268, MH63706); National Heart, Lung, and Blood Institute (US) (HL0876792 (STAMPEED program), HL085144, HL068041); National Institutes of Health (US) (Fogarty International Center Grant TW05596; National Center for Research Resources RR20649); National Public Health Institute, Helsinki, Finland; Netherlands Organisation for Scientific Research (NWO)/ Netherlands Organisation for Health Research and Development (ZonMw) (grants SPI 56-464-14192, 904-61-090, 904-61-193, 480-04-004, 400-05-717); Office of Population Studies Foundation, University of San Carlos, Philippines; Peninsula NIHR Clinical Research Facility (UK); Raine Medical Research Foundation; Rotterdam Homecare Foundation; South West National Health Service Research and Development (UK) Spinoza; St. Georg Hospital Leipzig; Stichting Astmabestrijding; Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR) Rotterdam; Technical University Munich; Telethon Institute for Child Health Research; Type 1 Diabetes Genetics Consortium; UFZ–Centre for Environmental Research Leipzig-Halle; University Hospital Oulu Biocenter, University of Oulu, Finland; University of Bristol; University of Leipzig; Wellcome Trust (grants 085301, 068545/Z/02, 076113/B/04/Z); Western Australian DNA Bank; Western Australian Genetic Epidemiology Resource; and the Wind Over Water Foundation.

Data exchange for the meta-analyses was facilitated by the SIMBioMS platform (http://simbioms.org).

Personal funding is as follows: R.M.F. by a Sir Henry Wellcome Postdoctoral Fellowship (Wellcome Trust grant 085541/Z/08/Z); E.W. by the Academy of Finland (grant 120315 and 129287); H.N.L. by US National Institutes of Health grant 1R01DK075787; E.H. by the Career Scientist Award, Department of Health, UK; C.M.L. by a Wellcome Trust Research Career Development Fellowship; A.R. by the UK Department of Health Policy Research Programme; B.M.S., B.A.K. and A.T.H. are employed as core members of the Peninsula NIHR Clinical Research Facility; J.F.W. by The Royal Society; L.P. by the Wellcome Trust (grant 89061/Z/09/Z); and V.W.V.J. by the Netherlands Organization for Health Research (ZonMw 90700303).

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Stage 1: GWA meta-analysis of birth weight; discovery samples, genotyping and imputation. We selected six population-based European studies with birth weight, gestational age and GWA data available by the beginning of May 2009 (combined $n = 10,623$): the Northern Finland 1966 Birth Cohort (NFBC1966; $n = 4,333$); Netherlands Twin Register (NTR; $n = 414$; singletons only); and subsamples from the 1958 British Birth Cohort (B58C-WTCCC, $n = 1,227$; B58C-T1DGC, $n = 2,037$), Generation R ($n = 1,194$) and Avon Longitudinal Study of Parents And Children (ALSPAC; $n = 1,418$). The B58C-WTCCC and B58C-T1DGC were analyzed separately because they were genotyped on different platforms at different times. However, there is no systematic phenotypic difference between these subsamples. Genotypes were obtained using high-density SNP arrays and then imputed for ~2.4 million HapMap SNPs (Phase II, release 21/22; see URLs). The basic characteristics, exclusions (for example, individuals of non-European ancestry), genotyping, quality control and imputation methods for each discovery sample are presented in **Supplementary Table 1**.

Statistical analysis within discovery samples. Multiple and preterm births (gestational age <37 weeks) were excluded from all analyses. Birth weight (BW) was transformed into a *z* score ([BW value – mean BW]/s.d. BW) to allow comparison of the data across studies. The overall (as opposed to sex-stratified) mean and s.d. from each study were used to create *z* scores. The association between each SNP and birth weight was assessed in each study sample using linear regression of birth-weight z score against genotype (additive model), with sex and gestational age as covariates. Imputed genotypes were used only where directly assayed genotypes were unavailable. In addition to this 'UNIFORM' analysis, a second analysis ('BEST') was performed, in which the analysis details were decided within each study. Details of the BEST analysis, GWA analysis software, and any additional corrections for study-specific population structure in the UNIFORM analysis are given in **Supplementary Table 1**.

Meta-analysis of discovery samples. Data exchange was facilitated by the SIMBioMS platform (http://simbioms.org)[33]. Prior to meta-analysis, SNPs with a minor allele frequency <1% and poorly imputed SNPs (proper_info ≤0.4 (SNPTEST); $r^2 \leq 0.3$ (MACH2QTL)) were filtered. Fixed-effects meta-analyses of the UNIFORM and BEST analyses were each run in parallel in two different study centers. Each was performed using different software packages: METAL (see URLs) and MetaMapper (developed in-house at Imperial College London, UK). Genomic control[34] was applied twice at the meta-analysis stage: first, to adjust the statistics generated within each cohort (see **Supplementary Table 1** for individual study λ values); and second, to adjust the overall meta-analysis statistics (λ = 1.032). The results from the UNIFORM analysis were meta-analyzed using the inverse-variance method, whereas for the BEST analysis a *z* score–weighted method that allows for differences in the units of β coefficients and standard errors was applied[35]. SNPs available for less than half of the total expected sample were excluded. Final meta-analysis results were obtained for 2,427,548 SNPs. Those SNPs that reached a *P*-value threshold of $<10^{-7}$ in the UNIFORM analysis ($n = 10$ SNPs, representing two distinct genomic regions on chromosome 3) were considered for further followup. The UNIFORM (reported here) and BEST analyses (data not shown) gave very similar results.

Checking for independent associations at the two loci. To test for the presence of additional association signals around the most strongly associated SNP in each region (rs900400 and rs9883204), we re-ran the UNIFORM association analysis on chromosome 3 in each discovery sample, including rs900400 and rs9883204 genotypes as additional covariates. Where these SNPs were imputed, genotype dosage was calculated from the genotype probabilities and used in the model. We meta-analyzed results using the inverse-variance method.

Stage 2: followup of two lead signals in additional samples; followup samples, genotyping and analysis. We used 17 study samples (combined $n = 30,098$) to follow up the two lead signals from the GWA meta-analysis (represented by index SNPs rs900400 and rs9883204). Details of these samples are presented in **Supplementary Table 2**. Thirteen of the samples (combined $n = 27,591$) were of European ancestry and were used for replication of the birth-weight associations. We also examined associations in four further non-European or admixed study samples (combined $n = 2,507$). Informed consent was obtained from all discovery and followup-study participants (or parental consent, as appropriate), and study protocols were approved by the local regional or institutional ethics committees. If the index SNP was unavailable, a closely correlated proxy was substituted (rs1482853 or rs900399 for rs900400 (HapMap $r^2 = 1$ and 0.96, respectively); rs2877716 or rs6798189 for rs9883204 (HapMap $r^2 = 0.95$ and 0.93, respectively)). In four of the replication studies, the index SNPs were imputed from genome-wide genotype data (see **Supplementary Table 2**). The UNIFORM birth-weight analysis (described above) was performed within each study sample. To investigate whether the associations were similar in the sexes, we repeated the analysis in males and females separately.

Meta-analyses. We performed fixed-effects inverse-variance meta-analyses of the UNIFORM results as follows: (i) including all 13 European replication samples; (ii) including all 19 discovery and replication samples of European descent, (iii) a sensitivity analysis, excluding the three studies without gestational age; and (iv) including all 23 study samples, regardless of ethnic background. We meta-analyzed the sex-stratified results from all European studies. All meta-analyses were performed in parallel at two different study centers, using different software packages (the METAN module, developed for Stata v.10 (ref. 36), MetaAnalyst (Beta 3.13)[37], RMeta in R (v.2.7.0)). We used the Cochran *Q* test and the I^2 statistic[15] to assess evidence of between-study heterogeneity of effect sizes.

Analysis of additional phenotypes: birth length, birth head circumference, ponderal index and small for gestational age. Where available, we created *z* scores ([value – mean]/s.d.) within each study for birth length, head circumference and ponderal index (birth weight/length^3). We used linear regression to assess the association between each outcome and each SNP (rs900400 or rs9883204, or proxies), with sex and gestational age as covariates. To examine the odds of small-for-gestational age (SGA) status, we created sex- and gestational age-adjusted birth-weight *z* scores (SDS) within 15 of the available European studies using Growth Analyser 3.0 (Dutch Growth Research Foundation, Rotterdam, The Netherlands; also see URLs). The reference was a cohort of 475,588 children born between 1977 and 1981 in Sweden[38]. Subsequently, each study defined SGA as below the tenth percentile of birth-weight SDS within their study population. We analyzed the associations between the two top hits and SGA using logistic regression. We combined the results across studies using fixed-effects inverse-variance meta-analysis.

Combined allele score. To estimate the birth-weight effect sizes attributable to the two loci in combination, we created an allele score using information from both SNPs, which was weighted by effect size. This allowed us to estimate the differences in birth weight between individuals with different numbers of birth weight–lowering alleles at the two loci. We used nine European replication samples in which gestational age was available ($n = 20,190$). After verifying that the two SNPs were statistically independent, we generated the score using the formula

$$s_j = 2 \times \sum_{i=1}^{2} w_i g_{i,j} / \sum_{i=1}^{2} w_i$$

where s_j is score for individual j, $g_{i,j}$ is number of birth weight–lowering alleles (0, 1, 2) for SNP i carried by individual j and w_i is effect size for SNP i from the UNIFORM analysis within the cohort. We performed linear regression of birth weight (in grams) against the allele score (additive model), with sex and gestation as covariates. We combined the coefficients from the nine studies using fixed-effects inverse-variance meta-analysis. We then rounded the weighted score to the nearest integer, grouping scores "0" and "1" together, and performed linear regression of birth weight against the rounded score as indicator variables, with sex and gestation as covariates. The β coefficients from the comparison of score 4 versus 0 or 1 in all nine studies were meta-analyzed (inverse variance, fixed effects).

Variance explained. To estimate the percentage of variation in birth weight explained by each of the associated loci, we obtained the adjusted R^2 from univariate linear regression of birth weight against genotype. We then calculated a mean value from all European studies, weighted by sample size. For comparison, we also calculated the variance explained by variables such as gestational age, maternal age and smoking.

Analyses of potential confounders. To assess whether the associations were independent of maternal genotype, we used mother-offspring pairs from the five studies with both maternal and fetal genotype available (see **Supplementary Table 4**; total $n = 8,880$ for rs900400; $n = 9,127$ for rs9883204). Within each study, we performed the UNIFORM analysis, with maternal genotype as an additional covariate. For direct comparison, we repeated this without maternal genotype, using only subjects for whom maternal genotype was available. We performed two inverse variance meta-analyses (fixed effects) for each SNP, combining regression coefficients for (i) fetal genotype and (ii) fetal genotype adjusted for maternal genotype.

To verify that the SNPs were not associated with maternal covariates of birth weight that could theoretically confound the observed associations with birth weight (including maternal age, BMI, parity, smoking, pre-eclampsia and education), we used linear or logistic regression to model the association between each covariate and genotype, using nine European replication cohorts with gestational age available. Where possible, we meta-analyzed results to assess overall evidence of association.

URLs. Growth Analyser 3.0, http://www.growthanalyser.org; HapMap, http://hapmap.ncbi.nlm.nih.gov/; METAL, http://www.sph.umich.edu/csg/abecasis/metal/index.html.

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doi:10.1038/ng.567