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Replication of *LIN28B* SNP association with age of menarche in young Filipino women

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Abstract

Age of menarche, or the timing of first menses in girls, is a physiological trait that shows substantial genetic heritability. Earlier age of menarche is associated with increased childhood adiposity and with adult risk of obesity and cardiovascular disease. In 827 young Filipino women from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), we observed nominal association with age of menarche ($\beta = -0.118$ years, 95% confidence interval = (−0.216, −0.020), $P = 0.019$) for the single nucleotide polymorphism (SNP) rs7759938 from the menarche locus *LIN28B* with an effect direction consistent with the previous report. We also tested whether childhood adiposity, as measured by body mass index (BMI) at age 8, mediated the relationship between rs7759938 and age of menarche. We observed suggestive evidence that the effect of the SNP on age of menarche was independent of childhood adiposity. These data confirm the strongest gene reported in Europeans (*LIN28B*) as a contributor to age of menarche in an Asian population.

Keywords

SNP association; age of menarche; mediation analysis; childhood adiposity; Filipinos

Introduction

Earlier age of menarche is associated with increased childhood adiposity and with adult risk of obesity and cardiovascular disease (1, 2). Nutrition and other environmental factors play substantial roles in the timing of first menses (3), yet approximately 50% of the variability in age of menarche is heritable (4, 5). The association at the *LIN28B* locus was the first menarche signal widely identified at the genome-wide significance level ($P < 5 \times 10^{-8}$) (6-10), but it has only been replicated in one association study of non-European subjects, that of 3,468 Hispanic-American women (11). To examine the genetic relationships with and between childhood adiposity and age of menarche in the *LIN28B* locus, we tested the index single nucleotide polymorphism (SNP) rs7759938 for association with age of menarche and for evidence of mediation by body mass index (BMI) at age 8 in 827 young adult Filipino women from the Cebu Longitudinal Health and Nutrition Survey (CLHNS).

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Enrolled in the CLHNS since their births in 1983–1984, these women have been part of a mostly urban population similar to other developing areas of Asia in terms of socioeconomic diversity (12, 13) and genetic ancestry (14). Notably, this cohort also showed a very high prevalence of stunting during early childhood, partially attributable to poor postnatal nutrition and hygiene (12).

Methods

During surveys at mean ages 11.5, 15.0, and 18.7 years, 997 non-twin female offspring from the CLHNS were asked to report their menarcheal status and the month and year of their first menstrual period (13). Of these, 966 were measured for BMI at age 8, prior to the onset of menarche. Genotypes for the *LIN28B* SNP rs7759938 (chr6:105,485,647; hg18/Build 36.3) were extracted from MetaboChip array data (Illumina, San Diego, CA, USA) generated at the UNC Mammalian Genotyping Core. Quality control checks were performed on the genotype data as described previously (14).

The SNP rs7759938 was tested for additive association with age of menarche as well as height, weight, and BMI at age 8 using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Each trait was normally distributed and therefore not transformed. Models were adjusted for covariates associated with the outcomes ($P < 0.05$) (Figure S1A): a standardized factor score representing household socioeconomic status at age 8 (13) and the first nine principal components of population substructure calculated previously (14). The menarche association models were tested with and without adjustment for BMI at age 8. Genotype, phenotype, and covariate data were available in 827 women. Quanto v.1.2.4 (http://hydra.usc.edu/gxe/) was used to calculate statistical power.

Mediation analysis was performed using the SAS macro INDIRECT (15). Using linear regressions, this method models how an independent variable (X) affects a dependent variable (Y) through an intervening variable (M) (Figure S1B). The total effect of X on Y (c) is the sum of X's direct effect (c') and its indirect effects ($a \times b$) through the mediator M. We reported the mean value of the indirect effect ($a \times b$) and its bias-corrected non-parametric bootstrap 95% confidence interval (CI) along with the unstandardized regression coefficients (a, b, c, and c'). Power for the mediation analysis was calculated empirically using published R code (16).

Results

Age of menarche was recorded for 827 Filipino women from the CLHNS (Table 1), with high concordance between three surveys taking place within short intervals of the event (13). Their mean age of menarche (13.1 years) was comparable to Europeans (12.4–13.6 years) (10). The T allele of *LIN28B* SNP rs7759938 (frequency = 0.610) showed nominal association with age of menarche ($\beta = -0.118$, $P = 0.019$) (Table 2), consistent with the previously reported direction of effect. Comparable results were observed when BMI at age 8 was included as a covariate. This SNP explained 0.7% of the phenotypic variance in age of menarche in the CLHNS. For comparison, a menarche study in Europeans reported that two SNPs at *LIN28B* and *TMEM38B* together explained 0.6% of the variance (6). Our study had ~68% power to replicate the association of a SNP explaining 0.7% of the variability in age of menarche at $\alpha = 0.025$ in a one-sided test and ~35% power to replicate a SNP explaining 0.3% of phenotypic variability.

We then tested rs7759938 for evidence of a mediating effect by BMI at age 8 on its associations with age of menarche (Table 2). We observed a significant direct effect ($c' = -0.106$, $P = 0.032$) and a significant total effect ($c = -0.118$, $P = 0.019$), but not a significant

mediation effect ($a \times b = -0.012$, 95% CI = (−0.034, 0.008)). Consistent with this result, rs7759938 was not associated with either weight or height at age 8 ($P \geq 0.05$). Based on the observed mediation coefficients, the study had moderate power (~64%) to detect a mediation effect on the association between rs7759938 and age of menarche at $\alpha = 0.05$.

Discussion

These genetic analyses in young Filipino women from the CLHNS provided evidence of association with age of menarche for the *LIN28B* SNP rs7759938, first identified in subjects of European ancestry. Despite a high level of early childhood undernutrition manifested by high stunting prevalence at age 2 (12), the women did not have substantially delayed menarche in comparison to well-nourished populations (17). A recent genome-wide association study of age of menarche in 3,468 Hispanic-American women (11) also showed nominal association ($P < 0.05$) for *LIN28B*, providing support for shared genetic signals across populations. The effect size of the *LIN28B* SNP in the CLHNS (0.12 years or ~6.2 weeks) was comparable to that in Europeans (10) and Hispanic-Americans (11) (6.4 and 8.8 weeks, respectively). Even in the context of a powerful environmental effect, the current study replicated the major menarche signal at *LIN28B* that in Europeans explained more phenotypic variance than any signals discovered later (10).

The *LIN28B* SNP also showed evidence of "direct-only non-mediation" by childhood adiposity (18), meaning that while rs7759938 did have a direct effect on age of menarche (c'), BMI at age 8 did not significantly mediate the relationship of this SNP with age of menarche ($a \times b$). This result suggests that *LIN28B* does not have an effect on age of menarche through the biological pathways represented by childhood adiposity, though this study was generally underpowered to detect such an effect. The mediation model may also have been missing other mediators representing relevant pathways, such as hormonal biomarkers or alternative measures of adiposity. Notably, this study avoided a key temporal issue because childhood adiposity was measured years before the onset of menarche. Furthermore, *LIN28B* has been associated with height (19) though not with BMI (20) in childhood.

In summary, these data replicated in an Asian population a *LIN28B* SNP association with age of menarche widely observed in Europeans. Mediation analyses in larger populations with pre-menarcheal adiposity data will be necessary to more fully address the relationship between the genetics of obesity and of menarche at this locus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Basic characteristics of 827 women from the CLHNS

		Correlations				
Trait	**Mean/Median**	**Age of menarche**	**BMI at age 8**	**Weight at age 8**	**Height at age 8**	**SES at age 8**
Age of menarche (years)	13.1 ± 1.0	-	−0.22	−0.42	−0.41	−0.28
BMI at age 8 (kg/m^2)	14.7 ± 1.3	< 0.0001	-	0.74	0.14	0.06
Weight at age 8 (kg)	20.3 ± 2.8	< 0.0001	< 0.0001	-	0.76	0.29
Height at age 8 (cm)	117.6 ± 5.5	< 0.0001	< 0.0001	< 0.0001	-	0.36
SES at age 8	5 (3, 6)	< 0.0001	0.089	< 0.0001	< 0.0001	-

Values are reported as mean ± standard deviation or as median (25th percentile, 75th percentile). SES is a standardized factor score that ranges from 1 to 10 (see Methods). On the right, Pearson correlations are reported above the diagonal with *P*-values for significance below the diagonal.

CLHNS, Cebu Longitudinal Health and Nutrition Survey; BMI, body mass index; SES, socioeconomic status.

Table 2

Associations of the T allele of *LIN28B* SNP rs7759938 with age of menarche and body size traits in 827 women from the CLHNS

Trait Outcome	β ± SE	L95	U95	*P*
Age of menarche unadjusted for BMI at age 8	−0.118 ± 0.050	**−0.216**	**−0.020**	**0.019**
Age of menarche adjusted for BMI at age 8	−0.106 ± 0.049	**−0.202**	**−0.010**	**0.032**
BMI at age 8	0.075 ± 0.064	−0.050	0.200	0.24
Weight at age 8	0.114 ± 0.137	−0.155	0.383	0.41
Height at age 8	−0.012 ± 0.257	−0.516	0.492	0.96
Mediation Model Coefficient	**β ± SE**	**L95**	**U95**	***P***
Total SNP effect on age of menarche (*c*)	−0.118 ± 0.050	**−0.216**	**−0.020**	**0.019**
Effect of SNP on BMI at age 8 (*a*)	0.075 ± 0.064	−0.050	0.200	0.24
Effect of BMI at age 8 on age of menarche (*b*)	−0.163 ± 0.027	**−0.216**	**−0.110**	**< 0.0001**
Direct SNP effect on age of menarche (c')	−0.106 ± 0.049	**−0.202**	**−0.010**	**0.032**
Mediation effect of BMI at age 8 on SNP association with age of menarche ($a \times b$)	−0.012	−0.034	0.008	-

SNP effect betas (β) are reported in terms of the T allele, which is the previously reported menarche-decreasing allele. The coefficients for the associations with age of menarche, weight, height are reported in years, kg, and cm, respectively. The mediation model coefficients *c*, *a*, *b*, and c' are not standardized and have units of years, kg/m^2, years, and years, respectively. Significant effects ($P < 0.05$ or a 95% confidence interval that does not include zero) are shown in boldface.

CLHNS, Cebu Longitudinal Health and Nutrition Survey; BMI, body mass index; SE, standard error; L95, lower bound of 95% confidence interval; U95, upper bound of 95% confidence interval.