

ORIGINAL ARTICLE

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Early pubertal maturation and risk of childhood asthma: A mendelian randomization and longitudinal study

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Abstract

Background: Studies on early puberty and incident asthma have reported inconsistent results and are mainly performed in females. In this longitudinal study, we investigated the causal relationship between pubertal maturation and asthma through Mendelian randomization (MR) and explored the joint effect of overweightness and early pubertal maturation on asthma.

Methods: We used data from the Taiwan Children Health Study with longitudinal follow-ups of 2991 children aged 11–17 years. Six puberty-related single-nucleotide polymorphisms (combined into a weighted allelic score) were used to yield genetic instrumental variables for early puberty. Early pubertal maturation was defined as reaching a certain pubertal stage earlier than the median age for that stage. Incident asthma cases were calculated by excluding children with a history of asthma prior to that age.

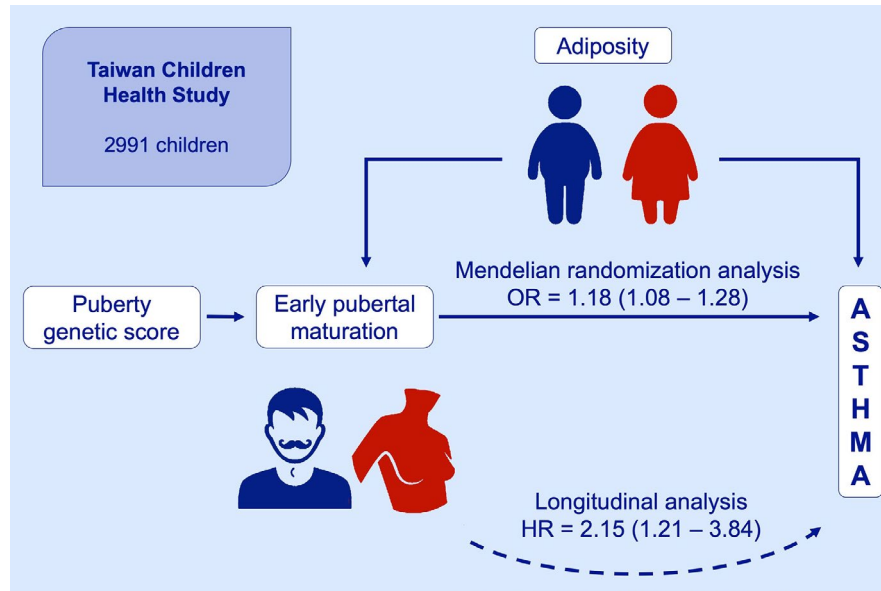
Results: The results of MR analysis revealed that early pubertal maturation was associated with active asthma (OR = 1.18; 95% CI: 1.08–1.28); this effect was significant in male children. Early pubertal maturation significantly increased the risk of incident asthma outcomes at 12 and 17 years of age in both sexes (hazard ratio = 2.15; 95% CI: 1.21–3.84). Taking non-overweight and non-early puberty children as the reference group, we observed a synergistic effect of overweightness and early pubertal maturation on asthma risk (OR = 1.08; 95% CI: 1.04–1.11) in children of both sexes.

Conclusions: Early screening and intervention for obesity are recommended to prevent future early pubertal onset and asthma occurrence.

KEYWORDS

asthma, early puberty, longitudinal study, Mendelian randomization analysis, overweightness

Abbreviations: 2SLS, two-stage least squares; BMI, body mass index; CI, confidence interval; DTHM, discrete-time hazard model; GWAS, genome-wide association study; HR, hazard ratio; IV, instrumental variable; MR, mendelian randomization; NCGM, National Center for Genome Medicine; OR, odds ratio; SE, standard error; SNP, single-nucleotide polymorphism; TCHS, Taiwan Children Health Study; TDCS, tanner derived composite stage.



GRAPHICAL ABSTRACT

Through Mendelian randomization analysis, we discovered that early pubertal maturation led to asthma in 2991 children from Taiwan Children Health Study. Early pubertal maturation significantly increased the risk of incident asthma outcomes at 12 and 17 years of age in both sexes. Childhood overweight and early pubertal maturation exerted synergistic effect on asthma risk.

1 | INTRODUCTION

A secular trend of early pubertal maturation in children of both sexes has been observed in developing and developed countries.^{1,2} Studies on early menarche and incident asthma have reported inconsistent results,^{3,4} and a meta-analysis of seven longitudinal studies reported a 37% higher risk of incident asthma in female children.⁵ However, studies on early puberty and asthma risk in male children are lacking. Given the considerable sex shift in asthma prevalence after puberty, longitudinal studies are necessary to investigate the effect of age at puberty on asthma risk in male children.

Conventional observational studies have been limited by unmeasured confoundings. Using Mendelian randomization (MR) analysis, Minelli et al⁶ provided evidence for a causal effect of early puberty on adult asthma (with the mean age at asthma onset of 40 years), rather than childhood asthma. However, because they used data from a UK Biobank (data collected at adulthood) with an invalid assessment of pubertal stages, their study was limited by strong recall bias. Moreover, childhood- and adult-onset asthma can result from different etiologies and the effect of early puberty on either childhood- and adult-onset asthma should be different. Prospective follow-up studies on adolescents from their transition to puberty up to young adulthood are necessary to appropriately assess whether early puberty is a causal risk factor for subsequent incident asthma.

The current state of knowledge indicates that early onset of puberty and obesity independently affect asthma risk;^{7,8} however, no study has investigated the synergistic or mediating effect of

adiposity on the relationship between early puberty and asthma in both sexes. Several observational studies have reported a stronger association between obesity and asthma severity or wheezing symptoms in women with early menarche.^{9,10} However, the role of obesity must be further investigated.

To determine the causal relationship between early pubertal maturation and asthma, we aimed to (a) investigate the causal direction between early pubertal maturation and asthma by performing MR analysis, (b) use longitudinal data to investigate the possible role of early pubertal maturation in incident asthma, and (c) explore the joint effect of overweightness and early pubertal maturation on asthma. In addition, we investigated the differential sex effect in these analyses.

2 | METHODS

2.1 | Cohort establishment and data collection

The Taiwan Children Health Study (TCHS) is a longitudinal, population-based cohort study focusing on atopic diseases, pubertal development, and adiposity growth in children. Detailed information on the TCHS was reported in a previous study.¹¹ In the TCHS, 10-year-old children have been enrolled since 2010 in an open-cohort design, and data on pubertal development have been collected since they were 11 years of age. We followed up children aged 11, 12, and 17 years. Both the parents or guardians of each participating children and the children themselves provided written informed consent; besides, they completed written questionnaires at school. A questionnaire completed by parents was used to categorize children's asthma

outcomes at age 11-12. We obtained the answer to the same question from children aged 17 years in the survey, rather than using a parent-reported questionnaire. The study protocol was approved by the Institutional Review Board of National Taiwan University Hospital and complied with the principles of the Declaration of Helsinki.¹²

2.2 | Definition of early pubertal maturation and asthma

A pubertal staging assessment was conducted using questionnaires completed by children aged 11-12 years. We used the validated Tanner derived composite stage (TDCS) to define pubertal stages.¹³ The TDCS contains schematic drawings of secondary sex characteristics of puberty (pubic hair and breast development for girls and pubic hair and genital development for boys). The median age for pubertal stages was referenced from two Chinese large-scale population-based studies.^{2,14} Children were defined as having early pubertal maturation if they reached a certain pubertal stage earlier than the median age for that stage.¹⁵ Children were defined as having early pubertal maturation if either their pubic hair or breast/genital development occurred at an earlier than the median age for that stage.

The question "Has a doctor ever diagnosed your child as having asthma?" was used to define the lifetime occurrence of physician-diagnosed asthma. Similarly, the question "Has your child ever experienced difficulty breathing or have you observed any wheezing or whistling from his/her chest in the past 12 months?" was used to define current wheeze. If the answer to both these questions was "Yes," then we classified the child as having active asthma.¹¹ Incident asthma cases among children aged 12 and 17 years were calculated by excluding those with a history of asthma prior to the age of 12 and 17 years, respectively.¹⁶

2.3 | Overweight definitions

Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2) and converted into age- and sex-specific percentiles according to growth charts for Taiwanese children and adolescents.¹⁷ We then defined overweight (including obese) children as those having a BMI of ≥ 85 percentile.

2.4 | Single-nucleotide polymorphism selection and genotyping

We chose MR genetic instrumental variables (IVs) for early pubertal maturation in accordance with the findings of large meta-analyses of genome-wide association studies (GWASs) conducted by Elks et al¹⁸ (32 studies, $n = 87\,802$), Perry et al¹⁹ (57 studies, $n = 182\,416$), and He et al²⁰ (2 studies, $n = 17\,438$). To prevent a potential pleiotropic effect, candidate single-nucleotide polymorphisms (SNPs) that were found to be associated with obesity or asthma were excluded. Moreover, the following criteria were used to identify candidate SNPs: (a) call rate exceeding 98% for all children, (b) minor allele frequency of

$\geq 10\%$, (c) no linkage disequilibrium with other selected SNPs, and (d) ability to fit our primer design test by using the National Genotyping Center of Academia Sinica platform.²¹ Finally, six candidate SNPs representing early pubertal maturation were selected. Tables S1 and S2 present detailed references for candidate gene selection. The details of genotyping procedures were reported in our previous study.²²

2.5 | Statistical analysis

Only children with complete data for pubertal stages and asthma outcomes were included. For those with missing data for variables, such as BMI, a multiple imputation method²³ was used, referencing previous cohort records. We employed a fully conditional specific regression method for multiple imputations, assuming that data were missing at random.

Under the IV assumption, a genotype should be (a) associated with a phenotype (early pubertal maturation), (b) independent of unmeasured confounding factors, and (c) independent of an outcome (asthma). Therefore, we examined the association of early pubertal genes with early pubertal maturation and asthma outcomes (Table S1), as well as with confounders (Table S2). We used the standard MR method, namely the two-stage least squares (2SLS) method, in which early pubertal maturation was regressed on puberty genetic scores and asthma outcomes were regressed on genetically predicted values for early pubertal maturation from the first regression.²⁴ To examine possible pleiotropic effects and provide a robust estimation of causality between early pubertal maturation and asthma, we also performed a sensitivity analysis for MR.²⁵ Individual MR estimates obtained using the 2SLS method for each early puberty gene and active asthma were pooled using the inverse variance-weighted method. Moreover, Egger's regression was used to detect small study and pleiotropic biases.²⁶ We also used a median-based method to estimate the causal effect of each genetic variant and then calculated the median estimate under the assumption that genetic variants representing over 50% of the weight in the analysis were valid instruments.²⁷

In addition to MR analysis, we performed a longitudinal follow-up survey at the age of 17 years to verify the temporal relationship between early pubertal maturation and new-onset asthma at the age of 12 and 17 years by using a discrete-time hazard model (DTHM). The DTHM is a survival analysis model that enables the estimation of hazard ratios (HRs) and 95% confidence intervals (CIs) for an event occurring in a discrete-time framework.²⁸ The model was adjusted for age, sex, parental education, and family income.

A multivariate logistic regression analysis was performed to examine the possible joint or mediation effect of overweightness on the relationship between early pubertal maturation and asthma by using non-overweight/non-early pubertal participants as the reference group. A mediation analysis was performed to further explore causal pathways from obesity to asthma. On the basis of the two-sided estimation, statistical significance was set at 5%. All model regression and sensitivity analyses were performed using R 3.3.2.

TABLE 1 Characteristics of participants in the Taiwan Children Health Study

Characteristics	Children at 11 y (n = 2869)	Children at 12 y (n = 2991)	Children at 17 y (n = 1386)
Age (years)	11.1 ± 0.3	12.1 ± 0.3	17.3 ± 0.5
Male sex	1433 (49.9)	1516 (50.7)	704 (50.7)
BMI (kg/m ²)	19.3 ± 3.8	20.4 ± 4.0	21.1 ± 3.5
Overweight	871 (32.0)	965 (34.5)	279 (21.4)
Male	487 (35.7)	536 (38.2)	160 (24.1)
Female	384 (28.3)	429 (30.7)	119 (18.5)
Parental education			
High school or below	1374 (47.9)	1547 (51.7)	609 (47.1)
College or university	1230 (42.9)	1195 (40.0)	544 (42.1)
Postgraduate school	265 (9.2)	249 (8.3)	140 (10.8)
Family income			
<600 000	1274 (44.4)	1435 (48.0)	567 (43.9)
600 001-1 000 000	1082 (37.7)	1049 (35.1)	496 (38.4)
>1 000 001	513 (17.9)	507 (17.0)	230 (17.8)
Tanner stage			
Male			
Tanner stage 1	416 (29.0)	183 (12.1)	-
Tanner stage 2	792 (55.3)	845 (55.7)	-
Tanner stage 3	200 (14.0)	362 (23.9)	-
Tanner stage 4	23 (1.6)	120 (7.9)	-
Tanner stage 5	2 (0.1)	6 (0.4)	-
Female			
Tanner stage 1	173 (12.0)	47 (3.2)	-
Tanner stage 2	984 (68.5)	695 (47.1)	-
Tanner stage 3	247 (17.2)	608 (41.2)	-
Tanner stage 4	30 (2.1)	115 (7.8)	-
Tanner stage 5	2 (0.1)	10 (0.7)	-
Early pubertal maturation	307 (10.7)	464 (15.5)	-
Male	166 (11.6)	365 (24.1)	-
Female	141 (9.8)	99 (6.7)	-
Physician-diagnosed asthma	271 (9.4)	276 (9.2)	155 (11.1)
Male	166 (11.6)	168 (11.1)	92 (13.1)
Female	105 (7.3)	108 (7.3)	63 (9.2)
Male:Female ratio	1.59	1.52	1.42
Current wheeze	151 (5.4)	181 (4.4)	10 (0.7)
Male	92 (6.6)	70 (5.3)	3 (0.4)
Female	59 (4.2)	48 (3.6)	7 (1.0)
Male:Female ratio	1.57	1.47	0.4
Active asthma	94 (3.3)	31 (1.0)	3 (0.1)
Male	62 (4.3)	14 (0.9)	2 (0.3)
Female	32 (2.2)	17 (1.2)	1 (0.1)
Male:Female ratio	1.95	0.75	3.0

Note: All data are presented as means ± standard deviation or numbers (%).

The number of participants did not add up to the total number because of missing data.

TABLE 2 Summary of coefficients of early pubertal maturation and asthma risk in MR analysis

Instrumental variables (IV)	Genetic score with intermediate trait		Genetic score with outcomes		Two-stage IV analysis	
	Coefficient (95% CI)	P value	Coefficient (95% CI)	P value	OR (95% CI)	P value
Physician-diagnosed asthma						
Puberty genes (6 SNPs)	20.82 (16.85, 24.79)	2.0×10^{-16}	8.25 (4.8, 11.70)	2.8×10^{-6}	1.49 (1.24, 1.78)	1.5×10^{-5}
Male	11.60 (4.60, 18.60)	1.2×10^{-3}	3.44 (−2.34, 9.22)	.24	1.34 (0.8, 2.27)	.27
Female	12.27 (4.46, 20.08)	2.1×10^{-3}	7.06 (−0.38, 14.51)	.06	1.78 (0.89, 3.56)	.10
Current wheeze						
Puberty genes (6 SNPs)	20.82 (16.85, 24.79)	2.0×10^{-16}	4.95 (2.36, 7.54)	1.8×10^{-4}	1.27 (1.11, 1.46)	4.0×10^{-4}
Male	11.60 (4.60, 18.60)	1.2×10^{-3}	4.42 (−0.04, 8.88)	.05	1.47 (0.94, 2.3)	.09
Female	12.27 (4.46, 20.08)	2.1×10^{-3}	−1.21 (−6.62, 4.20)	.66	0.91 (0.58, 1.41)	.67
Active asthma						
Puberty genes (6 SNPs)	20.82 (16.85, 24.79)	2.0×10^{-16}	3.36 (1.66, 5.05)	1.0×10^{-4}	1.18 (1.08, 1.28)	2.6×10^{-4}
Male	11.60 (4.60, 18.60)	1.2×10^{-3}	3.75 (0.82, 6.67)	.01	1.38 (1.01, 1.90)	.04
Female	12.27 (4.46, 20.08)	2.1×10^{-3}	1.27 (−2.23, 4.77)	.48	1.11 (0.83, 1.48)	.49

3 | RESULTS

In this study, the TCHS population comprised 2869 children aged 11 years, 2991 children aged 12 years, and 1386 children aged 17 years (Table 1). The average cohort follow-up rate from the age of 11 to 17 years was 64.6%. The proportion of male adolescents was 49.9% at the age of 11 years. One-third of the children were categorized as overweight. Overweight was more common among boys. The prevalence rates of early pubertal maturation were 10.7% and 15.5% among the children aged 11 and 12 years, respectively. A higher percentage of early pubertal maturation was observed in the male adolescents than in the female adolescents at the age of 11–12 years. At the age of 11 years, the prevalence of physician-diagnosed asthma was 9.4%, 5.4% of children exhibited the current wheezing symptom during the previous 12 months, and 3.3% had active asthma.

3.1 | Gender shift in asthma prevalence and incidence

As listed in Table 1, current wheeze and active asthma prevalence fall to 0.7% and 0.1% at the age of 17 years. The incidence of three asthma outcomes dropped from 4.3% at age 12 to 1.7% at age 17 (physician-diagnosed asthma); from 2.3% at age 12 to 0.5% at age 17 (current wheeze); and from 0.5% at age 12 to 0.2% at age 17 (active asthma) (Table S6). The male-to-female ratio of physician-diagnosed asthma prevalence was 1.59 at age 11 and 1.52 at age 12, and declined to 1.42 at age 17. Also in the prevalence of current wheeze, we even observed a marked gender shift²⁹ of male-to-female ratio from 1.57 at age 11 and 1.47 at age 12 to 0.4 at age 17.

3.2 | Early pubertal maturation increased asthma risk

Two of the six SNPs were significantly associated with early pubertal maturation (Table S1); however, adequate evidence was not found to establish an association between individual puberty SNPs and active asthma or confounders (Tables S1 and S2). Six SNPs for early pubertal maturation (rs6589964, rs2243803, rs7642134, rs1079866, rs12148769, and rs4452860) served as strong instruments, with F statistics of 105.5.³⁰ The weighted genetic scores of the six puberty SNPs were robustly correlated with early pubertal maturation (Table 2, $P = 2 \times 10^{-16}$). According to the results of the 2SLS analysis, early pubertal maturation was associated with the risk of physician-diagnosed asthma (OR = 1.49; 95% CI: 1.24–1.78), current wheeze (OR = 1.27; 95% CI: 1.11–1.46), and active asthma (OR = 1.18; 95% CI: 1.08–1.28). We determined that early pubertal maturation was significantly associated with active asthma in the male adolescents (OR = 1.38; 95% CI: 1.01–1.90). MR sensitivity analysis provided results similar to those of the 2SLS analysis (Table S3), indicating that no pleiotropy was detected. Only modest between-instrument heterogeneity was observed (I^2 : 21.6%, $P = .27$).

3.3 | Early pubertal maturation predicted long-term incident asthma risk

Figure 1 presents the results of the survival analysis for the effect of early pubertal maturation on incident asthma outcomes at the age of 12 and 17 years. Early pubertal maturation was determined to significantly increase the risk of incident physician-diagnosed asthma (HR = 1.57; 95% CI: 1.08–2.28) and incident current

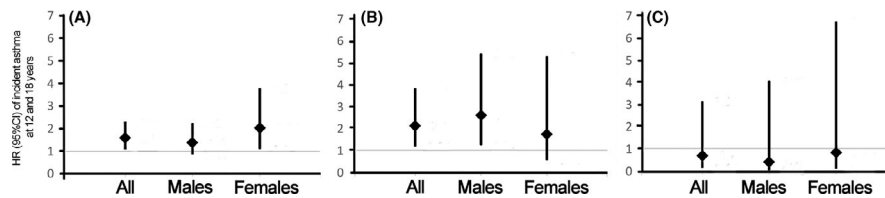


FIGURE 1 Hazard ratios of asthma at 12 and 17 y for early pubertal maturation at 11-12 y. A, Physician-diagnosed asthma; B, current wheeze; C, active asthma. Discrete-time hazard models were adjusted for age, sex, parental education, and family income

TABLE 3 Multivariate logistic regression analysis for asthma outcomes in children aged 11-12 y stratified by overweightness and early pubertal maturation status

Overweight/early pubertal maturation	Physician-diagnosed asthma		Current wheeze		Active asthma	
	Adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Overweight (-)/early pubertal maturation (-)	Reference		Reference		Reference	
Overweight (+)/early pubertal maturation (-)	1.01 (0.99, 1.02)	.41	1.02 (1.00, 1.03)	.03	1.01 (1.00, 1.01)	.23
Overweight (-)/early pubertal maturation (+)	1.02 (0.99, 1.05)	.32	1.02 (1.00, 1.04)	.10	1.02 (1.01, 1.03)	.03
Overweight (+)/early pubertal maturation (+)	1.08 (1.04, 1.11)	4.0×10^{-4}	1.04 (1.02, 1.07)	.01	1.01 (1.00, 1.03)	.21
P for trend		.001		.001		.02

Note: Models were adjusted for age, sex, parental education, and family income.

wheeze (HR = 2.15; 95% CI: 1.21-3.84). After further stratification by sex, the female adolescents who had early pubertal maturation appeared to have an increased risk of incident physician-diagnosed asthma (HR = 2.05; 95% CI: 1.10-3.81), and the male adolescents with early pubertal maturation had an increased risk of incident current wheeze (HR = 2.63; 95% CI: 1.27-5.43).

3.4 | Overweight and early pubertal maturation synergistically increased asthma risk

Table 3 demonstrated logistic regression analysis for asthma outcomes in children aged 11-12 years. Taking non-overweight and non-early pubertal maturation children as a reference group, the ORs of physician-diagnosed asthma and current wheeze gradually increased from the overweight/non-early puberty and non-overweight/early puberty groups to the overweight/early puberty group (OR: 1.01, 1.02, and 1.08, respectively, for physician-diagnosed asthma; OR: 1.02, 1.02, and 1.04, respectively, for current wheeze; *P* value for trend = .001). Compared with the children who were not overweight and did not have early pubertal maturation, the risk of physician-diagnosed asthma significantly increased 1.08-fold in the children who were both overweight and had early pubertal maturation (95% CI: 1.04-1.11). This synergistic effect of overweightness and early pubertal maturation on asthma risk was observed in both sexes (Table S4). Early pubertal maturation also appeared to act as a mediator between overweightness and asthma outcomes (Table S5).

4 | DISCUSSION

Through Mendelian randomization analysis, we discovered that early pubertal maturation has a causal effect on childhood asthma in 2991 TCHS children aged 11-12 years. The effect was significantly observed in the male adolescents. Furthermore, we examined real temporal relationships between early puberty and incident asthma at the age of 12 and 17 years in our cohort. After excluding children with a history of asthma, early pubertal maturation was found to significantly increase the risk of incident asthma outcomes at the age of 12 and 17 years in both sexes. The additional synergistic effect of overweightness and early pubertal maturation on asthma risk appeared in both sexes. These findings contribute to scientific evidence indicating that early pubertal maturation increases asthma risk in male adolescents. With the dramatically increasing prevalence of childhood obesity and early puberty, we also reflect the role of both overweightness and early puberty in asthma among adolescents.

4.1 | Gender shift of asthma prevalence and incidence

Our observations, current wheeze prevalence and incident asthma drop, were similar to previous studies. Using British cohort of 7225 subjects, Anderson et al³¹ reported that the prevalence of wheezing in past 12 months was 4.6% at age 11, but declined to 3.3 at age 16. In TRAILS (TRacking Adolescents' Individual Lives Survey) study, Vink et al³² discovered that the prevalence of asthma falls from 7%

at the age of 11.1 years to 5% at the age of 16.3 years. Nicolai et al²⁹ also proved that at age 20, only 24.5% of the initial asthma group still had symptoms. Similar phenomenon of the decline in incidence was observed by Anderson et al,³¹ in which asthma incidence was 1.1% at age 11 and became 0.76% at age 17. Moreover, the gender shift of asthma prevalence phenomenon was also reported in other studies.^{31,32} Anderson et al reported that the male:female asthma incidence ratio rose from 1.23 at 0-7 years to 1.48 at 12-16 years but reversed to 0.59 at 17-23 years. In TRAILS study, Vink et al also reported that the prevalence of asthma was similar in boys (7.7%) and girls (7.4%) at the age of 11 years. However, the prevalence of asthma was significantly higher in female (6.2%) than male (4.3%) at 16 years of age. The gender shift occurred between 12 and 17 years could be due to increased incidence of asthma in female.

4.2 | Possible mechanisms in the causal relationships between early puberty and asthma in both sexes

Several mechanisms might explain why early puberty leads to asthma development in females. First, hormonal changes at puberty might be related to immunity alterations.³³ Higher levels of estrogen and progesterone in girls with early menarche can act as inflammatory agents, triggering airway inflammation and obstruction.³⁴ Estrogen increases histamine release through immunoglobulin E-mediated mechanisms;³⁵ thus, pregnancy-induced aggravation of asthma severity was observed in a clinical study.³⁶ Second, poor pulmonary function, measured as forced expiratory volume in 1 second and forced vital capacity, was observed in girls with early menarche.³⁷ Moreover, the association between early menarche and poor pulmonary function was confirmed even after adjustment for overweightness and asthma. Third, the elevated insulin status and resistance that trigger puberty are independent risk factors for incident asthma. Kozyrskyj et al³⁸ found that persistently high insulin levels were the strongest determinants of nonatopic asthma in adolescent girls but not in boys. In summary, accumulating evidence supports the relationship between early puberty and asthma in girls. Common early fetal programming, such as low birth weight, has also been associated with both early menarche and asthma.

Evidence supporting the relationship between early puberty and asthma in male children is lacking. Studies have reported nonsignificant findings for the relationship between pubertal stages and asthma in male children.³² However, Minelli et al⁶ reported a 7% increased risk of adult asthma in male children, which is similar to our findings. Several mechanisms may be proposed to explain our findings. First, the effect of early pubertal maturation on asthma in male adolescents can result from overweightness as a comorbid factor. In our previous meta-analysis, we reported that obese boys had a 2-fold risk of incident asthma than did nonobese boys.⁸ In a large-scale population study, obesity was identified as a risk factor for early pubertal maturation in people of Chinese ethnicity.² Therefore, the causal relationship between early puberty and asthma can be precipitated by obesity. Second, an acute increase in the leptin level can

be a mechanism underlying both early puberty and asthma in male children. Prepubertal boys usually experience an approximately 3-year increase in the leptin level preceding the rise of levels of testosterone, growth hormone, and insulin-like growth factor.³⁹ This increase in the leptin level might be involved in the pathogenesis of asthma, particularly in boys.⁴⁰

4.3 | Synergistic effect of obesity and early puberty on childhood asthma

To our knowledge, no other studies have directly examined the additive effect of obesity and early puberty on childhood asthma. In one study, obesity and early puberty were regarded as independent predictors of asthma.⁷ When these two predictors coexisted, asthma risk was amplified, possibly due to a common biological pathway leading to asthma, such as leptin, insulin resistance, or pulmonary function. Our findings are supported by those of Varraso et al⁹ who discovered that BMI and early menarche acted synergistically to influence asthma severity in adult women. In addition, the role of obesity could either precede the early onset of puberty (childhood obesity → early puberty → childhood asthma) or be a consequence of early pubertal maturation (early puberty → adult obesity → adult asthma). We utilized our cohort to examine the first of these possibilities (Table S5). The average causal mediation effect from childhood obesity to childhood asthma, mediated through early puberty, was significant (causal mediation path coefficient = 9.6×10^{-4} ; 95% CI: 2.6×10^{-4} , 2.2×10^{-3} , $P \leq .01$ for physician-diagnosed asthma and 7.4×10^{-4} ; 95% CI: 7.7×10^{-5} , 1.5×10^{-3} , $P = .02$ for current wheeze). These findings indicate that early puberty is not simply involved in a joint effect with obesity but that it can be a crucial mediating pathway from obesity to asthma. Therefore, efforts to prevent early pubertal maturation can focus on blocking the causal pathway from obesity to asthma.²²

4.4 | Limitations

This study has several limitations. Limitations particular to MR analysis include population stratification, pleiotropy, and linkage disequilibrium. However, major population stratification was unlikely because the analysis was performed on unrelated individuals from the TCHS. Multiple genetic risk scores used in this study excluded candidate SNPs associated with both obesity and asthma. Therefore, the presence of shared pleiotropy or the effect of linkage disequilibrium-induced confounding was unlikely. An MR sensitivity analysis was also performed to prevent selection bias for genetic variants with pleiotropic effects on outcomes. We obtained consistent results supporting the hypothesis that early pubertal maturation leads to asthma. To further verify the temporal causal relationship between early puberty and incident asthma, we conducted a follow-up survey until the age of 17 years. This study was limited by being unable to use a GWAS to identify SNPs relevant to early pubertal maturation. Our candidate puberty genes were selected on the basis of puberty genes identified in other large-scale GWASs, which could still be satisfactory genetic IVs for MR analysis.

In summary, we confirmed that early pubertal maturation is a causal factor for childhood asthma in both sexes. Obesity was identified as a possible joint factor in aggravating childhood asthma, with early puberty acting as a mediator between obesity and childhood asthma. Bearing the considerable health burden represented by obesity, early puberty-related diseases, and childhood asthma, these findings have major clinical implications for disease prevention.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Dr. Yang-Ching Chen contributed to cohort data collection, hypothesis generation, data interpretation, and writing the manuscript. Mr. Hsien-Yu Fan assisted in the critical part of statistical analysis and in data interpretation. Dr. Yang Chen contributed to hypothesis generation and data interpretation. Prof. Yungling Lee reviewed the study design and data collection and interpretation, supervised the study, and critically revised the manuscript for essential intellectual content. All the authors approved the final manuscript for submission and publication and agreed to be accountable for all aspects of the work.

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REFERENCES

- Sørensen K, Aksglaede L, Petersen JH, Juul A. Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. *J Clin Endocrinol Metab*. 2010;95:263-270.
- Sun Y, Tao F, Su P-Y. National estimates of pubertal milestones among urban and rural Chinese boys. *Am J Hum Biol*. 2012;39:461-467.
- Nicolai T, Illi S, Tenbörg J, Kiess W, v Mutius E. Puberty and prognosis of asthma and bronchial hyper-reactivity. *Pediatr Allergy Immunol*. 2001;12:142-148.
- Lieberoth S, Gade E, Kyvik KO, Backer V, Thomsen SF. Early menarche is associated with increased risk of asthma: prospective population-based study of twins. *Respir Med*. 2015;109:565-571.
- Lieberoth S, Gade EJ, Brok J, Backer V, Thomsen SF. Age at menarche and risk of asthma: systematic review and meta-analysis. *J Asthma*. 2014;51:559-565.
- Minelli C, van der Plaats DA, Leynaert B, et al. Age at puberty and risk of asthma: a Mendelian randomisation study. *PLoS Med*. 2018;15:e1002634.
- Guerra S, Wright AL, Morgan WJ, Sherrill DL, Holberg CJ, Martinez FD. Persistence of asthma symptoms during adolescence: role of obesity and age at the onset of puberty. *Am J Respir Crit Care Med*. 2004;170:78-85.
- Chen YC, Dong GH, Lin KC, Lee YL. Gender difference of childhood overweight and obesity in predicting the risk of incident asthma: a systematic review and meta-analysis. *Obes Rev*. 2013;14:222-231.
- Varraso R, Siroux V, Maccario J, Pin I, Kauffmann F. Asthma severity is associated with body mass index and early menarche in women. *Am J Respir Crit Care Med*. 2005;171:334-339.
- Herrera-Trujillo M, Barraza-Villarreal A, Lazcano-Ponce E, Hernández B, Sanín LH, Romieu I. Current wheezing, puberty, and obesity among Mexican adolescent females and young women. *J Asthma*. 2005;42:705-709.
- Chen YC, Tu YK, Huang KC, Chen PC, Chu DC, Lee YL. Pathway from central obesity to childhood asthma. Physical fitness and sedentary time are leading factors. *Am J Respir Crit Care Med*. 2014;189:1194-1203.
- World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310:2191-2194.
- Taylor SJ, Whincup PH, Hindmarsh PC, Lampe F, Odoki K, Cook DG. Performance of a new pubertal self-assessment questionnaire: a preliminary study. *Paediatr Perinat Epidemiol*. 2001;15:88-94.
- Sun Y, Tao FB, Su PY, et al. National estimates of the pubertal milestones among urban and rural Chinese girls. *J Adolesc Health*. 2012;51:279-284.
- Wang Y. Is obesity associated with early sexual maturation? A comparison of the association in American boys versus girls. *Pediatrics*. 2002;110:903-910.
- Chen YC, Liou TH, Chen PC, et al. Growth trajectories and asthma/rhinitis in children: a longitudinal study in Taiwan. *Euro Respir J*. 2017;49:1.
- Chen W, Chang MH. New growth charts for Taiwanese children and adolescents based on World Health Organization standards and health-related physical fitness. *Pediatr Neonatol*. 2010;51:69-79.
- Elks CE, Perry JR, Sulem P, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet*. 2010;42:1077-1085.
- Perry JR, Day F, Elks CE, et al. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. *Nature*. 2014;514:92-97.
- He C, Kraft P, Chen C, et al. Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. *Nat Genet*. 2009;41:724-728.
- Liu YL, Wang SC, Hwu HG, et al. Haplotypes of the D-amino acid oxidase gene are significantly associated with schizophrenia and its neurocognitive deficits. *PLoS One*. 2016;11:e0150435.
- Chen YC, Fan HY, Huang YT, Huang SY, Liou TH, Lee YL. Causal relationships between adiposity and childhood asthma: bi-directional Mendelian randomization analysis. *Int J Obes (Lond)*. 2018;19:19.
- Pedersen AB, Mikkelsen EM, Cronin-Fenton D, et al. Missing data and multiple imputation in clinical epidemiological research. *Clin Epidemiol*. 2017;9:157-166.
- Palmer TM, Sterne JA, Harbord RM, et al. Instrumental variable estimation of causal risk ratios and causal odds ratios in Mendelian randomization analyses. *Am J Epidemiol*. 2011;173:1392-1403.
- Burgess SP, Bowden JP, Fall TP, Ingelsson EP, Thompson SGP. Sensitivity analyses for robust causal inference from mendelian randomization analyses with multiple genetic variants. *Epidemiology*. 2017;28:30-42.

26. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44:512-525.
27. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. 2016;40:304-314.
28. Richardson DB. Discrete time hazards models for occupational and environmental cohort analyses. *Occup Environ Med*. 2010;67:67-71.
29. Nicolai T, Pereszlenyiova-Bliznakova L, Illi S, Reinhardt D, von Mutius E. Longitudinal follow-up of the changing gender ratio in asthma from childhood to adulthood: role of delayed manifestation in girls. *Pediatr Allergy Immunol*. 2003;14:280-283.
30. Burgess S, Thompson SG. Use of allele scores as instrumental variables for Mendelian randomization. *Int J Obes (Lond)*. 2013;42:1134-1144.
31. Anderson HR, Pottier AC, Strachan DP. Asthma from birth to age 23: incidence and relation to prior and concurrent atopic disease. *Thorax*. 1992;47:537-542.
32. Vink NM, Postma DS, Schouten JP, Rosmalen JGM, Boezen HM. Gender differences in asthma development and remission during transition through puberty: the TRacking Adolescents' Individual Lives Survey (TRAILS) study. *J Allergy Clin Immunol*. 2010;126:498-504.
33. Salam MT, Wenten M, Gilliland FD. Endogenous and exogenous sex steroid hormones and asthma and wheeze in young women. *J Allergy Clin Immunol*. 2006;117:1001-1007.
34. Haggerty CL, Ness RB, Kelsey S, Waterer GW. The impact of estrogen and progesterone on asthma. *Ann Allergy Asthma Immunol*. 2003;90:284-289.
35. Cocchiara R, Albeggiani G, Di Trapani G, et al. Modulation of rat peritoneal mast cell and human basophil histamine release by estrogens. *Int Arch Allergy Immunol*. 1990;93:192-197.
36. Clifton V. Maternal asthma during pregnancy and fetal outcomes: potential mechanisms and possible solutions. *Curr Opin Allergy Clin Immunol*. 2006;6:307-311.
37. Macsali F, Real FG, Plana E, et al. Early age at menarche, lung function, and adult asthma. *Am J Respir Crit Care Med*. 2011;183:8-14.
38. Kozyrskyj AL, Zeng Y, Saurek-Aleksandrovska N, Sellers EAC, Ramsey CD, Becker AB. Insulin resistance, puberty, and nonatopic asthma in adolescent girls. *Am J Respir Crit Care Med*. 2014;190:474-477.
39. Shalitin S, Phillip M. Role of obesity and leptin in the pubertal process and pubertal growth—a review. *Int J Obes (Lond)*. 2003;27:869-874.
40. Guler N, Kirerleri E, Ones U, Tamay Z, Salmayenli N, Darendeliler F. Leptin: does it have any role in childhood asthma? *J Allergy Clin Immunol*. 2004;114:254-259.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Online supplement Appendix

Early Pubertal Maturation and Childhood Asthma: A Mendelian Randomization and Longitudinal Study

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Table E1. Associations of puberty genetic variants with early pubertal maturation and active asthma at 11-12 years

Rs number	Nearby Gene	Association with early pubertal maturation†				Association with active asthma		References
		CHR	MAF*	OR per allele (95%CI)	P value	OR per allele (95%CI)	P value	
rs6589964	BSX	11	0.43	1.00 (0.99, 1.02)	0.63	1.00 (1.00, 1.01)	0.81	Elks <i>et al</i>
rs2243803	SLC14A2	18	0.19	0.98 (0.96, 1.00)	0.03	0.99 (0.99, 1.00)	0.08	Perry <i>et al</i>
rs7642134	VGLL3	3	0.42	1.00 (0.98, 1.01)	0.68	1.00 (0.99, 1.01)	0.88	Elks <i>et al</i>
rs1079866	INHBA	7	0.21	1.00 (0.98, 1.02)	0.97	1.00 (0.99, 1.00)	0.56	Elks <i>et al</i>
rs12148769	MKRN3	15	0.35	1.00 (0.98, 1.01)	0.69	1.00 (0.99, 1.00)	0.52	Perry <i>et al</i>
rs4452860	VGLL3	9	0.48	1.01 (1.00, 1.03)	0.03	1.00 (1.00, 1.01)	0.11	He <i>et al</i>

*MAF: minor allele frequency

†Allele: Early pubertal maturation increasing allele / Early pubertal maturation decreasing allele

Table E2. P-values for the association of individual pubertal maturation SNP with confounding factors

Rs number	Nearby Gene	Gender		Parental Education		Family Income	
		Coefficient (95% CI)	P value	Coefficient (95% CI)	P value	Coefficient (95% CI)	P value
rs6589964	BSX	0.01 (-0.01, 0.03)	0.25	0.01 (-0.01, 0.03)	0.62	-0.01 (-0.03, 0.01)	0.39
rs2243803	SLC14A2	-0.4×10^{-2} (-0.02, 0.02)	0.74	0.01 (-0.03, 0.05)	0.46	-0.3×10^{-2} (-0.04, 0.04)	0.87
rs7642134	VGLL3	-0.01 (-0.03, 0.01)	0.56	-0.2×10^{-2} (-0.02, 0.02)	0.88	-0.01 (-0.05, 0.03)	0.51
rs1079866	INHBA	-0.01 (-0.03, 0.01)	0.25	0.05 (0.01, 0.09)	0.001	0.04 (0.001, 0.08)	0.03
rs12148769	MKRN3	0.01 (-0.01, 0.03)	0.30	0.01 (-0.01, 0.03)	0.55	0.01 (-0.03, 0.05)	0.51
rs4452860	VGLL3	0.3×10^{-2} (-0.02, 0.02)	0.73	-0.03 (-0.05, -0.01)	0.02	-0.02 (-0.04, -0.4×10^{-3})	0.25

These p-values have not been corrected for multiple testing. Under Bonferroni-correction the cut-off for significance is $\alpha = 0.05/6 = 0.008$

Table E3. Estimates of causal effect of early pubertal maturation on active asthma at 11-12 years by different Mendelian Randomization sensitivity analysis

Analysis Method	Male				Female			
	Coefficient*	Standard Error	OR (95% CI) for active asthma	<i>p</i> value	Coefficient*	Standard Error	OR (95% CI) for active asthma	<i>p</i> value
Inverse-variance weighted								
Fixed-effect	0.32	0.13	1.38 (1.08, 1.77)	0.01	0.11	0.15	1.11 (0.83, 1.48)	0.47
Random-effects	0.32	0.09	1.38 (1.17, 1.64)	1.9×10 ⁻⁴	0.11	0.13	1.11 (0.86, 1.43)	0.41
Egger regression, intercept								
Fixed-effect	0.00	0.00	1.00 (1.00, 1.01)	0.43	0.00	0.00	1.00 (1.00, 1.01)	0.35
Random-effects	0.00	0.00	1.00 (1.00, 1.01)	0.43	0.00	0.00	1.00 (1.00, 1.01)	0.35
Egger regression, slope								
Fixed-effect	0.45	0.19	1.56 (1.08, 2.25)	0.02	-0.12	0.26	0.88 (0.53, 1.48)	0.64
Random-effects	0.45	0.19	1.56 (1.08, 2.25)	0.02	-0.12	0.26	0.88 (0.53, 1.48)	0.64
Simple median	0.07	0.30	1.07 (0.59, 1.93)	0.82	0.42	0.29	1.52 (0.86, 2.71)	0.15
Weighted median	0.36	0.55	1.43 (0.49, 4.19)	0.52	0.00	0.45	1.00 (0.42, 2.40)	0.99

*Log OR for active asthma under early pubertal maturation.

Table E4. Multivariate analysis between stratified overweight status, early pubertal maturation status and asthma outcomes in children at 11-12 years, by genders

Overweight/ Early pubertal maturation	Physician-diagnosed Asthma		Current Wheeze		Active Asthma	
	Adjusted OR		Adjusted OR		Adjusted OR	
	(95% CI)	<i>p</i> -value	(95% CI)	<i>p</i> -value	(95% CI)	<i>p</i> -value
Males						
Overweight (-)/ Early pubertal maturation (-)	Reference		Reference		Reference	
Overweight (+)/ Early pubertal maturation (-)	1.00 (0.97, 1.02)	0.72	1.02 (1.00, 1.04)	0.06	1.00 (0.99, 1.02)	0.70
Overweight (-)/ Early pubertal maturation (+)	0.99 (0.96, 1.03)	0.77	1.01 (0.98, 1.04)	0.37	1.02 (1.00, 1.04)	0.07
Overweight (+)/ Early pubertal maturation (+)	1.07 (1.03, 1.13)	0.01	1.06 (1.02, 1.09)	0.02	1.02 (0.99, 1.04)	0.19
<i>P</i> for trend		0.07		0.01		0.05
Females						
Overweight (-)/ Early pubertal maturation (-)	Reference		Reference		Reference	
Overweight (+)/ Early pubertal maturation (-)	1.01 (0.99, 1.03)	0.35	1.01 (0.99, 1.02)	0.44	1.01 (1.00, 1.02)	0.31
Overweight (-)/ Early pubertal maturation (+)	1.03 (0.98, 1.08)	0.32	1.03 (0.99, 1.06)	0.27	1.02 (0.99, 1.04)	0.29
Overweight (+)/ Early pubertal maturation (+)	1.05 (1.00, 1.10)	0.08	1.02 (0.98, 1.06)	0.43	1.00 (0.98, 1.03)	0.90
<i>P</i> for trend		0.04		0.17		0.28

Models were adjusted for age, sex, parental education, family income

Table E5. Associations between adiposity measures, early pubertal maturation and asthma outcomes in children at 11-12 years (Mediation Analysis)

Asthma outcomes/Adiposity measures (z-score)	Average Causal Mediation Effect (O→P→A)		Average Direct Effect (O→A)		Total Effect (Direct + Mediation Effect)	
	Coefficient (95% CI)	<i>p</i> -value	Coefficient (95% CI)	<i>p</i> -value	Coefficient (95% CI)	<i>p</i> -value
Physician-diagnosed Asthma	9.6×10^{-4} (2.6×10^{-4} , 2.2×10^{-3})	<0.01	2.7×10^{-3} (-4.4×10^{-3} , 0.01)	0.49	3.7×10^{-3} (-3.3×10^{-2} , 0.01)	0.30
Male	7.3×10^{-4} (-2.5×10^{-4} , 1.5×10^{-3})	0.21	1.9×10^{-3} (-9.3×10^{-3} , 0.01)	0.74	2.6×10^{-3} (-8.8×10^{-3} , 0.01)	0.70
Female	1.5×10^{-3} (-2.5×10^{-4} , 3.2×10^{-3})	0.11	4.5×10^{-3} (-4.8×10^{-3} , 0.01)	0.35	0.25 (-1.89, 1.90)	0.31
Current Wheeze	7.4×10^{-4} (7.7×10^{-5} , 1.5×10^{-3})	0.02	3.9×10^{-3} (-1.9×10^{-3} , 0.01)	0.20	4.6×10^{-3} (-1.2×10^{-2} , 0.01)	0.14
Male	3.7×10^{-4} (-1.7×10^{-4} , 1.2×10^{-3})	0.32	9.1×10^{-3} (-6.6×10^{-5} , 0.02)	0.05	9.5×10^{-3} (4.5×10^{-5} , 0.02)	0.04
Female	5.1×10^{-4} (-2.8×10^{-4} , 2.4×10^{-3})	0.18	-9.7×10^{-4} (-7.9×10^{-3} , 6.0×10^{-3})	0.77	-4.6×10^{-4} (-6.8×10^{-3} , 6.7×10^{-3})	0.96
Active Asthma	7.2×10^{-4} (-1.4×10^{-4} , 1.7×10^{-3})	0.09	4.4×10^{-4} (-3.0×10^{-3} , 4.5×10^{-3})	0.82	1.2×10^{-3} (-2.2×10^{-3} , 5.2×10^{-3})	0.54
Male	7.8×10^{-4} (-4.6×10^{-4} , 2.0×10^{-3})	0.25	1.4×10^{-3} (-3.9×10^{-3} , 7.8×10^{-3})	0.63	2.2×10^{-3} (-3.4×10^{-3} , 8.4×10^{-3})	0.48
Female	5.0×10^{-4} (-6.3×10^{-4} , 2.2×10^{-3})	0.42	-2.2×10^{-4} (-4.2×10^{-3} , 4.7×10^{-3})	0.94	2.8×10^{-4} (-3.4×10^{-3} , 5.1×10^{-3})	0.81

Table E6. Incident asthma outcomes at age 12, 17 by sex.

Characteristics	Children at 12 y	Children at 17 y
	(n = 2991)	(n=1386)
Physician-diagnosed asthma	114 (4.3)	20 (1.7)
Male	62 (4.7)	10 (1.7)
Female	52 (3.9)	10 (1.8)
Current wheeze	54 (2.3)	6 (0.5)
Male	27 (2.3)	2 (0.3)
Female	27 (2.2)	4 (0.7)
Active asthma	14 (0.5)	2 (0.2)
Male	5 (0.4)	1 (0.2)
Female	9 (0.7)	1 (0.2)

Incident asthma cases among children aged 12 and 17 years were calculated by excluding those with a history of asthma prior to the age of 12 and 17 years, respectively.

Figure E1. Causal diagram of adiposity, early pubertal maturation, and childhood asthma

