Birth weight modifies the association between central nervous system gene variation and adult body mass index

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Genome wide association studies have identified ~ 100 loci associated with body mass index (BMI). Persons with low birth weight have an increased risk of metabolic disorders. We postulate that normal mechanisms of body weight regulation are disrupted in subjects with low birth weight. The present analyses included 2215 African American women from the Black Women’s Health Study, and were based on genotype data on 20 BMI-associated loci and self-reported data on birth weight, weight at age 18 and adult weight. We used general linear models to assess the association of individual single-nucleotide polymorphisms (SNPs) with BMI at age 18 and later in adulthood within strata of birth weight (above and below the median, 3200 g). Three SNPs (rs1320330 near TMEM18, rs261967 near PCSK1 and rs17817964 in FTO), and a genetic score combining these three variants, showed significant interactions with birth weight in relation to BMI. Among women with birth weight <3200 g, there was an inverse association between genetic score and BMI; beta-coefficient = −0.045 (95% confidence intervals (CI) −0.104, 0.013) for BMI at age 18, and −0.055 (95% CI −0.112, 0.002) for adult BMI. Among women with birth weight ≥3200 g, genetic score was positively associated with BMI; beta-coefficient = 0.110 (95% CI 0.051, 0.169) for BMI at age 18 (P for interaction = 0.0002), and 0.112 (95% CI 0.054, 0.170) for adult BMI (P for interaction < 0.0001). Because TMEM18, PCSK1 and FTO are highly expressed in the central nervous system (CNS), our results suggest that low-birth weight may disrupt mechanisms of CNS body weight regulation.

INTRODUCTION

Low birth weight, a marker of compromised fetal growth, has consistently been found to be associated with higher risk of type 2 diabetes (T2D) in adulthood.1-2 Although it was initially postulated that the association between low birth weight and metabolic disorders in adulthood was in part due to a higher risk of obesity,2-5 recent large-scale meta-analyses have reported that persons who had a low birth weight have in fact a lower adult body mass index (BMI) and a decreased risk of being overweight or obese later in life, compared with subjects with normal birth weight.6-8 Findings from our study of participants in the Black Women’s Health Study (BWHS) indicate that the association between low birth weight and adult risk of T2D is not mediated through BMI.9 Growing evidence suggests that alterations of the neuroendocrine system,10-13 deregulation of lipid metabolism14-16 and pancreatic dysfunction17-19 rather than increased risk of obesity may have a key mediating role between low birth weight and risk of T2D and other metabolic disorders in adulthood.

Genome wide association studies (GWAS)—in mostly European ancestry populations—have identified ~ 100 genetic loci belonging to multiple pathways, such as central nervous system (CNS) function, insulin secretion and action, energy metabolism and lipid biology and adipogenesis associated with variation in BMI and body weight.20-25 In African ancestry populations only eight of these loci show genome-wide significant association (P ≤ 5×10^{-8}) with BMI, and twenty loci have significant association at the gene-wide level (P ≤ 0.001).21 We postulate that because of the multiple alterations associated with low birth weight, normal genetic mechanisms of body weight regulation are not completely functional in persons who had a low birth weight. Thus, the association between BMI-associated gene variants and body weight would be modified among individuals with low birth weight. In particular, because pathway analysis shows a key role of the CNS in body weight regulation,25 we hypothesize that CNS gene variants are more likely to interact with birth weight in relation to adult BMI.

We tested this hypothesis in the BWHS, a prospective cohort study of 59 000 African American women.

MATERIALS AND METHODS

Study subjects

The present analyses were carried out in data from the BWHS. The BWHS began in 1995 when 59 000 African American women 21–69 years of age from across the continental US completed a 14-page postal questionnaire that included comprehensive questions on anthropometric measures, medical history, use of medications, demographic factors, reproductive history and behavioral factors.26 Participants were approximately equally distributed in the
Northeast, South, Midwest and West. Participants have been followed through biennial questionnaires to collect information on incident diseases and update information on risk factors. Follow-up through biennial questionnaires has been ~80% of the baseline cohort. DNA samples were obtained from BWHS participants by the mouthwash-swish method with all samples stored in freezers at −80 °C. Saliva samples were provided by ~50% of BWHS participants (26,800 women). The study protocol was approved by the Institutional Review Board of Boston University. Written informed consent was obtained from all subjects.

Subjects for the present analysis were BWHS participants who had previously been selected as controls for a nested case control study of genes and environment in relation to T2D and obesity risk. They were participants who had not been diagnosed with T2D, had provided a DNA sample and completed questions on birth weight on the 1997 questionnaire. The final analytic sample size included 2,215 subjects with information on birth weight and complete genotyping of 20 BMI-associated single-nucleotide polymorphisms (SNPs). This sample size allows us 80% power to identify an effect of 0.03 or higher of the genetic variants on BMI transformed residuals. This effect is within the range of genetic effects found in a recent GWAS meta-analysis of BMI in African ancestry subjects.

**Selection of SNPs and genotyping**

We selected the 20 SNPs that were found to be associated with BMI at the genome-wide level (P ≤ 0.001, including SNPs associated at the genome-wide level) in a recent GWAS meta-analysis in African ancestry subjects. DNA samples were genotyped on an Affymetrix Axiom 45-K custom array (Affymetrix, Santa Clara, CA, USA) designed to include genes and SNPs related to BMI and T2D, or related to relevant pathways such as adiponectin and leptin levels, fasting insulin and glucose, insulin resistance and fatty acid metabolism. In addition, the array included ~3K ancestral informative markers to estimate percentage of European ancestry. Genotyping was carried out at the Affymetrix laboratory, Santa Clara, CA. The genotype data passed Affymetrix quality control standards. Final mean calling rate for SNPs and subjects was 99.5%, mean reproducibility among blinded duplicates was 99.7% and mean concordance with HapMap samples was 99.5%.

**Assessment of BMI**

Information on weight at age 18, current weight, and current height, obtained from the baseline questionnaire (1995), were used to calculate BMI (kg m$^{-2}$). On the 1997 follow-up questionnaire, women were asked their birth weight in pounds and ounces, if known. We used information from both questions to create categories of birth weight (bottom 50% vs. top 50%; and low birth weight $\leq 2500$ g vs normal birth weight $\geq 2500$ g). Subjects with birth weight $\geq 2500$ g were classified as normal birth weight. Subjects with birth weight $< 2500$ g were classified as low birth weight. We then calculated a genetic risk score using the SNPs with a nominal significant interaction ($P \leq 0.05$) with birth weight in relation to BMI. The score is the sum of BMI increasing alleles. Association of the genetic score with BMI and interaction with birth weight was assessed using general linear models as described for the individual SNPs. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

**RESULTS**

Table 1 shows characteristics of the study participants by birth weight categories (bottom 50%: $< 3200$ g, top 50%: $\geq 3200$ g). Subjects with birth weight in the top 50% had higher BMI both at age 18 and adulthood compared with subjects in the bottom 50% of birth weight.

Table 1 Baseline (1995) characteristics of BWHS participants, by birth weight

| Characteristic | $< 3200$ g | $\geq 3200$ g |
|---------------|------------|--------------|
| Number of women | 1113 | 1102 |
| Gene score (mean) | 2.8 | 2.8 |
| European ancestry, % (mean) | 22.0 | 22.6 |
| Age, y (mean) | 39.9 | 40.3 |
| BMI at age 18, kg m$^{-2}$ (mean) | 20.9 | 21.5 |
| BMI, kg m$^{-2}$ (mean) | 27.1 | 27.9 |
| Energy intake (1995), kcal per day (mean) | 1482 | 1469 |

**Smoking, %**

| Smoking, % | $< 3200$ g | $\geq 3200$ g |
|------------|------------|--------------|
| Never | 63 | 64 |
| Past | 21 | 21 |
| Current | 16 | 15 |

**Vigorous exercise, %**

| Vigorous exercise, % | $< 3200$ g | $\geq 3200$ g |
|---------------------|------------|--------------|
| None | 31 | 31 |
| $< 1$ h per week | 18 | 18 |
| $1$–$4$ h per week | 40 | 38 |
| $\geq 5$ h per week | 12 | 13 |

**Education**

| Education | $< 12$ years | $12$–$15$ years | $16$ years | $\geq 17$ years |
|-----------|-------------|----------------|-----------|----------------|
| $\leq 12$ years | 13 | 14 | 36 | 35 |
| $13$–$15$ years | 26 | 23 | 24 | 28 |

Abbreviations: BMI, body mass index; BWHS, Black Women’s Health Study.
Table 2 List of selected SNPs

| SNP          | Gene | Alleles | EAF | GWAS Beta |
|--------------|------|---------|-----|-----------|
| rs12562499   | PTBP2| C/G     | 0.09| 0.048     |
| rs543874     | SEC16B| G/A     | 0.24| 0.060     |
| rs1320330    | TMEM18| G/T     | 0.88| 0.061     |
| rs7586879    | POMC | T/C     | 0.76| 0.042     |
| rs9816226    | ATV5 | T/A     | 0.80| 0.042     |
| rs10938397   | GNPD2| G/A     | 0.23| 0.053     |
| rs7708584    | GALNT1| A/G   | 0.31| 0.040     |
| rs261967     | PCSK1| C/A     | 0.39| 0.026     |
| rs974417     | KLHL32| C/T     | 0.65| 0.031     |
| rs987237     | TFAP2B| G/A     | 0.11| 0.051     |
| rs10968576   | LRRN6C| G/A     | 0.17| 0.037     |
| rs10501087   | BDNF | T/C     | 0.93| 0.081     |
| rs7138803    | FAIM2| A/G     | 0.18| 0.047     |
| rs10150332   | NXXN3| C/T     | 0.34| 0.034     |
| rs2241423    | MAP2K5| G/A   | 0.63| 0.028     |
| rs7359397    | SHZB1| T/C     | 0.08| 0.053     |
| rs17817964   | FTO  | C/T     | 0.12| 0.073     |
| rs12597579   | GP2  | C/T     | 0.90| 0.037     |
| rs6567160    | MCAR | C/T     | 0.22| 0.059     |
| rs2287019    | QCPLT| C/T     | 0.91| 0.066     |

The birth weight groups were similar with respect to the other characteristics—age, % European ancestry, energy intake, smoking, vigorous exercise and education.

Table 2 shows the list of 20 selected SNPs and their association with BMI at age 18, and adult BMI in the BWHS. Most of the SNPs (16 for BMI at age 18, and 19 for adult BMI) showed directionally consistent effects compared with previous GWAS results. The magnitude of the effects was also consistent for most of the examined variants.

Table 3 shows SNP–BMI association results stratified by birth weight (bottom 50%: <3200 g, top 50%: ≥3200 g). Two SNPs, rs1320330 near TMEM18 and rs261967 near PCSK1, showed significant interactions with birth weight in relation to both BMI at age 18 and adult BMI; and one SNP, rs17817964 in FTO, had a significant interaction with birth weight in relation to adult BMI only. For these three SNPs, the effect allele was associated with higher BMI among women with birth weight ≥3200 g, and either a weaker positive association or an inverse association with BMI among women with birth weight <3200 g. For example, the beta (95% CI) for the G allele in rs1320330 was 0.205 (0.085, 0.325) among women with birth weight ≥3200 g and 0.032 (−0.087, 0.150) among women with birth weight <3200 g for BMI at age 18 (P for interaction = 0.04); for adult BMI, it was 0.146 (0.029, 0.263) among women with birth weight ≥3200 g and −0.077 (−0.193, 0.039) among women with birth weight <3200 g (P for interaction = 0.008).

Table 4 shows the association of genetic score with BMI stratified by birth weight. The genetic score is the sum of BMI-increasing alleles of the three SNPs (rs1320330, rs261967 and rs17817964) found to have significant interactions with birth weight. The mean of the genetic score in the study sample was 2.8 alleles, with ranged 0–6 alleles. Genetic score was positively associated with BMI both at age 18 and adulthood in multivariate models adjusted for age, European ancestry, dietary energy intake, smoking, vigorous physical activity and education. The increase in BMI residuals per allele was 0.031 (95% CI 0.004, 0.058) for BMI at age 18 and 0.027 (95% CI 0.014, 0.040) for BMI at adulthood. The genetic score was positively associated with BMI at age 18 among individuals with birth weight in the top 50% (≥3200 g), beta = 0.110 (95% CI 0.051, 0.169); and tended to have a negative association with BMI among individuals with birth weight in the bottom 50% (<3200 g), beta = −0.045 (95% CI −0.105, 0.013; P for interaction = 0.0002). Similar results were observed for adult BMI; a positive association with BMI among women with birth weight ≥3200 g, beta = 0.112 (95% CI 0.054, 0.170), and a negative association with BMI among women with birth weight <3200 g, beta = −0.055 (95% CI −0.112, 0.002; P for interaction < 0.0001). In secondary analyses we categorized birth weight as low birth weight (<2500 g, n = 349), and normal birth weight (≥2500 g, n = 1866). There was no association of genetic risk score and BMI among persons with low birth weight for both BMI at age 18 and adulthood. A positive association was present among individuals with normal birth weight, beta = 0.040 (95% CI −0.006, 0.086) for BMI at age 18; and 0.038 (95% CI −0.007, 0.082) for adult BMI.

DISCUSSION
In the present study, we proposed and assessed the hypothesis that the association between variants in BMI-associated genes and body weight is modified by birth weight. In particular, because of the
Table 3 Beta-coefficients for the association of individual SNPs with BMI at age 18 and adulthood in the Black Women’s Health Study, overall and by categories of birth weight

| SNP          | Alleles | < 3200 g | ≥ 3200 g | P*  | < 3200 g | ≥ 3200 g | Pc  |
|--------------|---------|----------|----------|-----|----------|----------|-----|
| rs12562499   | C/G     | 0.030    | 0.103    | 0.46| -0.016   | 0.112    | 0.19|
| rs543874     | G/A     | 0.076    | 0.160    | 0.19| 0.099    | 0.118    | 0.07|
| rs1320330    | G/T     | 0.032    | 0.205    | 0.04| -0.077   | 0.146    | 0.008|
| rs7586879    | T/C     | 0.064    | 0.044    | 0.77| 0.021    | 0.064    | 0.50|
| rs9816226    | T/A     | 0.139    | 0.105    | 0.64| 0.097    | 0.112    | 0.83|
| rs10938397   | G/A     | -0.060   | 0.051    | 0.09| -0.007   | 0.012    | 0.77|
| rs7708584    | A/G     | 0.044    | 0.078    | 0.58| 0.027    | 0.123    | 0.10|
| rs261967     | C/A     | -0.099   | 0.073    | 0.002| -0.063   | 0.069    | 0.02|
| rs974417     | C/T     | -0.008   | 0.038    | 0.43| -0.038   | -0.059   | 0.71|
| rs987237     | G/A     | 0.021    | 0.145    | 0.16| 0.075    | 0.087    | 0.05|
| rs10968576   | G/A     | 0.114    | 0.071    | 0.56| 0.106    | 0.060    | 0.52|
| rs10501087   | T/C     | -0.059   | 0.045    | 0.34| 0.078    | 0.060    | 0.86|
| rs7138803    | A/G     | 0.011    | 0.037    | 0.71| 0.068    | 0.043    | 0.72|
| rs10150332   | C/T     | 0.040    | 0.001    | 0.50| 0.015    | -0.013   | 0.63|
| rs2241423    | G/A     | 0.051    | 0.064    | 0.82| 0.064    | 0.110    | 0.41|
| rs735997    | T/C     | 0.086    | 0.079    | 0.95| 0.063    | 0.065    | 0.98|
| rs17817964   | T/C     | -0.007   | 0.062    | 0.42| -0.020   | 0.168    | 0.03|
| rs12597579   | C/T     | -0.008   | 0.007    | 0.87| 0.116    | 0.011    | 0.25|
| rs6567160    | C/T     | 0.051    | 0.036    | 0.82| 0.074    | 0.054    | 0.75|
| rs2287019    | C/T     | 0.057    | 0.046    | 0.90| 0.038    | 0.028    | 0.92|

Abbreviations: BMI, body mass index; CI, confidence intervals; SNP, single-nucleotide polymorphisms.

Table 4 Beta-coefficients for the association of genetic scorea with BMI at age 18 and adulthood in the Black Women’s Health Study, overall and by categories of birth weight

| BMI at age 18 | Adult BMI |
|---------------|-----------|
| All subjects (n=2215) | 0.031 (-0.011, 0.073) | 0.027 (-0.014, 0.068) |

Abbreviations: BMI, body mass index; CI, confidence intervals.

aChange in BMI residuals for each copy of the effect allele. Adjusted for age, % European ancestry, dietary energy intake, smoking, vigorous exercise and education. Betas for BMI at age 18 were adjusted for % European ancestry only.

bP for interaction.
modified in these subjects. We tested 20 SNPs, located in or nearby genes expressed in a variety of tissues, which had previously been found to be associated with BMI in African ancestry subjects. Three of the SNPs (rs1320330 near TMEM18, rs261867 near PCSK1 and rs17817964 in FTO), and a genetic score calculated using these three variants, interacted with birth weight in relation to BMI. All three are located in or near genes that are highly expressed in the CNS.

Available evidence suggests that TMEM18, PCSK1 and FTO regulate body weight in part through their actions in the CNS and adipose tissue. TMEM18, which codes a transmembrane nuclear protein with a wide distribution of tissue expression, is downregulated in the hypothalamus of rats and adipose tissue of mice after high-fat feeding. In addition, TMEM18 expression is upregulated during in vitro human adipocyte differentiation, and downregulated in adipose tissue of obese subjects, suggesting TMEM18 action in adipose tissue too. PCSK1 codes for the neuroendocrine convertase 1 (PC1) that is involved in the processing of several hormones and neuropeptides that regulate feeding behavior and energy metabolism. FTO is a gene that is mostly expressed in neuroendocrine cells such as in brain and pituitary. Congenital deficiency of PC1 leads to a severe hormonal dysfunction and early-onset obesity. The FTO gene was one of the first loci found to be associated through GWAS with body weight and adiposity.

The present study has several strengths including its large size, and ability to control for important confounding variables. It also has some limitations. First, information on birth weight was self-reported many years after the fact, raising the possibility of non-differential exposure misclassification. However, we found high correlation between self-reported birth weight and birth registry data in our validation study. Second, although TMEM18, PCSK1 and FTO are highly expressed in the CNS, they are also expressed in other tissues. Therefore, we cannot rule out the possibility that the observed interactions are also mediated by gene activity in other tissues (for example, adipose and pancreas). Third, the SNPs that were assessed in the present study were selected because of their association with BMI in African ancestry subjects. It remains to be determined whether the same interactions would be observed in other populations such as European- or Asian-ancestry individuals. Finally, we did not have information about maternal characteristics during pregnancy (for example, maternal gestational diabetes and maternal malnutrition) that could affect both birth weight and adult body weight in the offspring and potentially confound our results. However, if gestational diabetes and maternal malnutrition affects adult body weight of the offspring mostly through birth weight, it is unlikely that these unmeasured maternal variables would have a major impact in our results.

In summary, our results show that birth weight modifies the association between BMI-associated gene variants and body weight. Specifically, the association between genetic polymorphisms and body weight was weaker or in the opposite direction in subjects having lower birth weights. The SNPs found interacting with birth weight are nearby genes highly expressed in the CNS, suggesting that normal CNS mechanisms of body weight regulation are altered in persons with low birth weight.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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