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Influence of common variants in *FTO* and near *INSIG2* and *MC4R* on growth curves for adiposity in African– and European–American youth

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Abstract Recent genome-wide association (GWA) studies identified several common variants for obesity: rs9939609 in *FTO*, rs7566605 near *INSIG2* and both rs17782313 and rs17700633 near the *MC4R* gene. This study aimed to assess the influence of these polymorphisms on development of adiposity in European– (EA) and African–American (AA) youth in two ongoing longitudinal studies including 986 and 606 participants with age ranges of 10–25.8 and 4.0–23.9 years, respectively. Individual growth curve modeling was conducted separately in the two studies. We tested the effect of the SNPs on levels and increase with age (i.e., slope) of weight, body mass index (BMI), waist circumference and skinfolds from childhood to adulthood, and potential moderation by ethnicity or gender. Beta coefficients computed in the two studies were pooled using meta-analysis. Rs9939609 was associated with logtransformed levels of BMI ($\beta = 0.021$, $P = 0.01$), weight ($\beta = 0.019$, $P = 0.04$) and waist circumference ($\beta = 0.012$, $P = 0.04$). Rs17782313 was associated with triceps ($\beta = 0.05$, $P = 0.02$). Significant interactions of rs17700633 with gender were observed on subscapular-, suprailiac- and sum of skinfolds, with significant associations limited to males ($P < 0.05$). No significant interactions with ethnicity were found. Only one effect on the slope was observed, rs17700633 showed a significant interaction with age on triceps ($\beta = 0.004$, $P = 0.04$). In two longitudinal studies of EA and AA youth, we replicated the effect of *FTO* and common variants near *MC4R* on general and central adiposity. These variants did not affect the increase with age of adiposity from childhood to adulthood with one exception. Common variants for obesity identified in GWA studies have detectable but modest effects on growth curves for adiposity in EA and AA youth.

Keywords Adiposity · Genetic · Growth curve model

Abbreviations

AA	African–American
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
DZ	Dizygotic
EA	Europe-American
GEE	Generalized estimating equations
Georgia CV	Georgia cardiovascular

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GWA	Genome-wide association
HWE	Hardy–Weinberg equilibrium
LD	Linkage disequilibrium
MAF	Minor allele frequencies
MZ	Monozygotic
SES	Social economic status
SNPs	Single nucleotide polymorphisms

Introduction

Obesity is becoming an increasingly important clinical and public health challenge worldwide and is associated with several comorbidities such as type 2 diabetes, cardiovascular diseases, metabolic syndrome and certain forms of cancer [1–3]. The complex etiology of obesity reflects effects of genes and environment as well as their interactions [4]. In this context, an understanding of the effects of environment and genes on obesity and also their interactions is important to provide a basis for determining the role they could have on the development and prevention of obesity.

In recent years, several independent genome-wide association (GWA) studies reported significant associations of common genetic variants near *INSIG2*(rs7566605) [5], in the *FTO* gene (rs9939609) [6–9] and near *MC4R* (rs17782313, rs17700633) [10] with body mass index (BMI) as a measure of general adiposity and for *MC4R* rs12970134 with waist circumference as a measure of central adiposity [11]. Associations of *FTO* and obesity have been broadly replicated in various populations including Caucasians, Asians and African–American children [7, 8, 12, 13], but could not be confirmed in an African population [14]. Similarly, the association of the common variants near *MC4R* with obesity were replicated in Caucasians [15–19], but two recent studies showed inconsistent findings in African–Americans [15, 19]. In contrast, the association of the common variant near *INSIG2* has not been consistently replicated [20, 21].

The rise of overweight and obesity in youth is of particular concern in the current obesity epidemic. Many overweight and obese children go on to become obese adults [22] and are at greater risk of developing cardiovascular disease and metabolic syndrome [1, 2]. Thus greater insight into the development of adiposity from childhood into adulthood is needed. However, most of the above mentioned studies were cross-sectional and do not offer information on the impact of genetic susceptibility on interindividual differences in development of obesity over time. To the best of our knowledge, the few available longitudinal studies [16, 23–25] have not investigated the influence of these common variants on growth curves for adiposity from childhood to adulthood, nor did they study the interaction of these common variants with ethnicity or gender.

The main purpose of the current study was to assess the effect of these common variants in *FTO*, near *INSIG2* and near *MC4R* on longitudinal development of general and central obesity from childhood to early adulthood in European–American (EA) and African–American (AA) youth available from the Georgia Cardiovascular (CV) Twin study [26] and the Blood Pressure (BP) Stress Cohort study [27, 28]. We further investigated whether the effects of these common variants on general and central obesity were moderated by ethnicity or gender.

Subjects and methods

Subjects

Subjects are among participants in two ongoing longitudinal studies, the Georgia CV Twin study [26] [($n = 986$ twins, with 480 monozygotic (MZ) (234 pairs and 12 singleton) and 506 dizygotic (DZ) twins (230 pairs and 46 singletons)) and the BP Stress study [27, 29] ($n = 606$, including 135 siblings). The twin longitudinal data encompass 4 assessments (ranged from 2 to 4) over a 13-year period (1996–2009). All twins were recruited from public middle and high schools in the Augusta, Georgia area and the cohort consisted of roughly equal numbers of AAs and EAs (56.5% EA, 47.9% male, mean age at entry [SD]: 14.3[2.7] years, ranged from 10.0 to 25.8 years). All twin pairs were reared together and zygosity was determined by genotyping 5 standard microsatellite markers using buccal swabs or buffy coat DNA [30]. The BP Stress study consisted of approximately equal numbers of EA and AA boys and girls (50.7% EA, 49.3% male, mean age at entry [SD]: 11.9[4.0], ranged from 4.0 to 23.9 years). The BP Stress study is an ongoing longitudinal study of the development of cardiovascular risk factors in which evaluations have been conducted annually from 1989 to 2007 encompassing up to 16 assessments (ranged 2–16). Information on subject recruitment, evaluation that started in 1989 and attrition rate have been previously described [29, 31]. The criteria for classifying subjects as AAs or EAs using self-identification of ethnicity have been described previously [32]. Subjects were apparently healthy, free of any acute or chronic illness on the basis of parental reports and were 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.

The data sets are complicated because not all subjects had the same number of visits, with subjects recruited into

the two studies at different ages and different years. In the current study, only subjects with at least 2 visits were included because the analyses depend on fitting polynomial regression curves to each subject's data. In the Georgia CV Twin study 65.2% of subjects had ≥3 visits, and in the BP Stress study 99.8% of subjects had ≥3 visits and 93.7% had ≥8 visits, making the data set very informative for the study of obesity changes over time. The annualized attrition rate has been <6%/year in the Georgia CV Twin study and <4%/year in the BP stress study, which has been primarily due to some of the subject moving out of the region. There have been no significant differences in age, ethnicity, or sex distributions between the dropouts and the subjects that remained in the study.

Anthropometrics assessment

Height and weight were measured by standard methods using a wall-mounted stadiometer and a digital scale, respectively. BMI was calculated as weight/height^2 (kg/m^2). Waist circumference (in cm) was measured twice at the center of the umbilicus over the T-shirt and the values were averaged. Skinfold thicknesses (i.e., triceps, subscapular, and suprailiac) were measured on the right side of the body with Lange calipers according to established protocols [33]. Three sets of measurements for each skinfold were recorded and averaged. The inter-correlations were >99%. BMI and the sum of the 3 skinfold thicknesses were used as measures of general adiposity, while waist circumference was used as a measure of central adiposity.

Genotyping

DNA was extracted from buffy coats by using the QiaAmp DNA Blood Mini Kit (Qiagen, Valencia, CA) or from buccal swabs by using QuickExtract DNA Extraction Kit (Epicentre, Madison, WI). The single nucleotide polymorphisms (SNPs) rs7566605, rs9939609, rs17782313 and rs17700633 were genotyped by allelic discrimination Taqman assays (Applied Biosystems, Foster City, CA). PCR were performed in a 96-well format in a total of 5 µl reaction volume using 10 ng of genomic DNA and FAM/VIC dye labeled allelic probes with the Taqman Universal Fast Master mix and subjected to 95°C for 15 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min on an ABI 9800 Fast Thermocycler (Applied Biosystems, Foster City, CA). The Taqman assay plates were transferred to an ABI 7500 Fast Real Time PCR system in which the fluorescence intensity in each well of the plate was recorded and genotypes were analyzed using Sequence Detection Software 1.3(Applied Biosystems, Foster City, CA). Genotyping quality control procedures included genotyping 10% duplicates for accuracy checking and inclusion of both positive and non-template controls in each 96-well plate. Genotyping success rates were 99.5% for rs7566605, 99.5% for rs9939609, 97% for rs17700633 and 93% for rs17782313. Genotyping accuracy for the four SNPs, as determined by concordance between duplicates, was 100%.

Social economic status

Social economic status (SES) was represented by father's education level divided into three categories: low education level (<12 years), medium education level (≥12 and <16 years) and high education level (≥16 years) [28]. As no significant interaction for any of the SNPs with SES on any of the adiposity variables was found (data not shown), and SES was not a covariate of interest in the current study, we did not include SES in the final models.

Statistical analysis

Growth curve modeling

All analyses in this study were conducted by using individual growth curve modeling within a multilevel framework, which is a data analysis technique especially designed for longitudinal data [27, 34]. In growth curve modeling, a curve is fitted for each individual subject. These individual growth curves in adiposity are characterized by their intercept (or level) and slope (rate of change). Addition of independent variables to the model, such as SNPs, aimed at explaining between-subject variation (in level and slope) of the growth curves.

Analytical strategy and software

There were 3 levels in our multilevel longitudinal data, repeated measures of the same subjects at level 1, within family (twins or sibs) at level 2 and between families at level 3. First, we started with the intercept-only model to test the random effect of intercept within families (level 2) and between families (level 3); furthermore, in the Georgia CV Twin study, we modeled MZ and DZ covariances separately to account for the different degrees of relatedness between MZ and DZ twin pairs [35]. Second, the effects of age and age^2 were modeled, i.e., linear (fixed and random) and quadratic trends (fixed).We did not fit the random quadratic term as the models were not robust. A likelihood ratio test was used to select the best-fitting model at a significance level of $P < 0.05$. Third, age at entry, ethnicity and gender were separately added to the growth curve model to test its effect on adiposity intercept. We further tested the effects of gender and ethnicity on the rate of change in adiposity modeled as interactions with age and age^2, as described in detail previously [27, 29]. The

interaction between ethnicity and gender was tested as well. Finally, after arriving at the most parsimonious full "environmental" model including only significant terms (shown in Supplementary Table 1), SNPs were added to the model to test their main effects on the level of the growth curve. Based on the previous findings [10, 36], we only considered an additive model in testing the associations between these SNPs and adiposity phenotypes except for the SNP near *INSIG2* for which we used a recessive model [5]. Effects on the slope of adiposity growth curve were modeled as interactions of SNPs with age. Analyses were done separately for each of the SNPs and followed with the test of SNP interaction with ethnicity and gender. Interactions of SNPs with ethnicity and gender were modeled to examine whether the effect of the SNP on adiposity was moderated by these factors. A likelihood ratio test was used to determine the significance of the effects that were added to the model in each of the analysis steps at a significance level of $P < 0.05$.

There were several distinct characteristics between the two longitudinal studies. For example, subjects were twins with a maximum of 4 visits in the Georgia CV Twin study, while <25% of subjects were sibs in the BP Stress study, and the follow-up period was much longer with up to 16 visits. Thus, the growth curve modeling was conducted in the Georgia CV Twin study and the BP Stress study separately, and β coefficients computed in the two studies were pooled in meta-analysis using the inverse variance-weighted method [37], the I^2 statistic,which describes the percentage of variation across studies that is due to heterogeneity rather than chance, was used to estimate between-study heterogeneity ($P > 0.1$) [38]. If there is very little variation between studies then I^2 will be low and a fixed effects model might be appropriate. Meta-analysis was performed using Stata 10 software (StataCorp, College Station, TX). A P value of ≤ 0.05 was considered to be statistically significant.

In the analyses, all variables except height were natural log transformed to obtain better approximations of the normal distribution. We only assessed the influence of *MC4R* rs17782313 on height based on Loos et al.'s findings [10]. In the Georgia CV Twin study and the BP Stress study, age was expressed as a deviation from the mean age of 17.5 and 18.2 years, respectively; and age at entry was centered at the mean age of 14.3 and 11.9 years at the first visit, respectively.

All multilevel modeling was performed using mixed linear models in Proc Mixed of the SAS/Stat software package (Release 9.1, 2002, SAS Institute Inc., Cary, NC, USA), which is robust to and appropriately handles unbalanced longitudinal data. Hardy–Weinberg equilibrium (HWE) and ethnic differences in allele and genotype frequencies were tested by a χ^2 test in only one member per family, which was chosen randomly to prevent inflated significance. Pairwise linkage disequilibrium (LD) between the two *MC4R* SNPs was tested by calculating D' as well as r^2 for AAs and EAs separately.

Results

Participant characteristics

Descriptive characteristics by ethnicity and gender at first visit of subjects with available genotype and phenotype are shown separately for the Georgia CV Twin and BP Stress studies in Table 1. Effects of ethnicity, gender and their interaction were tested using generalized estimating equations (GEE) with age as covariate. The mean age at first visit was 14.3 and 11.9 years in the Georgia CV Twin and BP Stress study, respectively. Many significant gender differences were observed, although some of these were limited to the EA group. Similarly, many significant ethnic differences were observed with some limited to females, as indicated in Table 1. The correlations between adiposity measures at the first visit in the two cohorts are shown in Supplementary Table 2.

Allele and genotype frequencies

All variants were common in both ethnic groups with minor allele frequency (MAF) from 20.9 to 52.1%. As indicated in Table 2, there were significant differences in genotype and allele frequencies of rs9939609 in *FTO* gene, rs17782313 near *MC4R* gene between EA and AA subjects in both studies, but not for *INSIG2* rs7566605 and *MC4R* rs17700633 (Table 2).

Furthermore, rs17782313 and rs17700633 near *MC4R* gene were not found to be in strong LD in both studies ($D' = 0.178$, $r^2 = 0.027$ for EAs, $D' = 0.378$, $r^2 = 0.10$ for AAs in the Georgia CV Twin study; $D' = 0.157$, $r^2 = 0.023$ for EAs, $D' = 0.505$, $r^2 = 0.14$ for AAs in the BP Stress study). All variants were in HWE in both ethnic groups except for rs9939609 in the AA group of the BP Stress study (Table 2).

Results of growth curve modeling analysis of common variants on adiposity-related phenotype levels

Supplementary Table 3 displays the results for the analysis of single locus effects on adiposity-related phenotype levels in the Georgia CV Twin and BP Stress studies separately based on the most parsimonious full "environmental" models (Supplementary Table 1) as well as the results of the meta-analysis. In the meta-analysis, we found that each copy of the *FTO* rs9939609 C allele was significantly

Table 1 General characteristics of study subjects' first visit in Georgia CV Twin and BP Stress study

	European–American				African–American				Ethnicity P^a	Gender P^b
	Males		Females		Males		Females			
	N	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD		
Georgia CV Twin study										
Age (years)[c]	279	14.2 ± 2.6	278	14.4 ± 2.7	193	14.1 ± 2.7	236	14.3 ± 2.7	0.37	0.11
Height (cm)	279	161.4 ± 13.5	278	157.3 ± 10.0	193	162.6 ± 13.4	236	159.2 ± 8.1	0.006	<0.001
Weight (kg)	279	56.6 ± 19.6	278	53.2 ± 14.7	193	59.1 ± 20.8	236	59.7 ± 20.1	<0.001	0.60
BMI (kg/m^2)	279	21.2 ± 5.1	278	21.3 ± 4.6	193	21.9 ± 5.4	236	23.3 ± 6.5	0.001	0.009
Waist circumference (cm)	276	74.4 ± 13.9	278	70.8 ± 11.4	192	72.6 ± 14.3	235	74.2 ± 14.0	0.011**	0.015[#]
Subscapular skinfold (mm)	279	11.5 ± 8.7	278	14.3 ± 8.0	193	12.7 ± 10.1	236	18.1 ± 10.7	0.001	<0.001
Triceps skinfold (mm)	277	13.9 ± 8.7	278	18.5 ± 7.7	192	13.1 ± 9.5	236	19.4 ± 9.9	0.201	<0.001
Suprailiac skinfold (mm)	279	14.3 ± 10.9	278	16.8 ± 8.6	192	13.4 ± 11.8	236	17.7 ± 10.4	0.284	<0.001
Sum of skinfolds (mm)	276	39.8 ± 26.7	278	49.5 ± 22.9	192	39.3 ± 30.3	236	55.2 ± 29.7	0.909	<0.001
BP Stress study										
Age (years)[c]	163	12.1 ± 4.2	144	11.4 ± 4.3	135	12.1 ± 3.8	164	12.0 ± 3.5	0.32	0.81
Height (cm)	161	151.5 ± 24.6	141	144.1 ± 22.0	134	151.7 ± 21.7	164	150.5 ± 17.0	<0.001**	0.002[#]
Weight (kg)	161	48.8 ± 22.2	141	44.3 ± 21.1	134	53.2 ± 24.5	164	55.0 ± 25.1	<0.001	0.71
BMI (kg/m^2)	161	19.9 ± 4.8	141	19.9 ± 5.4	134	21.9 ± 6.2	164	23.1 ± 7.6	<0.001	0.24
Subscapular skinfold (mm)	160	9.5 ± 7.1	141	12.1 ± 8.0	133	12.5 ± 10.4	163	16.9 ± 12.2	<0.001	<0.001
Triceps skinfold (mm)	161	13.3 ± 9.2	141	17.7 ± 9.5	134	14.0 ± 10.8	163	20.8 ± 13.2	0.89	<0.001
Suprailiac skinfold (mm)	160	13.5 ± 10.5	141	15.7 ± 9.8	134	15.6 ± 13.1	163	19.0 ± 13.0	0.84	<0.001
Sum of skinfolds (mm)	160	36.4 ± 25.6	141	45.6 ± 25.9	134	41.9 ± 33.0	164	56.7 ± 37.0	0.35	<0.001

The median follow up period was 5.38 years in the Georgia CV Twin study and 14.34 years in the BP Stress study

BMI body mass index, *SD* standard deviation

* Significant only in males; ** significant only in females, [#] significant only in European–American, [##] significant only in African–American

[a] *P* value for the difference between European–Americans and African–Americans, adjusted for age and gender

[b] *P* value for the difference between males and females, adjusted for age and ethnicity

[c] Number of subjects with phenotype and genotype data. Only subject with ≥2 observations were included

associated with BMI (increasing with 2.1% per allele, $P = 0.01$), weight (increasing with 1.9% per allele, $P = 0.04$), as well as with waist circumference (increasing with 1.2% per allele, $P = 0.04$) (Fig. 1; Supplementary Table 3). Figure 2 clearly shows the effect of *FTO* rs9939609 on the level of BMI in the Georgia CV Twin study (*P* for level = 0.02). Compared with the full environmental model, the additional explained percentages of variance were 0.31, 0.14 and 0.16% for BMI, weigh and waist circumference, respectively.

In the meta-analysis, significant associations were found between *MC4R* rs17782313 and triceps skinfold level (Fig. 3; Supplementary Table 3), increasing with 5.0% per allele ($P = 0.02$) and 0.25% explained variance. We also found a borderline significant association between rs17782313 and sum of skinfolds level ($P = 0.08$), increasing with 3.7% per allele and 0.16% explained variance. Moreover, we observed the C allele at rs17782313 was negatively associated with height ($P = 0.03$) (Supplementary Table 3).

Significant interactions of *MC4R* rs17700633 with gender on subscapular, suprailiac and sum of skinfolds were observed ($P_{inter} = 0.01$, 0.04 and 0.03, respectively) (Supplementary Table 4). We found this locus showed a significant effect in males only, with the A allele carriers having significantly higher skinfolds levels, increasing with 6.0, 6.3 and 6.3% per allele on subscapular, suprailiac and sum of skinfolds levels, respectively(Supplementary Table 3). Compared with the full environmental model, the locus explained additional 0.44, 0.35 and 0.44% between-subjects variation of triceps, subscapular and sum of skinfolds in males, respectively. Significant interactions of *MC4R* rs17700633 and rs17782313 with gender on weight were also observed (both $P = 0.002$). However, stratified analysis showed no significant effect on weight level in either gender group (Supplementary Table 3).

No significant associations were observed between rs7566605 near the *INSIG2* gene and any of the adiposity-related phenotypes ($P > 0.05$) (Supplementary Table 3).

Table 2 Genotype and allele frequencies of the four SNPs in EA and AA subjects

Study	SNP	Ethnicity	*N*	Genotype 11[a]	Genotype 12[b]	Genotype 22	*P**	MAF (%)	*P**	HWE test *P**
Georgia CV Twin study	*INSIG2* rs7566605	EA	292	140	122	30	0.11	31.2	0.06	0.65
		AA	225	123	89	13		25.6		0.55
	FTO rs9939609	EA	289	109	135	45	0.04	38.9	0.04	0.76
		AA	225	61	123	41		45.6		0.13
	MC4R rs17782313	EA	231	140	81	10	0.03	21.9	0.01	0.69
		AA	183	90	77	16		29.8		0.92
	MC4R rs17700633	EA	267	139	109	19	0.32	27.5	0.43	0.71
		AA	219	112	83	24		29.9		0.16
BP Stress study	*INSIG2* rs7566605	EA	230	112	95	23	0.37	30.7	0.17	0.66
		AA	240	132	89	19		26.5		0.47
	FTO rs9939609	EA	229	89	109	31	<0.001	37.3	<0.001	0.79
		AA	240	67	96	77		52.1		0.002
	MC4R rs17782313	EA	223	139	75	9	0.001	20.9	<0.001	0.78
		AA	238	111	102	24		31.6		0.92
	MC4R rs17700633	EA	228	110	94	24	0.74	31.1	0.57	0.56
		AA	237	118	99	20		29.3		0.92

rs7566605: G is the common allele and C is the minor allele; rs9939609: T is the common allele and A is the minor allele; rs17782313: T is the common allele and C is the minor allele; rs17700633: G is the common allele and A is the minor allele

N number of subjects (one of each twin or one child per family), *HWE test* Hardy–Weinberg equilibrium test, *MAF* minor allele frequency, *EA* European–American, *AA* African–American

* *P* values based on tests in one twin per pair or one child per family

[a] 1 represents the common allele

[b] 2 represents the minor allele

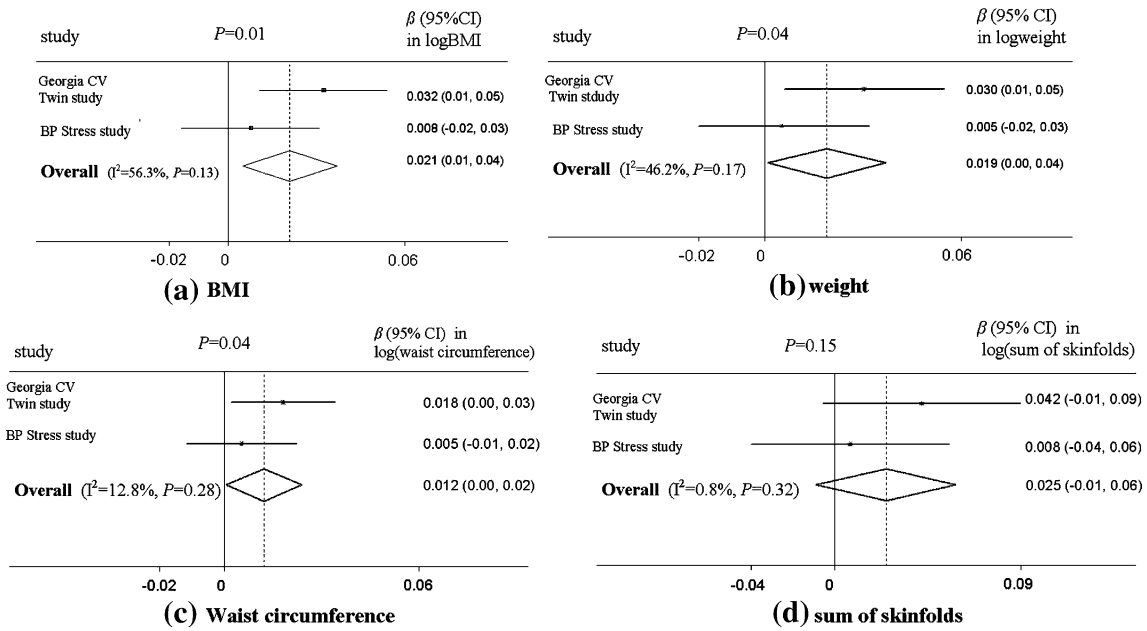

Fig. 1 Meta-analysis plots of *FTO* rs9939609 and adiposity levels. **a** BMI, **b** weight, **c** waist circumference, **d** sum of skinfolds

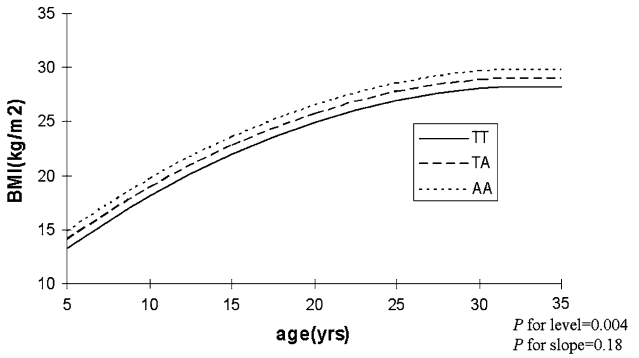

Fig. 2 Growth curves for BMI by *FTO* rs9939609 genotype in the Georgia CV Twin study. *Note*: *P* value presents significance of log transformed BMI

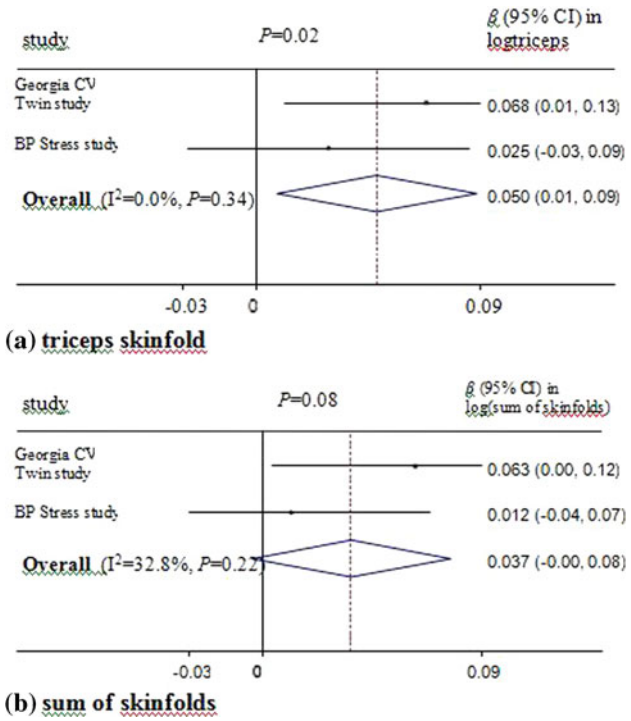

Fig. 3 Meta-analysis plots of *MC4R* rs17782313 and adiposity levels. **a** Triceps skinfold. **b** Sum of skinfolds

No significant interactions between these SNPs and ethnicity were observed for any of the adiposity-related phenotypes ($P > 0.05$) (Supplementary Table 5).

The growth curve modeling analysis of common variants and adiposity levels in EAs and AAs separately showed that rs9939609 was significantly associated with BMI, waist circumference and sum of skinfolds in EAs, but no significant associations were found in AAs. However, most of the effects were in the same direction (Supplementary Table 6).

Results of growth curve modeling analysis of common variants on increases with age (slope) of adiposity-related phenotype

None of the 4 SNPs showed significant interactions with age on any of the adiposity-related phenotypes (Supplementary Table 7), with one exception. *MC4R* rs17700633 showed a significant interaction with age on triceps skinfold (*P* for slope = 0.04). Figure 4 shows growth curves for triceps skinfold for the three genotype groups of rs17700633 in the BP Stress study (*P* for slope = 0.03), clearly indicating an increasing effect of genotype over time. For example, the per A-allele effect size was ~ -0.03 mm at 15 years and ~ 0.88 mm at 25 years.

Discussion

In the current study, we investigated the role of common variants in *FTO* and near the *INSIG2* and *MC4R* loci identified through GWA studies on development of obesity from childhood to adulthood. We performed growth curve modeling in 1592 EA and AA youth from two longitudinal studies separately, and pooled results through meta-analysis. There were five main findings in this study. The first was that we replicated the effect of *FTO* rs9939609 on levels of BMI (increasing with 2.1% per A-allele), weight (increasing with 1.9% per A-allele) and waist circumference (increasing with 1.2% per A-allele) in the meta-analysis with explained variances of 0.31, 0.14 and 0.16, respectively. The second was that *MC4R* rs17782313 was significantly associated with triceps skinfold (increasing with 5.0% per C-allele). The third was that no significant interaction between any of the SNPs and ethnicity on any of the adiposity-related phenotype was observed. The fourth was that significant interactions between *MC4R* rs17700633 and gender on the level of subscapular, suprailiac skinfold and sum of skinfolds were observed. Finally, a significant effect of *MC4R* rs17700633 on increase of triceps skinfolds with age (i.e., slope) was observed.

Since Frayling et al. [9] first reported the significant associations between the *FTO* variant rs9939609 and obesity-related phenotypes such as BMI, weight, waist circumference, %BF and skinfolds in both children and adults, these associations have been replicated in Europeans [7, 8, 24, 25, 39] and Asians [12] through both cross-sectional and longitudinal studies. In our longitudinal studies, we found that each copy of the *FTO* rs9939609 A allele was significantly associated with BMI with 2.1% increase per allele. For example, within a population with average BMI of 25 kg/m^2, the approximate per A-allele effect is ~ 0.5 kg/m^2, which is similar to the effect that Frayling reported in UK children at the age of 11 years (0.4 kg/m^2) and a little higher than that in Finnish children at the age of 14 years (0.1 kg/m^2) [9]. A cross-sectional

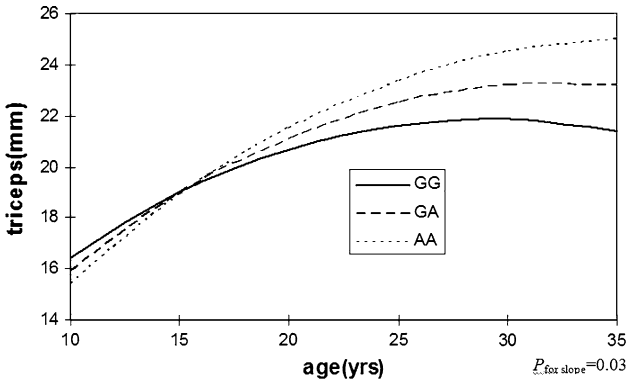

Fig. 4 Growth curves for triceps skinfold by *MC4R* rs17700633 genotype in the BP Stress study. *Note*: *P* value presents significance of log transformed triceps

sample of 1,600 subjects has 80% power at an alpha of 0.05 to detect an effect size of the SNP of 0.5% of the trait variance. Thus, our meta-analysis of two cohorts with repeated measures will have had sufficient power to detect even smaller effects. We found that the variance in BMI explained by rs9939609 was 0.31%, which is lower than the previously reported ~1.0% and probably due to sample variability [9]. In addition, we replicated the association of *FTO* rs9939609 with weight and waist circumference, which is consistent with Frayling et al.'s findings [9]. The effect of *FTO* rs9939609 on sum of skinfolds did not reach significance. This could be due to a more inaccurate measurement procedure compared to BMI, or that the measures represent slightly different aspects of adiposity. Furthermore, several longitudinal studies have been conducted to investigate the effect of rs9939609 on the development of obesity over time [24, 25, 40]. However, the results of the rs9939609 by age interaction were inconsistent. In the MRC National Survey of Health and Development (NSHD) cohort, a longitudinal birth cohort from 1946 and followed through to age 53 years, significant genotype-by-age interaction was observed for BMI, showing the association strengthened between age 2 and 20 years at a rate of 0.007 sex-specific standard deviation scores (SDS) (95% CI 0.003–0.01) per A-allele per year, reached a peak at age 20 years, and thereafter weakened with age from 20 to 53 years (rate of decline: −0.003SDS per A-allele per year). And similar pattern was observed for weight [40]. An increasing effect of genotype over time ($P = 0.047$) was observed in 1886 middle-aged females from the Cebu Longitudinal Health and Nutrition Survey (CLHNS) cohort [25] with a maximum of 8 measurements spanning 22 years. However, the interaction between rs9939609 and age was not significant in either a female cohort of about 2,200 nurses followed up from 1976 to 2002 (average age at entry: 44 years) or a male cohort of about 3,500 Health professional followed up from 1986 to 2002 (average age at entry: 56 years) ($P = 0.08$ and 0.20, respectively) [24], although in the male cohort the genetic effects appeared to decrease at older age. In our longitudinal study in youth, we did not find any significant interaction between rs9939609 and age for any of the obesity-related phenotypes. That is, rs9939609 did not affect the slope of the growth curves of these traits. Although the association between rs9939609 and adiposity has been broadly replicated in Europeans [7, 8, 24, 39] and Asians [12], it could not be confirmed in an African population [14]. In our study including both EA and AA youth, we did not find any significant interaction between rs9939609 and ethnicity on any adiposity-related phenotype. We also did not find any significant interaction between rs9939609 and gender, which was consistent with Hardy et al.'s finding of no differences between males and females in the genetic associations with body size across the life course [40].

Since Loos et al. [10] reported association between common variants near *MC4R* and fat mass, weight and BMI in both children and adults, several studies have replicated the finding in European populations [15–18]. In our longitudinal studies, we found the C allele carriers of rs17782313 showed higher weight, BMI and waist circumference compared to TT homozygotes, but the association did not reach statistical significance. However, we did find a significant effect of rs17782313 on triceps skinfolds level, and a borderline significant effect of rs177882313 on sum of skinfolds. The C allele at rs177882313 was found to be positively associated with height in European adults [10, 17]. On the contrary, we found rs17782313 was negatively associated with height in youth, and stratified analysis showed significant negative association only in AAs ($\beta = -0.9$, $P = 0.035$). Loos et al. [10] also found the per-C allele effect of rs17782313 on BMI in children was about twice that observed in adults. In the NSHD cohort, significant genotype-by-age interaction was observed for weight, showing the association strengthened between birth and 20 years at a rate of 0.005 SDS (95% CI 0.001–0.008) per C-allele per year, reached a peak at age 20 years, and thereafter weakened with age (rate of decline: −0.002SDS per C-allele per year) [40]. However, no significant genotype-by-age interaction was observed for BMI in the NSHD cohort [40]. In our longitudinal study, we did not find significant interaction between rs17782313 and age on any of the obesity-related phenotypes, which is in line with findings for BMI in the NSHD cohort [40]. A recent paper could not replicate the association of the common variants near *MC4R* and obesity in African-Americans [15]. In the current study including both EAs and AAs, we did not find any significant interaction between rs17782313 and ethnicity on any of these obesity-traits. Findings on the interaction between

rs17782313 and gender on adiposity in previous studies are inconsistent [40–42]. In 3885 Swedish adults, rs17782313 showed significant interaction with gender ($P_{inter} = 0.02$), with the association limited to females ($P = 0.003$) [42]. On the contrary, a study in French adults found stronger association of rs17782313 with adiposity and fat mass deposition in males than in females ($P = 0.003$ and 0.03, respectively) [41]. In the NSHD cohort, no significant interaction between rs17782313 and gender for adiposity phenotypes was observed ($P > 0.05$) [40]. In our study, rs17782313 significantly interacted with gender on weight, and the stratified analyses showed that neither of the associations was significant. Further investigations are needed to ultimately clarify whether the effect of rs17782313 on obesity is modified by gender.

Loos et al. [10] found that the association between rs17700633 and BMI seemed to be dependent on that of rs17782313. We did not observe any significant association between rs17700633 and weight, BMI or waist circumference, which is inconsistent with findings in a middle aged Danish population [17]. We found significant interaction between rs17700633 and age on triceps skinfolds, which means rs17700633 affected the slope of the triceps growth curve. The A allele carriers showed a steeper increase in triceps skinfold per unit increase of age as compared to the GG homozygotes. However, we cannot exclude the possibility that this significant interaction is a chance finding. We observed significant interaction between rs17700633 and gender for subscapular, suprailiac and sum of skinfolds, with significant associations limited to males. These findings indicate that the effect of rs17700633 on skinfolds might be modified by gender.

The association of *INSIG2* rs7566605 with BMI [5], has not been consistently replicated [20, 21]. In our study, we did not find any significant association between rs7566605 and any obesity-related phenotype levels, nor did we find any significant interaction with ethnicity, gender or age.

A major strength of our study is the longitudinal design of both cohorts with up to 16 assessments in one of these. Longitudinal studies offer information on determinants of interindividual differences in the development of obesity over time. These two cohorts thus allowed us to investigate the influence of common genetic variants in growth curve modeling to obtain better insight into the development of obesity from childhood into adulthood. Longitudinal designs have superior power to detect genetic effects on the trait levels compared to cross-sectional studies, because measurement of subjects at multiple time points provides much better precision in determining the phenotypes [43, 44]. As such, longitudinal studies such as this one require a fraction of the sample size of cross-sectional studies to achieve the same power. Another strength is the involvement of AA as well as EA youth, and the investigation of a potential interaction of these common variants with ethnicity. Several limitations of our study need to be mentioned as well. In these two studies, we used BMI and skinfolds as measures of general adiposity and waist circumference as a measure of central adiposity, which are convenient and low cost measurements, while more accurate measurements of percent body fat for general adiposity and visceral adipose tissue for visceral adiposity were not available. In these two studies, sexual maturation was not assessed, thus it could not be incorporated as a covariate.

In summary, in two longitudinal studies of EA and AA youth, we replicated the effect of *FTO* and common variants near *MC4R* on general and central adiposity. These effects were similar for EAs and AAs. Furthermore, these common variants did not affect the slope of adiposity development from childhood to adulthood with one exception. Common variants for obesity identified in genome-wide association studies have detectable but modest effects on growth curves for adiposity in African– and European–American youth.

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Conflict of interest The authors have indicated they have no financial relationship to this article to disclose. The authors declare no conflict of interest.

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