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The chromosome 3q25 genomic region is associated with measures of adiposity in newborns in a multi-ethnic genome-wide association study

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Newborns characterized as large and small for gestational age are at risk for increased mortality and morbidity during the first year of life as well as for obesity and dysglycemia as children and adults. The intrauterine environment and fetal genes contribute to the fetal size at birth. To define the genetic architecture underlying the newborn size, we performed a genome-wide association study (GWAS) in 4281 newborns in four ethnic groups from the Hyperglycemia and Adverse Pregnancy Outcome Study. We tested for association with newborn anthropometric traits (birth length, head circumference, birth weight, percent fat mass and sum of skinfolds) and newborn metabolic traits (cord glucose and C-peptide) under three models. Model 1 adjusted for field center, ancestry, neonatal gender, gestational age at delivery, parity, maternal age at oral glucose tolerance test (OGTT); Model 2 adjusted for Model 1 covariates, maternal body mass index (BMI) at OGTT, maternal height at OGTT, maternal mean arterial pressure at OGTT, maternal smoking and drinking; Model 3 adjusted for Model 2 covariates, maternal glucose and C-peptide at OGTT. Strong evidence for association was observed with measures of newborn adiposity (sum of skinfolds model 3 *Z*-score 7.356, $P = 1.90 \times 10^{-13}$, and to a lesser degree fat mass and birth weight) and a region on Chr3q25.31 mapping between *CCNL* and *LEKR1*. These findings were replicated in an independent cohort of 2296 newborns. This region has previously been shown to be associated with birth weight in Europeans. The current study suggests that association of this locus with birth weight is secondary to an effect on fat as opposed to lean body mass.

INTRODUCTION

Fetal growth has important short and long-term implications for the health of the newborn. In the short term, low birth weight is associated with increased neonatal morbidity, including an increased risk of respiratory distress syndrome, necrotizing enterocolitis, neurologic and developmental disabilities, and increased mortality during the first year of life, whereas large

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for gestational age babies are at increased risk of neonatal hypoglycemia and birth injuries (1,2). An important association between fetal growth and long-term health outcomes has also been described. Association of low birth weight with the risk of chronic disease [including coronary artery disease , hypertension, dyslipidemia, obesity and type 2 diabetes] in adult life has been found in men and women of many ethnic groups and geographic locations, and it has been proposed that intrauterine events make a major contribution to the global risk of chronic diseases (1,3–11). Interestingly, large for gestational age babies also appear to be at risk for adverse long-term health outcomes, including childhood and adult obesity and metabolic disease (12).

The fetus is imbued with a genetic potential for growth that is modified by an interaction with its intrauterine environment (13,14). Although the intrauterine environment has a major impact on fetal growth and size at birth, fetal genes also contribute significantly to fetal growth. Twin, intergenerational and family studies have estimated the heritability of birth weight to be between 30% and 70% (15–25). Considering birth weight as either a dichotomous trait or continuous variable gives similar estimates of heritability (15), suggesting that the contribution of genes to variation in birth weight is similar at the extremes of birth weight and across the normal distribution. A history of maternal and, to a lesser extent, paternal low birth weight increases risk of low birth weight and has been estimated to account for ∼9% and 3% of the variance in infant birth weight, respectively (26–28).

To date, limited information is available on genetic variation in the fetus that impacts the size at birth. A meta-analysis of genome-wide association data in individuals of Northern European ancestry demonstrated association of two loci on chromosome 3 with birth weight with a more recent meta-analysis identifying five additional loci associated with birth weight (29,30). The same consortium demonstrated association of two variants on chromosome 12 with the infant head circumference (31). To further define the genetic architecture underlying the newborn size at birth, we performed a genome-wide association study (GWAS) using DNA and newborn measures of fetal growth, including birth weight, birth length, head circumference and sum of skinfolds collected from four different ancestry groups by the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study, an observational epidemiologic investigation that examined associations of levels of glucose intolerance during pregnancy and risks of adverse neonatal outcomes (32,33).

RESULTS

Population demographics

We performed cohort-specific and meta-analyses of genome-wide SNP data to identify common genetic variants associated with newborn anthropometric traits, including the birth length, head circumference, birth weight, percent fat mass and sum of skinfolds, as well as newborn metabolic traits, including cord glucose and C-peptide. Basic phenotypic characteristics of the newborns and their mothers from the four different cohorts are presented in Table 1. We successfully genotyped 4281 newborns [1095 Afro-Caribbean (AC) newborns, 1363 Northern European (NE) newborns, 616 Mexican American (MA) newborns and 1207 Thai (TH) newborns] and tested for association with the above phenotypes.

Genetic analysis

Cohort-specific analyses were performed using linear regression with SNPTEST against an additive genotype variable and covariates defined in the original HAPO Study followed by meta-analysis using METAL, weighting each cohort by sample size.

Table 1. Demographic Characteristics

	NE*	MA	AC	Thai
Newborns (*n*) with genotype	1363	616	1095	1207
and birthweight	1358	616	1093	1186
Gestational age at OGTT	28.5 ± 0.04	26.9 ± 0.08	27.1 ± 0.05	28.2 ± 0.05
Age at OGTT	31.2 ± 0.14	28.9 ± 0.22	25.6 ± 0.17	27.7 ± 0.16
BMI at OGTT	28.5 ± 0.13	30.1 ± 0.23	27.8 ± 0.19	25.7 ± 0.10
MAP at OGTT (mmHg)	83.8 ± 0.21	84.1 ± 0.32	79.3 ± 0.23	79.7 ± 0.22
Fasting PG (mg/dl)	82.2 ± 0.18	83.9 ± 0.31	80.8 ± 0.24	79.9 ± 0.19
1-hr PG (mg/dl)	132.2 ± 0.79	137.6 ± 1.41	123.2 ± 0.85	148.0 ± 0.90
2-hr PG (mg/dl)	109.5 ± 0.59	111.5 ± 0.99	109.3 ± 0.68	119.4 ± 0.72
Gestational age at delivery	40.0 ± 0.03	39.7 ± 0.05	39.8 ± 0.04	39.4 ± 0.04
Maternal smoking (%)	13.79	0.00	0.46	0.66
Male offspring (%)	49.7	49.5	51.6	49.1
Primiparous births (%)	57.2	26.2	45.5	53.7
Offspring birth weight (gm)	3424 ± 13.5	3440 ± 17.4	3230 ± 13.5	3100 ± 11.2
Offspring birth length (cm)	50.6 ± 0.06	50.6 ± 0.07	49.3 ± 0.08	49.4 ± 0.04
Head circumference (cm)	34.9 ± 0.04	34.3 ± 0.05	33.8 ± 0.04	33.9 ± 0.03
Sum of skinfolds (mm)	13.0 ± 0.07	14.2 ± 0.12	11.5 ± 0.05	11.7 ± 0.07
Fat mass (%)	12.5 ± 0.10	13.1 ± 0.14	11.4 ± 0.10	9.9 ± 0.09

NE, Northern European Ancestry; MA, Mexican American; AC, Afro-Caribbean; MAP, mean arterial pressure
*Toronto: 604; Belfast: 445; Brisbane: 190; Newcastle: 124

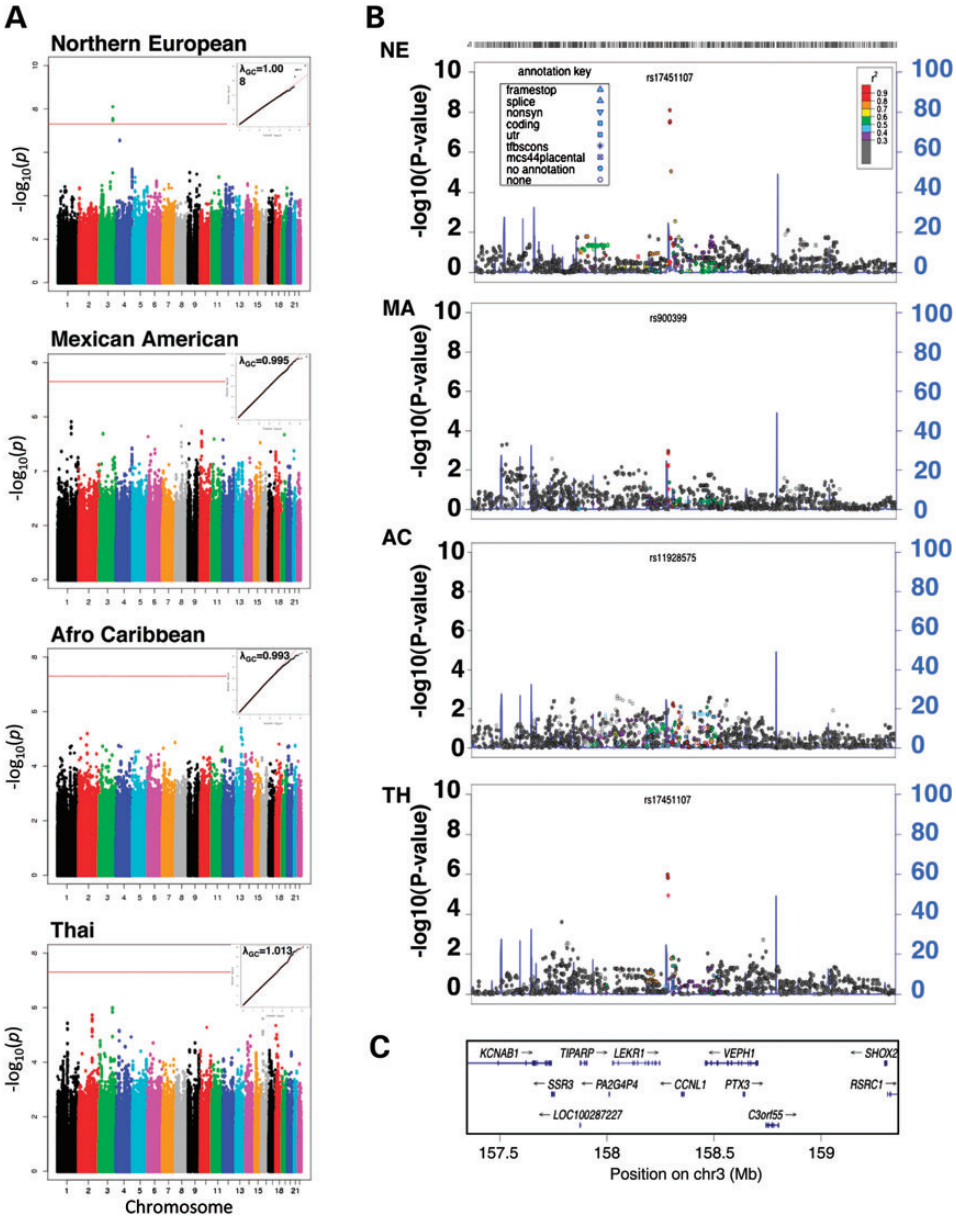

Figure. 1. (**A**) Genome-wide associations with the sum of skinfolds in HAPO newborns. Shown are the Manhattan plots for Model 2 for each of the four populations along with the corresponding QQ plot in the upper right corner. The redline indicates genome-wide significance ($-\log_{10}P = 7.3$). (**B**) Locus zoom plot of chromosome 3q25.32 locus for the sum of skinfolds for each cohort under Model 2. Each of the panels contains the population-specific association results and estimates of LD (r^2) from the SNP with the strongest evidence for association in the meta-analysis. The LD estimates are color coded as a heat map from purple to red ($0.9 \geq r^2 > 1.0$), while grey indicates $r^2 < 0.3$. These coincide with the recombination hotspots shown by the blue lines (recombination rate in cM/Mb from HapMap). (**C**) Map of the genes and their orientation (5′–3′) in this region of chromosome 3q25.31.

Genome-wide association study

We identified one locus on chromosome 3 that demonstrated genome-wide significance in the meta-analysis (Fig. 1A). This locus, located at 3q25.31 between *CCNL1* and *LEKR1*, was associated with the sum of skinfolds and was previously shown to be associated with birth weight in a meta-analysis of GWAS performed in babies of European descent (30). The most strongly associated SNP, rs17451107, was imputed in the NE, MA and AC ancestry cohorts and directly genotyped in the TH cohort. Rs17451107 demonstrated greatest evidence for association in NE newborns (Model 2, $\beta = 0.078$; $P = 7.92 \times 10^{-9}$; Fig. 1B, Table 2). Evidence for association was slightly reduced after adjusting for maternal glucose and C-peptide levels (Model 3, $\beta = 0.070$; $P = 1.39 \times 10^{-7}$; Table 2). The same SNP was also strongly associated with the sum of skinfolds in the TH cohort (Model 2, $\beta = 0.063$ mm; Model 3 $\beta = 0.063$; Table 2) but at a slightly reduced significance level ($P = 1.04 \times 10^{-6}$ for Model 2 and 6.08×10^{-7} for Model 3; Table 2) possibly resulting from a smaller sample size in the TH cohort. The evidence for association observed in the MA and AC cohorts was less significant than that observed in the European and Thai ancestry cohorts, although the effect size was similar to that in the NE

Table 2. Evidence for Association with measures of newborn size at the *LEKR1/CCNL1* locus

	NE Freq	β (*Z*-score)	*P*-value	MA Freq	β (*Z*-score)	*P*-value	AC Freq	β (*Z*-score)	*P*-value	TH Freq	β (*Z*-score)	*P*-value	META β (*Z*-score)	*P*-value
Newborn Trait Model 2														
rs10049088 (T)	0.39			0.42			0.27			0.45				
Sum of Skinfolds (√mm)		−0.0757	3.27×10^{-8}		−0.0709	0.00140		−0.00847	0.505		−0.0626	1.04×10^{-6}	(-7.275)	3.46×10^{-13}
Fat Mass (%)		−0.413	0.00155		−0.452	0.0111		−0.192	0.219		−0.519	1.19×10^{-5}	(-5.702)	1.18×10^{-8}
Birthweight (gm)		−41.3	0.0117		−45.5	0.0328		−29.8	0.141		−40.5	0.00275	(-4.570)	4.88×10^{-6}
rs1482853 (C)	0.61			0.58			0.73			0.55				
Sum of Skinfolds (√mm)		0.0744	3.10×10^{-8}		0.0703	0.00136		0.00769	0.542		0.0621	1.39×10^{-6}	(7.225)	5.02×10^{-13}
Fat Mass (%)		0.408	0.00146		0.451	0.0105		0.182	0.239		0.513	1.61×10^{-5}	(5.658)	1.54×10^{-8}
Birthweight (gm)		41.4	0.0100		46.0	0.0297		27.5	0.171		39.9	0.00334	(4.532)	5.85×10^{-6}
rs17451107 (T)	0.59			0.58			0.65			0.55				
Sum of Skinfolds (√mm)		0.0784	7.92×10^{-9}		0.0610	0.00619		0.0183	0.119		0.0626	1.04×10^{-6}	(7.686)	1.52×10^{-14}
Fat Mass (%)		0.399	0.00205		0.345	0.0535		0.249	0.0839		0.518	1.20×10^{-5}	(5.671)	1.42×10^{-8}
Birthweight (gm)		47.5	0.00350		35.7	0.0959		41.9	0.0251		40.5	0.00277	(5.000)	5.72×10^{-7}
rs900400 (T)	0.61			0.58			0.73			0.55				
Sum of Skinfolds (√mm)		0.0743	2.90×10^{-8}		0.0717	0.00109		0.00779	0.536		0.0620	1.48×10^{-6}	(7.253)	4.08×10^{-13}
Fat Mass (%)		0.408	0.00139		0.488	0.00557		0.189	0.221		0.511	1.81×10^{-5}	(5.757)	8.55×10^{-9}
Birthweight (gm)		41.6	0.00951		50.7	0.0163		28.4	0.158		39.7	0.00355	(4.642)	3.46×10^{-6}
Newborn Trait Model 3														
rs10049088 (T)	0.39			0.42			0.27			0.45				
Sum of Skinfolds (√mm)		−0.0671	5.22×10^{-7}		−0.0695	0.00152		−0.00730	0.561		−0.0633	6.12×10^{-7}	(-6.993)	2.68×10^{-12}
Fat Mass (%)		−0.350	0.00662		−0.447	0.0112		−0.191	0.220		−0.522	9.29×10^{-6}	(-5.475)	4.39×10^{-8}
Birthweight (gm)		−33.7	0.0370		−43.8	0.0385		−28.0	0.166		−41.0	0.00240	(-4.279)	1.88×10^{-5}
rs1482853 (C)	0.61			0.58			0.73			0.55				
Sum of Skinfolds (√mm)		0.0661	4.88×10^{-7}		0.0688	0.00149		0.00633	0.612		0.0628	8.34×10^{-7}	(6.934)	4.09×10^{-12}
Fat Mass (%)		0.346	0.00627		0.446	0.0107		0.179	0.245		0.517	1.27×10^{-5}	(5.424)	5.84×10^{-8}
Birthweight (gm)		34.0	0.0322		44.2	0.0351		25.46	0.204		40.3	0.00292	(4.235)	2.29×10^{-5}
rs17451107 (T)	0.59			0.58			0.65			0.55				
Sum of Skinfolds (√mm)		0.0699	1.39×10^{-7}		0.0595	0.00677		0.0160	0.169		0.0633	6.08×10^{-7}	(7.356)	1.90×10^{-13}
Fat Mass (%)		0.336	0.00853		0.340	0.0548		0.234	0.104		0.522	9.35×10^{-6}	(5.389)	7.09×10^{-8}
Birthweight (gm)		39.9	0.0126		34.0	0.110		38.8	0.0374		40.9	0.00241	(4.676)	2.93×10^{-6}
rs900400 (T)	0.61			0.58			0.73			0.55				
Sum of Skinfolds (√mm)		0.0660	4.57×10^{-7}		0.0703	0.00116		0.00624	0.617		0.0627	8.89×10^{-7}	(6.959)	3.44×10^{-12}
Fat Mass (%)		0.346	0.00599		0.484	0.00556		0.185	0.231		0.514	1.43×10^{-5}	(5.521)	3.38×10^{-8}
Birthweight (gm)		34.1	0.0307		49.1	0.190		26.1	0.192		40.1	0.00310	(4.344)	1.40×10^{-5}

NE, Northern European Ancestry; MA, Mexican American; AC, Afro-Caribbean

babies and in the same direction (Model 2, β: 0.061 and 0.018, respectively, *P*-value: 6.2×10^{-3} and 0.012, respectively; Model 3, β: 0.060 and 0.016, respectively, *P*-value: 6.8×10^{-3} and 0.017, respectively; Table 2).

Meta-analysis of all four populations demonstrated strong association between rs17451107 and the sum of skinfolds (Model 2 *Z*-score 7.69, $P = 1.52 \times 10^{-14}$; model 3 *Z*-score 7.36, $P = 1.90 \times 10^{-13}$; Table 2). Furthermore, the meta-analysis identified three additional SNPs in the 3q25 genomic region that reached genome-wide significance in the combined cohorts (rs10049008, $P_{model2} = 3.46 \times 10^{-13}$, $P_{model3} = 2.68 \times 10^{-12}$; rs1482853, $P_{model2} = 5.02 \times 10^{-13}$, $P_{model3} = 4.09 \times 10^{-12}$; rs900400, $P_{model2} = 4.08 \times 10^{-13}$, $P_{model3} = 3.44 \times 10^{-12}$; Table 2). The results for rs10049008 and rs1482853 were generated from imputed genotypes while rs900400 was directly genotyped.

Given the multiple ancestry groups included in the GWAS, an alternative analytic approach that accounts for the trans-ethnic nature of the cohort was also employed. To that end, we performed a trans-ethnic meta-analysis using MANTRA (34). The trans-ethnic meta-analysis using MANTRA closely mirrored the results from the standard meta-analysis using METAL (Supplementary Material, Figs. S1and S2) when compared genome-wide. Finer-grain investigation of the trans-ethnic meta-analysis results for the chromosome 3 locus supports the evidence from standard meta-analysis (Table 3). Each SNP in the region is more strongly associated with the sum of skinfolds than with birth weight, and the allelic effects are generally consistent across the four populations. There is some evidence for heterogeneity in the association of each SNP, other than rs17451107, with the sum of skinfolds as indicated by the posterior probabilities >0.5. At the heterogenous SNPs, the posterior mean allelic effect in the AC is approximately 20% that observed in the NE, MA and TH populations (a decrease from ∼0.06 mm per copy of the reference allele to ∼0.01 mm per copy of the reference allele). This suggests rs17451107 tags the effect better than the remaining SNPs as no heterogeneity was observed for this SNP (posterior probability of association = 0.46), although there is still an attenuation of the effect from ∼0.06 (√mm) to ∼0.03 (√mm) per copy of the reference allele.

While not reaching genome-wide significance the *CCNL1/LEKR1* genomic region was also nominally associated with percent fat mass (Supplementary Material, Fig. S3) in newborns and to a lesser degree birth weight (Supplementary Material, Fig. S3 and Table S2). Evidence for association with birth weight from the meta-analysis was the strongest with rs17451107 (*Z*-score 5.00–4.68; $P = 0.572–2.93 \times 10^{-6}$; Table 2). rs17451107 is in strong linkage disequilibrium with the SNP identified by Freathy *et al*. (rs900400) which was shown to be strongly associated with birth weight in a cohort of 10 623 European ancestry offspring (30).

Replication study

157 SNPs from the GWAS fulfilled our selection criteria (see Methods) and were genotyped in a cohort of 2296 NE newborns (Supplementary Material, Table S3). One-hundred and forty-eight SNPs passed QC and were used in an association study of 2296 NE newborns using the same analysis models as in the GWAS. The strongest evidence for association remained

Table 3. Trans-ethnic meta-analysis results for the CCNL1/LEKR locus on chromosome 3.

	NE		MA		AC		TH		log10 Bayes Factor Favoring Association	Posterior Probability of Association	Total Sample Size	Effect Direction
	Posterior Mean Allelic Effect(1)	Posterior s.d.(2)	Posterior Mean Allelic Effect	Posterior s.d.	Posterior Mean Allelic Effect	Posterior s.d.	Posterior Mean Allelic Effect	Posterior s.d.				
rs10049088 (T)												
Birthweight (gm)	-30.2	10.2	-30.2	10.2	-30.1	10.2	-30.2	10.2	2.999	0.463	4138	− − − −
Sum of Skinfolds (√mm)	-0.064	0.010	-0.063	0.010	-0.012	0.016	-0.063	0.010	10.301	0.926	4131	− − − −
rs1482853 (C)												
Birthweight (gm)	35.0	11.0	35.0	11.0	35.0	11.1	35.0	11.0	3.338	0.484	4138	+ + + +
Sum of Skinfolds (√mm)	0.062	0.010	0.062	0.010	0.010	0.016	0.062	0.010	9.587	0.930	4131	+ + + +
rs17451107 (T)												
Birthweight (gm)	34.9	12.7	34.9	12.7	34.9	12.8	34.9	12.7	2.120	0.455	4138	+ + + +
Sum of Skinfolds (√mm)	0.060	0.012	0.060	0.012	0.025	0.017	0.060	0.011	10.642	0.741	4131	+ + + +
rs900399 (G)												
Birthweight (gm)	-33.3	5.2	-33.4	5.1	-33.4	5.1	-33.4	5.1	4.032	0.474	4138	− − − −
Sum of Skinfolds (√mm)	-0.063	0.010	-0.063	0.010	-0.010	0.015	-0.063	0.010	9.429	0.940	4131	− − − −
rs900400 (T)												
Birthweight (gm)	24.0	7.9	24.1	7.9	23.9	8.0	24.0	7.9	2.765	0.458	4138	+ + + +
Sum of Skinfolds (√mm)	0.063	0.010	0.063	0.010	0.010	0.015	0.063	0.010	10.430	0.950	4131	+ + + +

1. Aligned to effect allele
2. Standard deviation

Table 4. Results of Replication of association with rs1482853 at *LEKR1/CCNL1* gene region

	Population	Allele	Freq	β (*Z*-score)	SE	*P*-value
Newborn Trait Model 2						
Sum of Skinfolds	NE2*	C	0.620	0.055	0.010	1.01×10^{-7}
	Meta2**	C	–	(8.97)	–	2.85×10^{-19}
Fat Mass	NE2	C	0.620	0.438	0.093	2.59×10^{-6}
	Meta2	C	–	(7.35)	–	2.03×10^{-13}
Birthweight	NE2	C	0.620	43.2	11.4	1.63×10^{-4}
	Meta2	C	–	(5.89)	–	3.92×10^{-9}
Newborn Trait Model 3						
Sum of Skinfolds	NE2	C	0.620	0.055	0.0103	1.20×10^{-7}
	Meta2	C	–	(8.72)	–	2.71×10^{-18}
Fat Mass	NE2	C	0.620	0.447	0.094	1.85×10^{-6}
	Meta2	C	–	(7.20)	–	6.08×10^{-13}
Birthweight	NE2	C	0.620	43.5	11.5	1.59×10^{-4}
	Meta2	C	–	(5.65)	–	1.59×10^{-8}

NE2*, European replication cohort; Meta2**, Meta analysis including NE2, NE, MA, AC, and TH cohorts

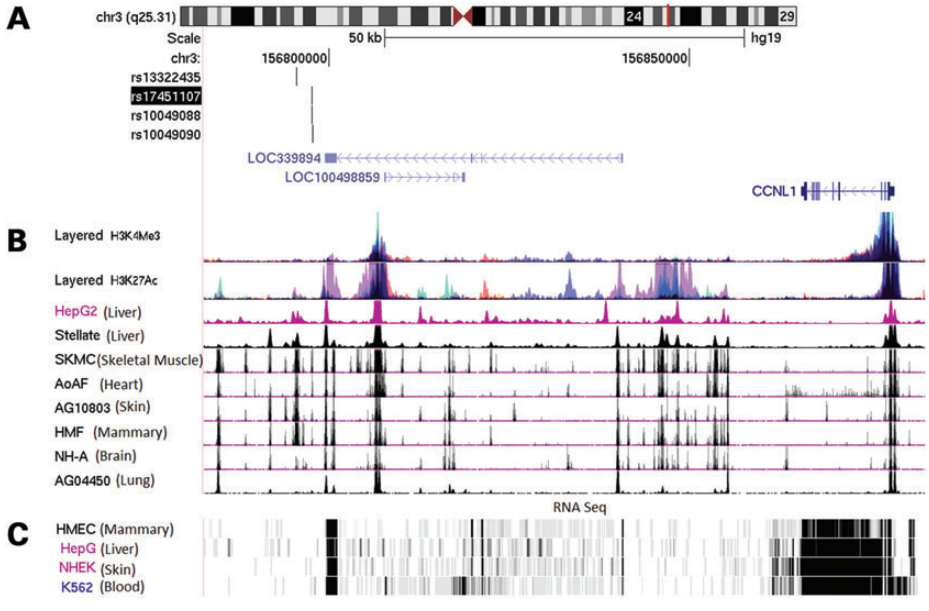

Figure 2. Schematic of the region of chromosome 3q25.31 adiposity associated region showing aligned SNPs, noncoding RNAs and genes, active enhancer marks, open chromatin regions and expression profiles of RNAs mapping to this region. (http://genome.ucsc.edu Build 37)

with the chr3q25.31 region and the sum of skinfolds in both the replication cohort (rs1482853, $\beta = 0.055$ mm, $P = 1.01 \times 10^{-7}$, model 2, Table 4) and the meta-analysis of the four discovery cohorts plus the replication cohort (rs1482853, *Z*-score = 8.97, $P = 2.85 \times 10^{-19}$, model 3, Table 4). Similarly evidence for association between rs14828853 and fat mass also reached genome-wide significance under both Models 2 and 3 (Table 3).

Potentially interesting loci that did not fulfill our criteria for replication are the chr.13q12.12 association with the sum of skin folds in the MA cohort (Fig. 1A), the chr.2p14 association with birth weight in the AC cohort (Supplementary Material, Fig. S3B), the chr.2p24.2 association with birth length in the MA cohort (Supplementary Material, Fig. S3D), the chr.12q23.1 association with cord glucose levels in the AC cohort and the chr.313.33 association with cord glucose in the TH cohort (Supplementary Material, Fig. S3F). The significance of these findings needs to be further investigated in appropriate populations.

Characterization of the 3q25.31 locus

To better understand the biological relevance of the 3q25.31 locus, we queried functional genomic datasets produced by the ENCODE project for regulatory function in the associated region. The four variants associated with the sum of skinfolds overlap regions with experimental evidence of regulatory function in the region (Fig. 2). The associated SNPs fell into two clusters. One SNP, rs13322435, overlapped with 11 open chromatin regions in cell types that include primary and representative cells from the liver, skin, lung, skeletal muscle and the central nervous system. The SNP also overlapped regions with histone marks indicative of an active regulatory element. The second

cluster included four variants, rs17451107, rs1482853, rs10049088 and rs10049090, that together overlap a more restricted set of open chromatin and active histone regions that include cells from skin, heart, mammary tissue and skeletal muscle as shown in Fig. 2B. All four SNPs are in linkage disequilibrium with each other.

These sites may impact the regulation of nearby noncoding RNAs (ncRNAs) including *LOC339894* and *LOC100498859*. Although the function of these ncRNAs is unknown, they are highly expressed in cells from mammary tissue, skin, blood and the liver. These sites may also be responsible for regulating nearby genes including *CCNL1*. *CCNL1* is involved in transcription via the regulation of mRNA splicing. As shown in Fig. 2C, *CCNL1* is highly expressed along with the two ncRNAs as shown. The lead SNP reported by Freathy *et al*. (30) also demonstrated association in our study, but was not located on open chromatin, suggesting that the SNPs we identified may be the causal variants.

Evidence for association with previously identified birth weight loci in the HAPO cohorts

In addition to the chr3q25.31 locus, six other loci were reproducibly associated with birth weight in a meta-analysis of up to 69 308 individuals of European ancestry; rs9883204 in *ADCY5*, rs6931514 in *CDKAL1,* rs1042725 in *HMGA2*, rs724577 in *LCORL*, rs1801253 in *ADRB1* and rs4432842 on chromosome 5q11.2 (29). While none of these loci reached genome-wide levels of significance in the present study, we investigated whether these SNPs or SNPs over a 20 kb region surrounding the lead SNP were more nominally associated with birth weight. The strongest evidence for association was observed with multiple SNPs in *ADRB1* in the MA cohort (rs2429511, rs10787516, rs3813720, rs10885531) and the meta-analysis (Table 5). Less strong evidence for association was also evident in the NE and TH cohorts, but in the AC population no variants at this locus were associated with birth weight. We observed no evidence for association between the *ADCY5* locus and birth weight in the NE, MA, and TH cohorts and only minimal evidence for association in the AC cohort ($P = 0.019-0.042$) (Table 5). Multiple SNPs in the *LCORL* region were modestly associated with birth weight in the NE and TH cohorts and the meta-analysis under all models tested although the previously identified SNP, rs724577, was not associated with birth weight in our study (Table 5). No evidence for association of SNPs in the 5q11.2 locus with birth weight was evident in the our cohorts, including with the previously reported SNP, rs4432842 (Table 5). The observation that the strongest evidence for association at these loci was primarily in the European ancestry cohort is consistent with the limitation of the recent meta-analysis to populations of European origin.

Failure of SNPs within the above loci to reach statistical significance is likely due to reduced power in the HAPO cohorts compared with the meta-analysis published by Horikoshi *et al*. (29). Interestingly, for those loci with nominal evidence for association in the HAPO study, the effect sizes were generally >35 g, while in Horikoshi *et al*. effect sizes tended to be substantially smaller (16–35 g). Furthermore, whenever an SNP was nominally associated with birth weight, the effect was in the same direction in the HAPO cohort as in the Horokoshi study (29).

In addition to loci associated with birth weight, two loci associated with the infant head circumference at 18 months (range 6–30 months) in Europeans have also been reported (31). The two loci map to rs7980687 on chromosome 12q15 and rs1042725 on chromosome 12q24. We did not observe any significant evidence for association with the head circumference at these loci (Supplementary Material, Fig. S3). The strongest evidence for association with the head circumference in our cohorts was to a 70 kb region mapping to chr9p21.1 (rs11787683, rs10969138, rs10969139, rs10969148, rs10813083, rs10969156, rs10969189, rs10969194, rs10969197 and rs10813103; *P*-values in the NE cohort $\sim 10^{-8}$) and a 1.5 kb region mapping to chr7q35 (rs1860681, *P*-value in the NE cohort $= 3.46 \times 10^{-9}$ and rs10487937, *P*-value in the NE cohort $3.52 \times \sim 10^{-9}$). However, for both the regions all the associated variants are rare [minor allele frequency (MAF) <3%].

Biological pathways

To determine whether variants within genes in biological pathways were associated with the size at birth, we implemented meta-analysis gene-set enrichment of variant associations or MAGENTA with the genetic association data for birth weight and sum of skinfolds under Model 2 in each ancestry cohort separately as well as in the joint meta-analysis. For the sum of skinfolds, multiple pathways reached significance [nominal $P < 0.05$ and false discovery rate (FDR) <0.20], however only the PANTHER_BIOLOGICAL_PROCESS Other_nucleoside,_nucleotide_and_nucleic_acid_metabolism pathway remains significant in the combined data (META) (Supplementary Material, Table S4). Likewise, multiple signaling pathways fulfilled our criteria for significance for birth weight—however only the Ingenuity Cell.Cycle.G2.M.DNA.Damage.Checkpoint.Regulation pathway remains significant in the combined data (META) (Supplementary Material, Table S4).

DISCUSSION

The goal of the present study was to identify genetic variants associated with measures of fetal size at birth. By performing a GWAS using DNA and phenotype data from newborns from four different ancestry groups, we demonstrated genome-wide significance with measures of newborn adiposity. As described, recent meta-analysis of GWAS performed in individuals of Northern European ancestry identified seven loci associated with birth weight (29,30). In our GWAS, we observed nominal evidence for association with birth weight in one or more ancestry groups at six of the previously identified loci (*ADCY5*, *ADRBI, LCORL, CDKAL1*, 5q11.2 and *HMGA2*) and highly significant association with the sum of skinfolds and, to a lesser extent, percent fat mass and birth weight at the 3q25.31 locus which is intergenic between *CCNL1* and *LEKR1*. In addition to the recent meta-analyses, the lead SNP in the 3q25.31 locus, rs900400, has been shown to be associated with birth weight in a Danish population (35), while a meta-analysis of preterm newborns (mean gestational age of 29–35 weeks in the different studies) demonstrated marginal

Table 5. Association results in HAPO cohorts for previously identified birth weight loci (29).

Locus	Marker	Allele		NE Model 1	NE Model 2	NE Model 3	MA Model 1	MA Model 2	MA Model 3	AC Model 1	AC Model 2	AC Model 3	TH Model 1	TH Model 2	TH Model 3	META Model 1	META Model 2	META Model 3
ADCY5	rs9857714	C	Frequency	NA			NA			0.92			NA					
			β	NA	NA	NA	NA	NA	NA	-66	-61	-58	NA	NA	NA	1.96	1.87	1.78
			P-value	NA	NA	NA	NA	NA	NA	0.051	0.061	0.074	NA	NA	NA	0.051	0.061	0.074
	rs9861812	C	Frequency	NA			NA			0.92			NA					
			β	NA	NA	NA	NA	NA	NA	-66	-62	-58	NA	NA	NA	1.96	1.87	1.79
			P-value	NA	NA	NA	NA	NA	NA	0.050	0.061	0.074	NA	NA	NA	0.050	0.061	0.074
	rs9824181	A	Frequency	NA			NA			0.98			NA					
			β	NA	NA	NA	NA	NA	NA	-138	-126	-123	NA	NA	NA	-2.13	-2.01	-1.97
			P-value	NA	NA	NA	NA	NA	NA	0.033	0.045	0.049	NA	NA	NA	0.033	0.045	0.049
	rs9883204*	T	Frequency	0.25			0.34			NA			NA					
			β	4	5	3	2	-3	-1	NA	NA	NA	NA	NA	NA	0.203	0.151	0.101
			P-value	0.845	0.783	0.881	0.943	0.891	0.966	NA	NA	NA	NA	NA	NA	0.839	0.880	0.920
LCORL	rs6840334	T	Frequency	0.82			0.84			NA			0.72					
			β	56	45	45	-35	-28	-27	NA	NA	NA	33	27	27	2.33	2.02	2.10
			P-value	0.011	0.030	0.027	0.246	0.320	0.335	NA	NA	NA	0.052	0.090	0.080	0.020	0.043	0.036
	rs7668933	G	Frequency	0.18			0.16			NA			0.28					
			β	-56	-45	-45	30	26	25	NA	NA	NA	-30	-25	-26	2.33	1.97	2.05
			P-value	0.011	0.030	0.027	0.324	0.347	0.375	NA	NA	NA	0.071	0.116	0.104	0.020	0.049	0.040
	rs13151620	T	Frequency	0.85			0.87			0.91			0.72					
			β	48	36	37	-32	-24	-21	-51	-51	-53	31	25	26	0.908	0.618	0.688
			P-value	0.048	0.112	0.097	0.328	0.433	0.492	0.106	0.097	0.083	0.066	0.108	0.096	0.364	0.537	0.492
	rs16896210	G	Frequency	0.18			0.16			NA			0.28					
			β	-56	-45	-45	30	26	24	NA	NA	NA	-30	-25	-26	2.34	1.98	2.07
			P-value	0.011	0.029	0.026	0.328	0.352	0.382	NA	NA	NA	0.071	0.116	0.104	0.019	0.048	0.039
	rs724577*	C	Frequency	0.75			0.67			0.65			0.62					
			β	-9	-16	-17	3	16	18	7	13	17	-34	-24	-23	1.20	0.743	0.578
			P-value	0.644	0.377	0.363	0.893	0.474	0.417	0.721	0.475	0.353	0.027	0.098	0.113	0.231	0.458	0.564
	rs16896215	T	Frequency	0.82			0.84			0.58			0.72					
			β	56	45	45	-35	-28	-27	-18	-19	-21	33	27	27	1.53	1.21	1.21
			P-value	0.011	0.031	0.027	0.251	0.323	0.330	0.329	0.287	0.242	0.053	0.091	0.081	0.127	0.228	0.225
5q11.2	rs4432842*	T	Frequency	0.70			0.43			0.21			0.59					
			β	15	6	7	-6	-8	-7	-18	-18	-18	-2	-2	-2	-0.119	-0.474	-0.398
			P-value	0.424	0.739	0.674	0.801	0.688	0.731	0.423	0.405	0.401	0.891	0.864	0.874	0.906	0.636	0.690
	rs13355565	T	Frequency	0.95			0.67			0.74			0.71					
			β	9	-12	-10	-15	-16	-17	39	36	34	6	3	3	0.997	0.507	0.468
			P-value	0.822	0.744	0.776	0.529	0.466	0.422	0.065	0.079	0.92	0.728	0.868	0.861	0.319	0.613	0.640
CDKAL1	rs2206734	T	Frequency	0.18			0.20			0.24			0.30					
			β	-50	-51	-51	-17	-8	-10	2	1	3	-0.05	-0.3	-3	-1.43	-1.44	-1.53
			P-value	0.030	0.017	0.016	0.539	0.773	0.707	0.936	0.949	0.882	0.998	0.984	0.862	0.154	0.150	0.127
	rs11753081	T	Frequency	0.83			0.78			NA			NA					
			β	44	46	47	9	-4	-3	NA	NA	NA	NA	NA	NA	1.74	1.64	1.73
			P-value	0.060	0.036	0.031	0.744	0.869	0.919	NA	NA	NA	NA	NA	NA	0.082	0.101	0.083
	rs3749925	G	Frequency	0.14			0.25			0.04			NA					
			β	57	50	49	15	12	11	9	13	12	NA	NA	NA	-1.84	-1.76	-1.73
			P-value	0.028	0.040	0.038	0.553	0.603	0.657	0.866	0.799	0.802	NA	NA	NA	0.066	0.079	0.083
	rs1040558	G	Frequency	0.17			0.22			NA			0.39					
			β	-44	-46	-47	-9	4	2	NA	NA	NA	2	-2	-4	1.30	1.40	1.57
			P-value	0.060	0.036	0.031	0.743	0.870	0.920	NA	NA	NA	0.906	0.876	0.751	0.192	0.163	0.118

ADRB1	rs2429511	T	Frequency	0.53			0.55			0.72			0.44					
			β	-44	-32	-32	-76	-64	-67	6	-1	0.9	-6	-11	-10	-2.83	-2.75	-2.74
			P-value	0.0099	0.044	0.045	$6.8x10^{-4}$	0.0021	0.0011	0.749	0.949	0.963	0.667	0.450	0.474	0.0046	0.0060	0.0061
	rs1801253*	G	Frequency	0.28			0.83			NA			0.25					
			β	-48	-42	-46	48	46	48	NA	NA	NA	-32	-31	-32	2.02	1.95	2.11
			P-value	0.013	0.020	0.010	0.107	0.101	0.083	NA	NA	NA	0.072	0.061	0.050	0.043	0.051	0.035
	rs3813720	T	Frequency	0.63			0.74			0.51			0.73					
			β	56	47	49	51	46	51	14	21	23	33	30	30	3.97	3.98	4.23
			P-value	0.0014	0.0037	0.0022	0.044	0.053	0.299	0.455	0.236	0.195	0.055	0.059	0.056	$7.2x10^{-5}$	$6.9x10^{-5}$	$2.4x10^{-5}$
	rs10787516	T	Frequency	0.50			0.46			0.15			0.56					
			β	44	32	32	70	58	62	-34	-27	-27	12	11	9	2.41	2.07	2.12
			P-value	0.012	0.050	0.045	0.0015	0.0045	0.0024	0.192	0.294	0.288	0.429	0.456	0.508	0.016	0.039	0.034
	rs10885531	T	Frequency	0.50			0.46			0.20			0.56					
			β	44	31	32	71	59	62	-12	-4	-6	12	10	9	2.81	2.49	2.50
			P-value	0.011	0.050	0.046	0.0012	0.0040	0.0022	0.602	0.849	0.789	0.452	0.483	0.537	0.0049	0.013	0.012
HMGA2	rs1351394	T	Frequency	0.48			0.34			0.39			0.14					
			β	43	44	44	12	3	8	-0.5	-4	-0.7	-2	4	3	1.53	1.56	1.74
			P-value	0.013	0.0072	0.0062	0.603	0.871	0.711	0.977	0.809	0.967	0.907	0.855	0.892	0.126	0.119	0.081
	rs867633	G	Frequency	0.59			0.42			NA			0.19					
			β	34	34	31	2	-1	2	NA	NA	NA	3	13	14	-1.38	-1.80	-1.81
			P-value	0.057	0.038	0.052	0.919	0.958	0.922	NA	NA	NA	0.880	0.452	0.423	0.166	0.073	0.071
	rs1042725*	T	Frequency	0.50			0.63			0.37			0.79					
			β	-41	-40	-40	-20	-11	-17	-14	-16	-18	4	-7	-7	-1.99	-2.30	-2.50
			P-value	0.015	0.011	0.0097	0.366	0.580	0.415	0.453	0.384	0.329	0.827	0.680	0.668	0.047	0.021	0.013
	rs8756	C	Frequency	0.48			0.34			0.40			0.13					
			β	47	45	45	13	5	10	3	0.7	4	4	10	9	-1.97	-2.01	-2.17
			P-value	0.0056	0.0040	0.0034	0.566	0.811	0.640	0.861	0.969	0.834	0.856	0.619	0.671	0.048	0.045	0.030
	rs11175982	T	Frequency	0.19			0.38			0.14			0.68					
			β	-39	-33	-33	6	13	13	-36	-38	-37	0.5	-12	-13	-1.58	-1.85	-1.85
			P-value	0.074	0.102	0.104	0.793	0.535	0.521	0.176	0.142	0.149	0.976	0.424	0.398	0.115	0.064	0.065
	rs7970350	T	Frequency	0.50			0.63			0.37			0.79					
			β	-41	-40	-40	-20	-11	-17	-15	-16	-18	5	-7	-7	-2.00	-2.31	-2.50
			P-value	0.014	0.011	0.0095	0.37	0.58	0.417	0.425	0.371	0.321	0.800	0.688	0.674	0.046	0.021	0.012
	rs7968902	T	Frequency	0.42			0.33			0.62			0.16					
			β	45	38	37	21	13	16	9	11	12	-11	0.5	2	1.76	1.87	2.00
			P-value	0.0095	0.019	0.020	0.357	0.561	0.453	0.624	0.542	0.501	0.558	0.978	0.916	0.078	0.061	0.045

* Birth weight associated marker (29)

NE, Northern European Ancestry; MA, Mexican American; AC, Afro-Caribbean; TH, Thai; META, Meta analysis including NE, MA, AC, and TH

association of rs900400 with birth weight (36). Using ultrasound examinations to assess fetal growth throughout gestation, another study found that the same SNP demonstrated marginal association with the head circumference and femur length in the second trimester, association with the head and abdominal circumference, femur length and estimated fetal weight in the third trimester and association with birth weight (37). Together, these data suggested that the genetic variation at rs900400 was associated with symmetrical growth changes from early pregnancy onward (37).

Birth weight is a function of fat and lean body mass, and some have suggested that fetal fat accretion is largely under maternal metabolic control, whereas lean body mass is under genetic control (38). Freathy *et al*. (30) demonstrated significant association of the 3q25.31 locus with the ponderal index, which has been shown to have a weak ($r^2 = 0.15$), albeit significant, correlation with fat mass in newborns (39). This suggested greater association of the 3q25.31 locus with fat mass as opposed to lean body mass. However, the association of this locus with the ponderal index has not been consistently observed (35,37). The present study more definitively demonstrates that the 3q25.31 locus is highly associated with newborn body fat through its association with the sum of skinfolds, a directly measured phenotype reflective of newborn body fat. Moreover, the trans-ethnic meta-analysis suggested that one of the SNPs in this region, rs17451107, tags the association with the sum of skinfolds better than the remaining SNPs, consistent with its localization in a site of open chromatin and active histones.

Significant subcutaneous fat at birth is of interest as, among primates and mammals more generally, it is present at much higher levels in humans (40). Whereas humans typically have ∼10%–15% body fat at birth, with the exception of harp seals and guinea pigs, most mammals, including nonhuman primates, have 1%–4% body fat at birth (40). This accumulation of body fat in humans represents primarily white adipose tissue and is thought to serve as an energy source to support growth of the large human newborn brain (40), presumably by providing ketone bodies to the brain during times of starvation and/or by providing fatty acids and glycerol as substrates for energy metabolism in organs other than brain and thereby making glucose available to the brain. Given the importance of fetal fat accretion as an energy source for the growing brain during the early postnatal period, it has been speculated that the acquisition of significant body fat at birth was an important evolutionary adaptation to support growth of the large human brain (40). Fetal fat accretion occurs largely during the third trimester of gestation, presumably in anticipation of the transition from dependence upon umbilical flow to lactation (40–42), and maternal factors, e.g., maternal glucose and triglyceride levels as well as maternal adiposity, significantly impact fetal fat accretion (43,44). We have now demonstrated that in addition to maternal metabolic factors, fetal genetic variation is also important for fetal fat accretion. Moreover, we have demonstrated that the 3q25.31 locus is important across multiple race groups and not solely Europeans.

Given the role of maternal metabolic factors in fetal fat accretion, association of maternal genotypes at the 3q25.31 locus with maternal fasting glucose or C-peptide or 1 and 2 h glucose levels was examined with no evidence for association (data not shown). Similarly, the SNPs at 3q25.31 were not associated with newborn cord glucose or C-peptide. Thus, there was no evidence to suggest that the SNPs at this locus affect fetal fat accretion through an impact on maternal or fetal metabolic traits. Consistent with this, Freathy *et al*. showed that rs900400 was not associated with type 2 diabetes or adult glycemic traits in the MAGIC consortium (30). However, in a Danish population, the birth weight-lowering allele of rs900400 was associated with increased insulin release and disposition index and lower fasting plasma glucose (35). Given the role of metabolic factors in fetal fat accretion, the impact of adjusting for maternal glucose and C-peptide levels on the observed association was examined in the present study. Although there was some attenuation of the observed association, the association remained highly significant after adjusting for maternal glucose and C-peptide levels, suggesting that the underlying effect of the locus on fetal fat accretion was largely independent of maternal glucose levels and insulin secretion.

The mechanism by which the locus at 3q25.31 may impact fetal fat mass is not known. The nearest gene, *CCNL1*, encodes cyclin L1, a member of the cyclin family thought to be involved in the regulation of mRNA splicing as opposed to cell cycle control (45). To address the potential biological role of the locus, functional genomic datasets produced by the ENCODE project were queried for regulatory function in the associated region. Of interest, in addition to *CCNL1*, there are two noncoding RNAs, *LOC339894* and *LOC100498859*, present in the region. Based upon RNAseq data, both are expressed in a variety of cell types. Similarly, *CCNL1* is also expressed in multiple cell types. One SNP in the region, rs13322435, is present within open chromatin marks in many tissues and is also associated with CTCF binding and histone modifications consistent with a repressive domain. Four other SNPs in a second cluster, rs10049088, rs10049090, rs1482853, and rs17451107, are within 100 bp of each other on the genome and have open chromatin around them, as well as a histone modification, histone 3 lysine 9 methylation, characteristic of enhancers and promoters (46). Thus, the region appears to be transcriptionally active and includes sites of potential enhancers or promoters. Future studies will be required to determine the role of these or other mRNA species regulated by this region and their association with fetal fat accretion.

We carried out pathway analysis on the association data for birth weight and sum of skinfolds. Peptide and hormone signaling pathways were significantly associated with birth weight in different ancestry groups, but the findings were not consistent across the different populations and were not confirmed using the dataset from the meta-analysis, suggesting that no one pathway has a primary effect on birth weight. Interestingly, in contrast to the findings with birth weight, there was no evidence that biological pathways as such contribute significantly to the sum of skinfolds. Whether this is due to a greater impact of environmental factors on the sum of skinfolds and/or variation in a more limited number of genetic loci contributing to the phenotype will require additional studies.

In summary, we have used a non-biased genome-wide approach to identify loci associated with measures of fetal growth in four different ancestry groups and have demonstrated that a locus on chromosome 3 in the region of *CCNL1*, 3q25.31, previously shown to be associated with birth weight was associated with measures of fetal fat accretion, sum of skinfolds

and percent fat mass. These data suggest that the previously demonstrated association of this locus with birth weight was likely driven primarily by newborn fat mass as opposed to lean body mass. Identification of this locus and, ultimately, definition of the mechanism by which it regulates fetal fat accretion will provide new insight into this human-specific trait.

PARTICIPANTS AND METHODS

HAPO project and participants

HAPO was an international, multi-center epidemiologic study conducted at 15 centers in nine countries with IRB/Ethics Committee approval at all sites. An external Data Monitoring Committee provided oversight. Study methods have been published (32,33,47,48). A brief overview is presented here. All pregnant women at each field center were eligible to participate, unless they had one or more exclusion criteria (47). Gestational age and the expected date of delivery were determined as previously described (47).

Oral glucose tolerance test (OGTT)
All participants underwent a standard 75 g OGTT between 24 and 32 weeks gestation and as close to 28 weeks as possible. Plasma glucose samples were collected at fasting and 1 and 2 h after the glucose load. Samples for serum C-peptide were collected at fasting and 1 h time points (Table 1). Cord blood was collected at delivery for newborn glucose and C-peptide analyses (47).

Anthropometric measurements
Maternal height, weight and blood pressure were measured at the OGTT visit using standardized procedures and calibrated equipment. Newborn weight, length, head circumference and flank, triceps and subscapular skinfolds were measured within 72 h of birth using methods and equipment standardized across all field centers (47). Personal and demographic data were collected using standardized questionnaires.

DNA sample collection and preparation

Mother DNA was isolated from blood collected during the OGTT clinical visit. Newborn DNA was isolated from blood collected from the umbilical cord within five minutes of delivery. DNA was extracted by the Center for Genetic Medicine of Northwestern University Feinberg School of Medicine using the Gentra Puregene Blood kit (Qiagen). Nine thousand eight hundred and fourteen mother and offspring HAPO samples [2581 AC, 3152 northern Europeans (NEs), 1615 MA and 2466 Thai (TH)], along with 126 HapMap control samples were submitted for genotyping, of which 9008 (2278 AC, 2797 NE, 1498 MA, and 2435 TH passed quality control (QC) (Supplementary Material, Table S1). Mother and baby DNAs were genotyped and data cleaned together to minimize the introduction of batch effects.

Genotyping

AC and MA DNA samples were genotyped using the Illumina Human1M-Duov3_B SNP array and the BeadStudio calling algorithm (version 3.1.3.0) and NE DNA samples were genotyped using the Illumina Human 610 Quad v1 B SNP array and the BeadStudio calling algorithm at the Broad Institute Center for Genotyping and Analysis (CGA). Mother/offspring pairs were kept on the same plate, and each plate contained a member of the HapMap CEU control trios. For the 1M-Duov_B SNP array, a member of HapMap YRI trio was plated in a random well. Suspect intensity data from scans were reviewed using Illumina BeadStudio. Genotype cluster definitions were reviewed for the SNPs with the strongest signal. SNPs were designated technical failures if any of the following criteria pertained: call rate (over scans) $<97\%$, number of replicate sample genotype discordances >2, number of sample trio inheritance errors >1, BeadStudio GenTrain score <0.6, and/or cluster separation <0.4.

TH DNA samples were genotyped using the Illumina HumanOmni1-Quad v1−0 B SNP array at the Center for Inherited Disease Research (CIDR). Mother–offspring pairs were plated together, distributing the singletons on each plate to match the instance of singletons in the overall study. Intensity data from scans were reviewed using Illumina BeadStudio, and genotype cluster definitions were reviewed. Each plate contained two random wells with HapMap subjects. Fifty total HapMap datasets were released encompassing 4 trios and 26 unique subjects from CEU, YRI, JPN and CHB populations. All mitochondrial and Y SNPs were manually reviewed and reclustered or dropped as appropriate. An SNP was considered a technical failure if the call rate was $<85\%$, there were any HapMap replicate discordances, or cluster separation <0.2.

Quality control procedures

Genotypic data that passed QC at the genotyping center (Broad or CIDR) were released to the GENEVA Coordinating Center (CC), the NCBI dbGaP team and the HAPO study team who collectively performed further QC procedures as described in detail by Laurie *et al*. (49) and briefly outlined below. In the AC cohort, 2278 out of 2581 samples (88%) passed all QC measures, and 946 608 SNPs out of 1 199 187 SNPs (82%) passed all QC measures and had an MAF $\geq 2\%$. For the NE dataset, 2797 out of 3152 samples (89%) passed all QC measures, and 531 407 SNPs out of 592 532 SNPs (90%) passed all QC measures and had an MAF $\geq 2\%$. In the MA study set, 1498 out of 1615 samples (93%) passed all QC measures, and 911 633 SNPs out of 1 154 691 SNPs (79%) passed all QC measures and had an MAF $\geq 2\%$. In the HAPO TH cohort, 2435 out of 2466 samples (99%) passed all QC measures, and 663 028 SNPs out of 1016423 SNPs (65%) passed all QC measures and had an MAF $\geq 2\%$. Sample and SNP genotyping efficiencies are provided in Supplementary Material, Tables S1 and S2, respectively.

Gender checks
The mean intensities of the SNP probes on the X and Y chromosomes along with X chromosome heterozygosity were used to verify that the annotated gender from the HAPO database and the genetic gender were consistent. Evaluation of X and Y chromosome SNP probe intensities identified 51 (28 AC, 4 MA and 19 NE) samples with gender discrepancies that were excluded from subsequent analyses (Supplementary Material, Table S1).

Chromosomal anomalies

Gross chromosomal anomalies, such as aneuploidy and large insertion/deletion events, were detected by analyzing relative intensity ('LogRRatio') and a measure of allelic imbalance ('BAlleleFreq'). To identify aneuploid or mosaic samples systematically, we calculated, for each sample, the variance of the BAF values for heterozygous SNPs in a sliding window along the genome. Chromosome scans for all sample–chromosome combinations that had one or more windows for which the heterozygous BAF variance was greater than four standard deviations from the mean of all variances for that window and samples that were outliers in the mean probe intensity on each chromosome relative to other chromosomes for the same sample (i.e. more than three interquartile ranges from the upper or lower quartiles) were examined visually (49).

Screening for chromosomal anomalies identified two study participants with an autosomal anomaly affecting the entire chromosome, while five study participants each had a large partial autosomal anomaly. Different autosomes are affected in each of the cases, and these regions of the genome for these individuals were excluded from the analysis. Several sex chromosome anomalies were also detected including six XXX/XX females, four XX/XO females, three XXY males and five XXY/XY males. A total of three mixed samples were also detected and excluded from the analysis.

Relatedness

The relatedness between each pair of participants was evaluated by estimating the coefficients corresponding to the probability that two, one or zero pairs of alleles were identical-by-descent (IBD). Any two alleles at a locus are either identical by descent or not and this gives rise to variation of IBD around the expected values (50). IBD coefficients were estimated independently in each of the four cohorts using 78 039 (AC), 107 980 (NE), 92 733 (MA) and 114 722 (TH) autosomal SNPs and the Method of Moments procedure used by PLINK 4, but implemented in R. The relationship of 51 (46 AC, 5 MA) pairs could not be verified, and the identity of 75 (28 AC, 47 NE) subjects could not be correctly verified. The data for these subjects have been removed from the analysis.

Ancestry: To investigate the population structure, we used principal components analysis (PCA), essentially as described by Patterson *et al*. (51). PCA was used to identify population group outliers and as covariates in the statistical model used for association testing to account for the population structure. Population group outliers among the HAPO study participants were detected using the known HapMap population anchor points. To detect population group outliers, we analyzed each of the four HAPO populations separately with and without 89 HapMap samples (CEU, CHB, JPT and YRI samples genotyped external to the HapMap control samples genotyped with the HAPO study participants) with a subset of autosomal SNPs [160,189 (AC), 90,712 (NE), 109,901 (MA), and 96,836 (TH) SNPs] (49). For each population, we analyzed: (i) all unrelated samples from each population in the presence of the HapMap reference samples, (ii) the mothers and babies together in the presence of the HapMap reference samples and (iii) the mothers and babies separately in the presence of the HapMap reference samples. Population outliers were removed from the analysis. For each population, the first two eigenvectors from the PCA of the mother or baby samples after the removal of outliers and without the HapMap reference samples were used as covariates in the association tests.

Population substructure analyses identified 16 NE babies who fell outside five standard deviations from the mean eigenvector in the offspring sample set. In the TH cohort, five baby population outliers were identified but since the removal of these participants did not significantly alter the numerical values of the eigenvectors they were not excluded from the analysis.

Imputation

We performed genotype imputation separately in each of the four QC cleaned and filtered genotyping sets using BEAGLE (52) and a HapMap 3 reference panel (53). We used a combined reference panel of unrelated individuals from multiple HapMap Phase III populations for imputation, based on the PCA described above. In the AC cohort, this consisted of Utah residents with Northern and Western European ancestry from the CEPH collection (CEU), Toscani from Italia (TSI), Yoruba in Ibadan, Nigeria (YRI); Luhya in Webuye, Kenya (LWK); Maasai from Kinyawa, Kenya (MKK) and African ancestry in southwestern USA (ASW). For the European cohort, we used a combined reference panel of two HapMap 3 populations: Utah residents with Northern and Western European ancestry from the CEPH collection (CEU) and Toscani in Italia (TSI). For the MA cohort, we used Utah residents with Northern and Western European ancestry from the CEPH collection (CEU), Toscani from Italia (TSI), Yoruba from Ibadan Nigeria (YRI), Los Angeles residents with Mexican Ancestry (MXL), Japanese from Tokyo, Japan (JPT), Han Chinese from Beijing, China (CHB), Gujarati Indians from Houston, Texas (GIH) and Maasai from Kinyawa, Kenya (MKK). For the TH cohort, we used Japanese from Tokyo, Japan (JPT) and Han Chinese from Beijing, China (CHB). Imputation runs were conducted separately in the mothers and offspring within each of the four HAPO cohorts. We used a conservative allelic r^2 threshold of 0.9 to remove questionable imputed SNPs which then added 565808 (AC), 2181130 (NE), 2651672 (MA) and 2877419 (TH) imputed SNPs.

Association Tests

The genotype call probabilities from the filtered BEAGLE output were used in a linear regression model between each of the phenotypes and the genotype probabilities under an additive model adjusting for the set of model-specific covariates. We used the frequentist approach in SNPTEST v2 to estimate the regression coefficient (β) and standard errors for each regression model and assess the significance of the association between the SNP and the phenotype of interest. Three models were evaluated: Model 1 adjusted for field center, ancestry using PCA, newborn gender, gestational age at delivery, parity and maternal age at OGTT. Model 2 included the covariates from Model 1 plus maternal BMI, height and mean arterial pressure at OGTT, and maternal smoking and drinking status (yes/no). Model 3 included the covariates from Model 2 plus maternal fasting glucose and fasting C-peptide during the OGTT. The

complete list of summary association results is being deposited to dbGAP.

Meta-analysis

The regression coefficients (β) and standard errors were combined across the four cohorts using meta-analysis under a fixed-effects model weighting each stratum by sample size. METAL (54) calculates a *z*-statistic which summarizes the magnitude and direction of effect for the association of a reference allele selected at each marker. After aligning the SNPTEST output from each of the four cohorts to the same reference allele, a weighted sum of the individual cohort results was used to calculate an overall z-statistic and *P*-value. The square root of the cohort specific sample size was used as the proportional weight, and these squared weights summed to 1.

Trans-ethnic meta-analysis

For each of the four ancestry-specific GWAS, we collated the number of study subjects genotyped or imputed at each SNP, the effect allele frequency, the effect (β) for the effect allele and the standard error from the most restrictive of our models, Model 3, for use in a trans-ethnic meta-analysis using MANTRA (34). In contrast to the standard fixed-effects meta-analysis described above, the trans-ethnic approach in MANTRA accommodates between-population heterogeneity of associated variants and their effect sizes by allowing for allelic effects to be most similar between the most closely related populations. Default settings were used in MANTRA breaking chromosomes into segments of 50 000 SNPs or less and relatedness between the four populations was estimated by the mean allele frequency differences between each pairwise population using the dmatcal command in the MANTRA package (34). The $\log_{10}$(Bayes' factor) and posterior probability for association, as well as the direction of the effect, were then compared with the results from the fixed-effects meta-analysis.

Replication study

We designed a custom iSelect array to replicate the strongest associations from the GWAS in an independent cohort of 2296 NE newborns (NE2). The criteria for inclusion on the array were an SNP must (i*)* have *P*-values $<10^{-5}$ in either the discovery NE cohort or the meta-analysis, (ii) have an MAF $>2\%$ and (iii) be independent of other SNPs in the replication array ($r^2 <$ 0.5). The array also included 100 ancestry informative markers that were used to correct for population stratification by adjusting for the first two principal components in the association tests, as was performed with the GWAS data.

Pathway analysis

Pathway analyses were conducted using the gene-set enrichment analysis (GSEA) approach for genotype data implemented in Magenta software (version 2.4) (55). All available pathway databases in Magenta were examined using default parameter settings and database-specific FDR computation. The results are reported for GSEA nominal $P < 0.05$ and FDR < 0.20.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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