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Original article

Associations Between Menarche-Related Genetic Variants and Pubertal Growth in Male and Female Adolescents

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A B S T R A C T

Purpose: Previous studies have identified novel genetic variants associated with age at menarche in females of European descent. The pubertal growth effects of these variants have not been carefully evaluated in non-European descent groups. We aimed to examine the effects of 31 newly identified menarche-related single-nucleotide polymorphisms (SNPs) on growth outcomes in African-American (AA) and European-American (EA) children in a prospective cohort.
Methods: We analyzed longitudinal data collected from 263 AAs and 338 EAs enrolled between ages 5 and 17 years; the subjects were followed semiannually for an average of 6 years. The associations between the SNPs and growth-related outcomes, including weight, height, and body mass index (BMI), were examined using mixed-effect models.
Results: Longitudinal analyses revealed that 4 (near or in genes *VGLL3*, *PEX2*, *CA10*, and *SKOR2*) of the 14 menarche-only–related SNPs were associated with changes in weight and BMI in EA and AA ($p \leq .0032$), but none of them was associated with changes in height. Of the eight menarche-timing and BMI-related SNPs, none was associated with changes in height, but three (in or near genes *NEGR1*, *ETV5*, and *FTO*) were associated with more rapid increases in weight and/or BMI in EA ($p \leq .0059$). Among the nine menarche-timing and height-related SNPs, four (in or near genes *ZBTB38*, *LOC728666*, *TBX2*, and *CABLES*) were associated with changes in weight or height in EA and AA ($p \leq .0042$).
Conclusions: Genetic variants related to age at menarche were found to be associated with various growth parameters in healthy adolescents. The identified associations were often race and sex specific.

IMPLICATIONS AND CONTRIBUTION

This study examines the growth effects of menarche-related variants in individuals of European and non-European ancestries. The findings indicate that genetic variants responsible for age at menarche are associated with pubertal growth in healthy adolescents. Data suggest shared genetic influences on menarcheal age and pubertal growth.

Puberty, the period of transition from childhood to adulthood, is characterized by accelerated growth and sexual maturation. The regulation of puberty is complex and partly influenced by individual's genetic background [1]. Genetic variation affecting events associated with puberty may relate to future disease risks. For example, previous studies have linked younger menarcheal age to increased later-in-life risks of obesity, type-2 diabetes, breast and endometrial cancer, and cardiovascular diseases [2–5]. During puberty, timing of menarche is in general well synchronized with somatic change. A higher childhood body mass index (BMI) has been shown to associate with early onset of menarche [6,7], suggesting a direct influence of adiposity on the timing of sexual maturity. The influence, however, may not be

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unidirectional as data suggest that female puberty is often accompanied by rapid gains in body weight and body fat [8,9]. Although the causal direction remains uncertain, data are consistent on the correlation between menarcheal timing and body fat accumulation, as reflected by increased BMI; this correlation points to the possible existence of common regulating mechanisms shared by these two processes. It is thought that a significant portion of the shared genes account for the correlation between increased childhood adiposity and early sexual maturation as indicated by the onset of menarche [10].

Menarche, the occurrence of the first menstrual cycle, is considered a landmark event of female puberty. Genetic factors are thought to account for approximately 50% of the observed variation in timing of menarche [11,12]. Previous genome-wide association studies (GWAS) identified variants in *LIN28B* that associated with age at menarche in women of European ancestry [13–16]. The growth effects of genetic variants in *LIN28B* have been investigated in three studies in the European and European-American (EA) populations [14,17,18]. However, the growth effects of these genetic compositions in nonwhite populations are less studied. African-American (AA) women are known to have menarche earlier than white women [19,20], and AA children of both sexes tend to have earlier pubertal growth spurts than children of European ancestry [21]. In addition, genetic variants in *LIN28B* have also been found to associate with the development of male sexual characteristics (pubic hair and voice change) and pubertal growth in boys [14,17,18], suggesting shared genetic mechanisms in boys and girls. It is therefore logical to question whether menarche-related genetic variations relate to growth outcomes similarly in boys and girls and in blacks and whites.

The goal of this study was to explore the genetic influences on pubertal growth. Specifically, we investigated the growth effects of menarche-related genetic variation using 31 single-nucleotide polymorphisms (SNPs) newly identified in an expanded meta-analysis of GWAS of age at menarche among women of European ancestry [22]. Using prospectively collected data from a cohort of AA and EA children and adolescents [23], we evaluated the genetic effects of 31 menarche-related SNPs on various growth measures, including height, weight, and BMI. By assessing the growth effects of these SNPs, we attempted to determine whether there were shared genetic influences underlying menarcheal age and pubertal growth characteristics. Despite the moderate sample size, the frequently repeated measurements and rich phenotypes have provided a unique opportunity to disseminate the growth effects of these SNPs.

Methods

Study design and subjects

The study subjects were participants in a long-standing prospective cohort study that was established in 1986 to investigate blood pressure development in children and adolescents. The study design and data collection process have been described elsewhere [23]. Briefly, healthy children between ages 5 and 17 years were recruited from schools in Indianapolis, IN, and were followed at 6-month intervals prospectively. The cohort included 601 children, 338 EAs, and 263 AA, who had contributed blood samples for genetic analysis. Data on growth-related outcomes were assessed during the course of follow-up. The study was approved by a local institutional review board. Study subjects or their parents provided informed consent or assent as appropriate.

Outcome assessment

Height and weight were measured every 6 months during follow-up [24]. BMI, defined as weight (kilograms)/height (square meters), was calculated for each follow-up visit. On average, each subject contributed 11 repeated measurements. Information on race was ascertained based on the subject's self-report.

Single nucleotide polymorphisms selection and genotyping

We selected 31 independent SNPs that have been reported to be associated with age at menarche in a recent meta-analysis of GWAS of women of European ancestry [22]. Those SNPs were either strongly associated with age at menarche (with reported $p \leq 1 \times 10^{-8}$) or associated with both age at menarche and another adult growth outcome (weight or height) from this report. Based on their associations with age at menarche and other correlated growth traits, we further categorized these SNPs into three groups: group I included 14 SNPs associated with age at menarche only; group II included eight SNPs associated with both age at menarche and adult BMI, and group III included nine SNPs associated with both age at menarche and adult height. Detailed descriptions of the SNPs are listed in Table 1.

DNA was extracted for all participants with blood sample available. Genotypes of the candidate SNPs were determined using the Sequenom MassArray iPLEX Platform (Sequenom, San Diego, CA). The genotyping success rate for each SNP was greater than 95%. Samples with percentage of missing genotypes greater than 2% were removed from the study. In the retained samples, all SNPs were in Hardy–Weinberg equilibrium ($p > .05$). The allele frequency for each SNP in our data was consistent with that reported for populations of European and African descent in the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/).

Statistical analysis

Weight and height data spanned between ages 5 and 21 years were used in the analyses. Longitudinal analyses were performed using mixed-effect models, which incorporated genetic effects on growth outcomes as fixed effects at the population level; and subject-specific random effects were incorporated into the model to accommodate the potential correlations among measures from the same subject. The association between each SNP and each measure of growth outcome (weight, height, and BMI) was assessed with adjustment for age, race, and sex, assuming a linear age effect on those outcomes. The models included the main effects of SNP, age, and the SNP $\times$ age interactions. The main effect of SNP represented the intercept of the regression line corresponding to the genotype (shift); the age effect represented the rate of growth of the reference genotype (slope); and the SNP $\times$ age interaction tested whether the growth rate differed by genotype. Because the true underlying genetic model was unknown, we tested dominant, recessive, and additive genetic models. We then tested three-way interactions SNP $\times$ age $\times$ race in data after adjusting for the effect of sex and SNP $\times$ age $\times$ sex in data after adjusting for the effect of race. These interaction terms allowed us to determine the heterogeneity of the genotype effect on growth rate between males and females and between