Common Variants Near Melanocortin 4 Receptor Are Associated with General and Visceral Adiposity in European- and African-American Youth

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Objective Recent genome-wide association studies found common variants near the melanocortin 4 receptor gene associated with obesity. This study aimed to assess the influence of the identified single nucleotide polymorphisms rs17782313 and rs17700633 on general and visceral adiposity in European- and African-American youth.

Study design In 1890 youth (49.1% European-American, 45.6% male, mean age 16.7 years), we examined the associations of the rs17782313 and rs17700633 with anthropometry, percent body fat, visceral adipose tissue, and subcutaneous abdominal adipose tissue. Interaction of the single nucleotide polymorphisms with ethnicity or sex was investigated and haplotype analyses conducted.

Results Rs17782313 was significantly associated with weight ($P = .02$) and waist circumference ($P = .03$) in all subjects and with body mass index ($P = .002$) in females. In females rs17700633 was significantly associated with percent body fat ($P = .001$), visceral adipose tissue ($P < .001$), and subcutaneous abdominal adipose tissue ($P < .001$). Rs17700633 was significantly associated with fasting insulin and homeostasis model assessment, but the significance attenuated after adjustment for percent body fat. These findings were confirmed by haplotype analysis. No significant interactions of the variants with ethnicity were found for any of these phenotypes.

Conclusions The relatively large effect of these common variants near melanocortin 4 receptor on general and visceral adiposity in childhood, especially in girls, could prove helpful in elucidating the molecular mechanisms underlying the development of obesity in early life. *(J Pediatr 2010;156:598-605)*.

Obesity in childhood and adolescence is epidemic worldwide and is associated with several comorbidities such as hypertension, dyslipidemia and type 2 diabetes.[1,2] Identification of genetic determinants of such a complex disease by linkage analysis or candidate-gene–based association approach has only been modestly fruitful, despite extensive efforts. The emergence of the genome-wide association study (GWAS) approach has provided more clues to the allelic architecture of complex diseases and traits.[3]

Loos et al[4] carried out a large-scale meta-analysis of GWAS data available for 16 876 adult samples of European descent. A cluster of single nucleotide polymorphisms (SNPs) on chromosome 18q21 (55700-56400) was shown to be associated with body mass index (BMI) as a measure of general adiposity. This region seemed to contain at least 2 independent association signals (rs17782313 and rs17700633). The strongest association signal (rs17782313, $P = 2.9 \times 10^{-6}$) was mapped 188 kb downstream of the melanocortin 4 receptor (MC4R). Haplotype analysis for rs17782313 and rs17700633 in EPIC-Norfolk confirmed that rs17782313 drives the association. Loos et al[4] also found that the effect of rs17782313 on BMI in children was about twice of that observed in adults, which indicated relevance for early-onset obesity characteristics. This finding may be an important clue for the design of functional studies of these variants. Another GWAS in adults of Indian Asian and European descent found that common variants near MC4R were associated with waist circumference and insulin resistance (IR).[5] However, further replication studies in populations of different ethnic origin with better measures of adiposity are necessary to establish a definite relationship between these variants and obesity-related phenotypes, especially in children.

AA	African-American	HTR	Haplotype trend regression
APEX	Adiposity Prevention through EXercise	HWE	Hardy-Weinberg equilibrium
%BF	Percent body fat	IR	Insulin resistance
BMI	Body mass index	LACHY	Lifestyle, Adiposity and Cardiovascular Health in Youths
CI	Confidence interval	LD	Linkage disequilibrium
DXA	Dual-energy X-ray absorptiometry	MAF	Minor allele frequencies
EA	Europe-American	MC4R	Melanocortin 4 receptor
FTO	Fat mass and obesity-associated	MRI	Magnetic resonance imaging
GWAS	Genome-wide association study	SAAT	Subcutaneous abdominal adipose tissue
HOMA	Homeostasis model assessment	SNP	Single nucleotide polymorphism
HOMA 2-%B	Homeostasis model assessment 2 β-cell function	VAT	Visceral adipose tissue

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Thus the aim of this study is to assess whether the previously identified SNPs near *MC4R* (rs17782313 and rs17700633) by GWAS are associated with adiposity and insulin resistance in African-American (AA) and European-American (EA) youth available from the Georgia Cardiovascular Twin study,[6] the Lifestyle, Adiposity and Cardiovascular Health in Youths (LACHY) study,[7] and the Adiposity Prevention through EXercise (APEX) study.[8]

Methods

This study included 1890 subjects from 3 cohorts, the Georgia Cardiovascular Twin study (n = 1179 twins, with 565 monozygotic [282 pairs and 1 singleton] and 614 dizygotic twins [279 pairs and 56 singletons]), the LACHY study (n = 527, including 38 sib-pairs), and the APEX study (n = 184, including 21 sib-pairs). All twins in the Georgia Cardiovascular Twin study were recruited from public middle and high schools in the Augusta, Georgia, area, and the cohort[6] consisted of roughly equal numbers of AA and EA boys and girls (55.1% EA, 47.5% male, mean age [SD]: 18.1[3.7] years). All twin pairs were reared together, and zygosity was determined by genotyping 5 standard microsatellite markers with buccal swabs or buffy coat DNA.[9] The LACHY study consisted of approximately equal numbers of EA and AA boys and girls (52.8% EA, 44.2% male) aged 14 to 18 years recruited from high schools in the Augusta, Georgia, area.[7] In the APEX study, subjects were AA boys and girls only (37.5% male), aged 8 to 12 years recruited from local elementary schools. Subjects eligible for the study were only those that weighted <136.1 kg and were not taking any medication known to affect body composition or fat distribution.[8] The criteria for classifying subjects as AAs or EAs with self-identification of ethnicity have been described previously.[10] Subjects in all the 3 studies were overtly healthy, free of any acute or chronic illness on the basis of parental reports, and taking no medication that could influence the results. The Institutional Review Board at the Medical College of Georgia approved the studies. Informed consent was obtained from all subjects and by parents if subjects were <18 years of age.

Height and weight were measured by standard methods with a wall-mounted stadiometer and a digital scale, respectively. BMI was calculated as weight/height^2 (kg/m^2). Waist circumference (in centimeters) was measured twice at the center of the umbilicus over the T-shirt, and the values were averaged. Skinfold thicknesses (ie, triceps, subscapular, and suprailiac) were measured on the right side of the body with Lange calipers according to established protocols.[11] Three sets of measurements for each skinfold were recorded and averaged. The intercorrelations were >99%. Measurements of skinfold thickness were available in 1888 subjects. BMI and the sum of the 3 skinfold thicknesses were used as measures of general adiposity, and waist circumference was used as a measure of central adiposity.

The blood was frozen quickly and glycolysis was immediately inhibited. Fasting glucose and insulin concentrations were measured at the NIDDK supported Clinical Nutrition Research Unit Core Laboratory at the University of Alabama. Glucose was measured in 10 μL of sera using an Ektachem DT II system (Johnson and Johnson Clinical Diagnostic, Rochester, New York). Insulin was assayed in duplicate 100-μL aliquots of serum by specific radioimmunoassay (Linco Research, Inc., St Charles, Missouri). Cross-reactivity with proinsulin is <0.2%. Assay sensitivity was 3.41 mU/mL. The intraassay coefficient of variation was 3.7%. Fasting glucose and insulin were only available in a subsample of twins because twins coming on afternoon visits were not required to fast. On the basis of fasting glucose and insulin, we used the homeostasis model assessment (HOMA) 2 to calculate insulin resistance (HOMA2-IR) and β-cell function (HOMA2-%B) with a nonlinear computer model as specified in the HOMA2 software (http://dtu.ox.ac.uk/homa).

In the LACHY study, percent body fat (%BF) was measured by use of dual x-ray absorptiometry (DXA) (Hologic QDR-4500 W, software version 6.0; Hologic, Bedford, Massachusetts). DXA provides reliable values for %BF[7]. Repeat measurements were performed with the QDR-4500 W machine with 219 adolescents and the intraclass correlation coefficient for %BF was found to be 0.99. For some subjects, DXA values were only available from the Hologic QDR-1000 W, but not from the Hologic QDR-4500 W model. For these individuals, %BF values were derived from prediction equations on the basis of 284 youths who were assessed on both instruments, by use of linear regression; ethnicity, sex and QDR-1000 W measurement were the predictor variables. The multiple R^2 value for %BF was 0.99.[12] In the APEX study, all %BF measurements were obtained with a Hologic QDR-1000 (Hologic) as previously described, the intraclass correlation coefficient for %BF was >0.998 between 2 scans.[13] DXA calibration was done each day, as specified by Hologic. DXA scans were not performed in the Georgia Cardiovascular Twin study.

In both the LACHY and APEX studies, visceral adipose tissue (VAT) and subcutaneous abdominal adipose tissue (SAAT) was determined with magnetic resonance imaging (MRI; 1.5 T General Electric Medical Systems, Milwaukee, Wisconsin) as described previously.[14] Briefly, with subjects in the supine position, a series of five 1-cm–thick transverse images was acquired beginning at the inferior border of the fifth lumbar vertebra and proceeding toward the head. A gap is left between the slices to avoid cross-talk. To calculate volumes for VAT and SAAT, the cross-sectional area (cm^2) from each slice was multiplied by the slice width (1 cm); the 5 individual volumes (cm^3) were then summed as described previously.[15] VAT and SAAT were measured in the Department of Radiology on equipment dedicated to patient care. VAT and SAAT measures were obtained in those subjects who underwent testing on days when the MRI equipment was available for the research study. Eventually, VAT and SAAT measurements were available for 397 subjects, respectively. No measurements of VAT and SAAT were available in the Georgia Cardiovascular Twin study and males in the APEX study.