

## SHORT COMMUNICATION

## The chromosome 3q25 locus associated with fetal adiposity is not associated with childhood adiposity

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Increased newborn adiposity is associated with later adverse metabolic outcomes. Previous genome-wide association studies (GWAS) demonstrated strong association of a locus on chromosome 3 (3q25.31) with newborn sum of skinfolds, a measure of overall adiposity. Whether this locus is associated with childhood adiposity is unknown. Genotype and sum of skinfolds data were available for 293 children at birth and age 2, and for 350 children at birth and age 6 from a European cohort (Belfast, UK) who participated in the Hyperglycemia and Adverse Pregnancy Outcome GWAS. We examined single nucleotide polymorphisms (SNPs) at the 3q25.31 locus associated with newborn adiposity. Linear regression analyses under an additive genetic model adjusting for maternal body mass index were performed. In both cohorts, a positive association was observed between all SNPs and sum of skinfolds at birth ( $P = 2.3 \times 10^{-4}$ ,  $\beta = 0.026$  and  $P = 4.8 \times 10^{-4}$ ,  $\beta = 0.025$ ). At the age of 2 years, a non-significant negative association was observed with sum of skinfolds ( $P = 0.06$ ;  $\beta = -0.015$ ). At the age of 6 years, there was no evidence of association ( $P = 0.86$ ;  $\beta = 0.002$ ). The 3q25.31 locus strongly associated with newborn adiposity had no significant association with childhood adiposity suggesting that its impact may largely be limited to fetal fat accretion.

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## INTRODUCTION

Fat at birth is unique to humans among primates, suggesting that a recently evolved genetic component accounts for this difference.<sup>1</sup> Body fat accumulated during intrauterine development in humans is thought to serve as an energy source to support growth of the large human newborn brain in the early neonatal period.<sup>1</sup> As such, subcutaneous fat provides adaptive advantages and is thought to be under positive selection. However, high or low body fat at birth is associated with increased morbidity in the first year of life as well as an increased susceptibility to poor metabolic and/or cardiovascular health later in life.<sup>2,3</sup> While maternal fuels such as glucose and triglycerides impact fetal fat accretion, genetic factors also contribute.<sup>4–8</sup> A locus between *CCNL1* and *LEKRI* on chromosome 3 (3q25.31) was previously shown to be associated with birthweight,<sup>5</sup> but our recent genome-wide association study (GWAS) performed in a multi-ancestry cohort of newborns revealed this locus was much more strongly associated with newborn sum of skinfolds ( $P = 1.52 \times 10^{-14}$ ), a measure of newborn adiposity, than with birthweight ( $P = 5.72 \times 10^{-7}$ ).<sup>8</sup> The goal of the present study was to determine whether this same locus is associated with adiposity at later points during childhood.

## MATERIALS AND METHODS

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study was an international multi-center epidemiologic study that

examined levels of maternal glucose tolerance and risk of adverse pregnancy outcomes. Study methods have been previously described.<sup>9</sup> Briefly, eligible women underwent a 75 g oral glucose tolerance test between 24 and 32 weeks gestation. Using standardized methods and procedures, maternal height, weight, and blood pressure were measured, and maternal demographic information was collected. At delivery, cord blood was collected for measurement of newborn glucose, C-peptide and DNA extraction. Using standardized methods and equipment, neonatal anthropometric measurements were obtained within 72 h of birth, including head circumference, birth length, birthweight and skinfold thickness measured at the flank, triceps and subscapular regions. HAPO participants and their offspring from the Belfast, Northern Ireland center were invited to participate in the HAPO Family Study in which offspring were examined at the ages of 2 and 6 years. Measurements included weight, height, head circumference, mid-arm circumference, and triceps, subscapular, and suprailiac skinfold thickness.<sup>10</sup> For genetic analyses, individual skinfold measurements and their sum were log transformed.

In the GWAS examining newborn size, HAPO newborns from four ancestries were genotyped using Illumina genomewide single nucleotide polymorphism (SNP) arrays (Illumina, Inc., San Diego, CA, USA). Details of genotyping platform selection, genotype calling algorithms, quality control and imputation procedures, and estimating population substructure were previously reported.<sup>8</sup>

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We examined the association of four SNPs at this locus with sum of skinfolds, a measure of adiposity, among Belfast HAPO offspring at 2 and 6 years of age using linear regression analyses (SNPTSTv2) with an additive model, adjusting for ancestry (principal components 1 and 2), newborn gender, gestational age at delivery, parity, and maternal age, body mass index (BMI), height, mean arterial blood pressure, smoking status and alcohol intake at the time of oral glucose tolerance test. Results are reported as a nominal *P* value and a  $\beta$  regression coefficient. All other analyses were conducted in SAS 9.3 (SAS Institute, Cary, NC, USA).

## RESULTS

Genotype and phenotype data were available at birth and age 2 for 293 children and at birth and age 6 for 350 children (Table 1); 270 children were common between the two cohorts. Four SNPs at the chromosome 3q25.31 locus that are in strong linkage disequilibrium (lowest pairwise  $r^2 = 0.93$ ), rs10049088, rs1482853, rs17451107 and rs900400, were previously shown to be the SNPs most highly associated with sum of skinfolds in a cohort of HAPO newborns.<sup>8</sup> The association and direction of effect between one of these SNPs, rs900400, and sum of skinfolds as well as individual skinfold thicknesses in the cohorts of 2- and 6-year-olds from the Belfast HAPO Family Study are shown in Table 2.

A positive association was observed between rs900400 and newborn sum of skinfolds among the 293 children in the cohort of 2-year olds ( $P = 4.8 \times 10^{-4}$ ;  $\beta = 0.025$ ), and 350 children in the cohort of 6-year olds ( $P = 2.8 \times 10^{-4}$ ;  $\beta = 0.024$ ). In contrast, at age 2 years and 6 years no association was observed between sum of skinfolds and rs900400 ( $P = 0.06$ ,  $\beta = -0.015$  at age 2;  $P = 0.86$ ,  $\beta = 0.002$  at age 6). Although rs900400 demonstrated a marginal, albeit non-significant, association with sum of skinfolds at age 2 years, the direction of effect was opposite to that observed in the newborns.

Similar analyses were performed examining the association of rs900400 with measurements of the flank/suprailiac, triceps and subscapular skinfolds (Table 2). A positive association was observed between rs900400 and individual skinfold measurements at birth

among the 293 children in the cohort of 2-year olds (triceps,  $P = 3.4 \times 10^{-3}$ ; subscapular,  $P = 2.0 \times 10^{-4}$ ; flank,  $P = 5.0 \times 10^{-3}$ ), and 350 children in the cohort of 6-year olds (triceps,  $P = 1.8 \times 10^{-3}$ ; subscapular,  $P = 5.0 \times 10^{-4}$ ; flank,  $P = 1.0 \times 10^{-3}$ ). For the suprailiac and subscapular skinfold thicknesses, an association with rs900400 was not seen at either age 2 ( $P = 0.14$  and  $P = 0.52$ , respectively) or age 6 ( $P = 0.70$  and  $P = 0.98$ , respectively). However, in the cohort of 2-year olds, rs900400 was weakly associated with triceps skinfold thickness at age 2 ( $P = 0.03$ ). However, the direction of effect was again opposite to that observed in newborns. There was no evidence for association of rs900400 with triceps skinfold thickness at age 6 ( $P = 0.97$ ).

## DISCUSSION

Excess adiposity at birth, which is recognized as a risk factor for peri- and postnatal complications as well as childhood obesity and adverse metabolic health outcomes during adulthood, is influenced by both environmental and genetic factors.<sup>11,12</sup> Multiple maternal factors influence the intrauterine environment, including maternal BMI, height, blood pressure, nutrition, alcohol consumption, smoking and socioeconomic background, with maternal metabolic factors also having an important impact on fetal adiposity.<sup>11,13</sup> In addition to maternal metabolism, fetal genetics also plays an important role in fetal fat accretion. We and others have previously used GWAS to identify genes associated with birthweight and/or newborn sum of skinfolds.<sup>5,6,8</sup> A highly significant and reproducible finding was the association of SNPs within the chromosome 3q25.31 locus with newborn adiposity as reflected by newborn sum of skinfolds or ponderal index.<sup>5,6,8</sup> In this report, we now demonstrate that association of this locus with measures of adiposity is no longer evident at ages 2 and 6 years, consistent with the recent observation that this locus is no longer associated with birthweight by age 2 months.<sup>6</sup> Association of this locus with sum of skinfolds, driven primarily by skinfold thickness at the triceps, approached significance in the cohort of 2-year olds, but this association was in the opposite direction to that observed in newborns. In the cohort of 6-year olds, there was no evidence of association of rs900400 with any of the individual skinfolds. Together with the results of previous studies, these new findings suggest that genetic variation within the 3q25.31 locus contributes to fetal fat accretion but not to early childhood adiposity.

As in newborns, childhood adiposity is under both environmental and genetic control. Both low and normal birthweight newborns with rapid postnatal weight gain during the first 2 years of life have higher body fat and lower lean body mass and, during adulthood, higher body weight and fat.<sup>14,15</sup> Prevention of rapid postnatal growth in such individuals may prevent development of an obese adult phenotype.<sup>2</sup> Genetic variation is another important determinant of childhood adiposity. Through GWAS and related approaches, association of variants within 22 different genetic loci with childhood BMI or body weight has been demonstrated,<sup>16,17</sup> but SNPs in the chromosome 3q25.31 locus have not been reported. Consistent with that, meta-analyses performed by the Early Growth Genetics (EGG) Consortium ([www.egg-consortium.org](http://www.egg-consortium.org)) in European ancestry children failed to demonstrate association of rs900400 with childhood obesity (defined as BMI  $\geq 95^{\text{th}}$  percentile;  $P = 0.90$ ).<sup>17</sup> Similarly, a previous analysis of data from the GIANT consortium showed no evidence of association of the 3q25.31 locus with adult BMI.<sup>6</sup>

Despite the presence of high-quality genotype and phenotype data, our study had certain limitations. We have examined a small cohort of offspring from only one HAPO ancestry and, as a result, our power to demonstrate an association at age 2 and 6 years was limited. Thus, we can not rule out an association of SNPs at the chromosome 3q25.31 locus with early childhood adiposity; however, if this locus is associated with childhood adiposity, the association is much weaker than that observed during fetal life

**Table 1.** Belfast HAPO mother and offspring population characteristics

	Cohort with genotype and sum of skinfolds data at birth and 2 years	Cohort with genotype and sum of skinfolds data at birth and 6 years
Sample size	293	350
Gestational age at OGTT (week)	29.1 $\pm$ 1.2	29.0 $\pm$ 1.2
Age at OGTT (year)	30.3 $\pm$ 5.2	29.9 $\pm$ 5.3
BMI at OGTT (kg m <sup>-2</sup> )	28.5 $\pm$ 4.6	28.2 $\pm$ 4.2
MAP at OGTT (mm Hg)	83.7 $\pm$ 7.9	83.3 $\pm$ 8.0
Fasting plasma glucose (mg dl <sup>-1</sup> )	83.3 $\pm$ 6.5	83.1 $\pm$ 6.1
1-h plasma glucose (mg dl <sup>-1</sup> )	134.9 $\pm$ 29.2	135.1 $\pm$ 29.9
2-h plasma glucose (mg dl <sup>-1</sup> )	109.7 $\pm$ 22.1	109.3 $\pm$ 22.3
Gestational age at delivery (week)	40.1 $\pm$ 1.1	40.1 $\pm$ 1.0
Maternal smoking (%)	59 (20.1%)	88 (25.1%)
Male offspring (n, %)	132 (45.1%)	169 (48.3%)
Primiparous births (n, %)	158 (53.9%)	175 (50.0%)
Offspring birth weight (g)	3455.1 $\pm$ 505.1	3457.3 $\pm$ 503.9
Offspring birth length (cm)	50.9 $\pm$ 2.1	50.8 $\pm$ 2.2
Head circumference (cm)	35.1 $\pm$ 1.3	35.2 $\pm$ 1.3
Fat mass (%)	12.1 $\pm$ 3.5	12.1 $\pm$ 3.5
Sum of skinfolds at birth (mm)*	12.7, 3.2	12.6, 3.1
Sum of skinfolds at 2 years (mm)*	25.4, 7.1	
Sum of skinfolds at 6 years (mm)*		22.5, 9.9

Abbreviations: BMI, body mass index; MAP, mean arterial blood pressure; OGTT, oral glucose tolerance.  $\pm$  indicates standard error. \*Medians and interquartile ranges are reported for sum of skinfolds measurements because of skewed distributions.

**Table 2.** Association of the T Allele of rs900400 with Belfast offspring sum of and individual skinfold thicknesses at birth, 2 years and 6 years

Skinfold	2-years old cohort (n = 293)						6-years old cohort (n = 350)				
	Newborn			2 years			Newborn			6 years	
	AF	P-value	Beta (s.e.)	P-value	Beta (s.e.)		AF	P-value	Beta (s.e.)	P-value	Beta (s.e.)
Sum	0.64	$4.8 \times 10^{-4}$	0.0248 (0.0071)	0.06	-0.0149 (0.0077)		0.63	$2.8 \times 10^{-4}$	0.0235 (0.0064)	0.86	0.0019 (0.0108)
Triceps	0.64	$3.4 \times 10^{-3}$	0.0215 (0.0073)	0.03	-0.0160 (0.0074)		0.63	$1.8 \times 10^{-3}$	0.0206 (0.0065)	0.97	-0.0004 (0.0091)
Subscapular	0.64	$2.0 \times 10^{-4}$	0.0288 (0.0077)	0.52	-0.0064 (0.0099)		0.63	$5.0 \times 10^{-4}$	0.0246 (0.0070)	0.98	0.0003 (0.0128)
Flank/Suprailiac	0.64	$5.0 \times 10^{-3}$	0.0232 (0.0083)	0.14	-0.0170 (0.0114)		0.63	$1.0 \times 10^{-3}$	0.0246 (0.0076)	0.70	0.0056 (0.0105)

Abbreviation: AF, allele frequency (T allele of rs900400). Beta-regression coefficient with logarithmically transformed sum of skinfolds as dependent variable and SNP included in an additive genetic model. Covariates included in the model were age, ancestry, gender, gestational age at delivery, parity, maternal smoking status and alcohol intake during pregnancy, maternal age, body mass index, height and mean arterial blood pressure at the time of oral glucose tolerance test.  $\exp(\beta)$  represents the multiplicative effect on sum of skinfolds associated with each copy of the allele after adjustment for all covariates.

and does not alter our conclusion that the chromosome 3 locus primarily influences fetal fat accretion. Similarly, we were not able to assess directly whether this locus is associated with measures of obesity in older children and adolescents, but previous studies of the genetics of childhood obesity have failed to demonstrate an association.<sup>16,17</sup>

In summary, we have demonstrated that SNPs within the chromosome 3q25.31 locus, which exhibit strong association across multiple ancestry groups with measures of newborn adiposity,<sup>5,6,8</sup> do not have a clear association with early childhood adiposity among European ancestry HAPO offspring.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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