Obesity Research & Clinical Practice 12 (2018) 445–451

Contents lists available at ScienceDirect

Obesity Research & Clinical Practice

journal homepage: www.elsevier.com/locate/orcp

Birthweight, time-varying adiposity growth and early menarche in girls: A Mendelian randomisation and mediation analysis

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ARTICLE INFO

Article history:
Received 11 March 2018
Received in revised form 21 June 2018
Accepted 13 July 2018

Keywords:
Birthweight
Adiposity growth
Mendelian randomisation
Mediation analysis
Early menarche

ABSTRACT

Objective: To explore the causal effect of time-varying z-BMI growth on early menarche using Mendelian randomisation (MR); to identify critical adiposity predictors of early menarche; to compare the effects of birthweight and time-varying z-BMI growth as mediators of the path from genes to early menarche using mediation analysis.
Methods: We used data from the Taiwan Children Health Study with 21 obesity-related single-nucleotide polymorphisms (SNPs) to yield genetic (instrumental variable)IVs for adiposity. Children with available data on genotyping, birthweight, adiposity, and menarcheal age were included.
Results: In MR analyses, results based on the time-varying z-BMI growth show more statistical power and capture more information of adiposity growth ($p = 0.01$) than those based on single point z-BMI ($p = 0.02$). Among adiposity measures, critical predictors of early menarche are fat free mass (RR = 1.33, 95% CI 1.07–1.65) and waist/height ratio (RR = 1.27, 95% CI 1.03–1.56). Other potential predictors of early menarche are sum of skinfold (RR = 1.24, 95% CI 1.03–1.48) and total body fat (RR = 1.20, 95% CI 1.05–1.38). In both one-mediation and multi-mediation analyses, time-varying z-BMI growth in the prepubertal years plays a crucial mediator in the pathway from the genes to early menarche.
Conclusions: This study discovered that greater prepubertal adiposity growth is a crucial mediator in the path from genes to early menarche. For girls with genes positively associated with obesity; and/or of lower birthweight, a strategy to prevent childhood adiposity should be implemented in order to avoid early menarche development.

Introduction

Childhood obesity has been linked to early menarche, which further leads to health events in later life such as fertility impairment [1], cardiometabolic diseases [2], breast cancer [3] and mortality [4]. Many developed countries have rising trends of childhood obesity [5] and declining trends of age at menarche [6], leading to speculation that adiposity and sexual maturation may be causally related. While most observational studies have found that girls with large body weight in childhood experienced an earlier age of menarche [7–9], the pathway from genes to early menarche via birthweight and adiposity growth is less clear.

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https://doi.org/10.1016/j.orcp.2018.07.008
1871-403X/

Mendelian randomisation (MR) analysis may be a desirable approach for causal inference analysis. Causal inference analysis in observational studies may be particularly susceptible to the effects of biases such as residual or unmeasured confounding and reverse causation [10], and adiposity exposures are difficult to study using randomised control trials (RCTs). Mumby et al. conducted a MR study using a proxy measure of adiposity (BMI at age 20) [11] but their study was limited in that only BMI data from a single arbitrary time point was used, and therefore it was unable to adequately capture time-varying adiposity growth. Although current studies have measured BMI at some time points [8,9], the use of such irregular and sparse longitudinal data may undervalue relationship inferences, not merely due to the measurement error in the exposure, but also due to a failure to capture its long-term change [12]. Therefore, current methodical studies have proposed a new approach, principal analysis by conditional expectation (PACE) [12,13], wherein original time points are recovered and smoothed, the cumulative effect of the time-varying exposure variable is calculated, and an MR analysis is performed.

Incomplete measurements of adiposity throughout infant and childhood may underestimate or overestimate the influence of birthweight and childhood adiposity on menarcheal timing [8]. For example, many studies report that lower birthweight is a risk for early menarche [8,14]; but that girls of a low fetal and early childhood body weight had a lower risk of early menarche [8,9]. However, their studies were limited by not assessing prepubertal body weight at multiple time points and misattributing sequential causation. Thus, studies with complementary measures of adiposity during prepubertal years are needed to determine whether the processes that relate prepubertal adiposity to timing of menarche.

Our study has three aims: (1) to explore the effect of single point z-BMI and time-varying z-BMI growth on early menarche using MR; (2) to discover critical adiposity predictors of early menarche using MR; (3) to further explore the mediating pathway from genes, birthweight and prepubertal time-varying z-BMI growth to early menarche and thereby reveal the critical factors influencing early menarche development.

Subjects, materials and methods

Cohort description

The Taiwan Children Health Study (TCHS) is a nationwide school-based cohort study consisting of two cohorts. Containing a menarcheal outcome, the second cohort with an open cohort design was used in this study. The menarcheal outcome and demographic information about girls and their family were reported from interviews in school. Adiposity measures were conducted by our trained members. Oral mucosa samples were collected to extract genomic DNA for identifying BMI SNPs. These data and samples were collected in 2010. Detailed procedures have been described in previous articles [15].

Definitions

Maternal gestational exposures are defined as girls whose was in was in fetal conditions [8]. The prenatal exposure was defined as exposures at birth. Prepubertal exposures are defined as adiposity measures before/at the age of menarche [35]. The menarche outcome is defined as the first menstrual bleeding in girls.

Menarcheal outcome

The Chinese version of the Puberty Category Score (PCS) was used to define early menarche [16]. Menarche was asked as either yes or no. When children answered the question with “yes”, each was asked an open question (age at menarche) at the end. Probit analysis based on the status quo method was used to observe a distribution of age of menarche [17]. The ages of 25th percentile [18] for menarche were 10.8 years, which was defined as early menarche.

Adiposity measures

General and abdominal adiposity measurements were obtained annually [19]. To standardise measurements, each was converted into age sex-specific z-scores according to our cohort reference [19]. BMI was converted into BMI z-scores according to the WHO Growth Standards [20]. Adiposity indices, including body fat, fat free mass, sum of skinfolds, waist circumference, hip, waist/hip ratio, and waist/height ratio, were described in the supplemental information.

Genotyping

Obesity-related SNPs were chosen from either the Asian Genome-Wide Association Study (GWAS) meta-analysis [21], or the Chinese child study (see the supplemental information) [22]. FTO genes are strong adiposity-related genes in the Chinese population [23]. Therefore, the 21 candidate SNPs were grouped as FTO genes (2 SNPs) and Non-FTO genes (19 SNPs).

Confounders

We identified potential confounders by reference to previous literature [24], and included maternal gestational factors (e.g., age, weight gain, and diabetes), breastfeeding, birthweight, parental education and household cigarette smoke. These confounders were adjusted for generalised estimating equations (GEE) analysis.

Data analysis

A weighted genetic risk score (GRS) based on the allele dosages and the coefficients was calculated [25]. A principal analysis through conditional expectation (PACE) was used to handle our time-varying individualised growth (see supplemental information) [12,13]. The strength of the association between genetic variants with time-varying z-BMI growth and single point z-BMI was compared using linear regression analysis with assumptions (see Fig. S1 and Table S3).

Basic characteristics of each SNP, including minor allele frequency and F statistics were determined with Plink v2.0. Linear regression analyses were used to examine relationships between genetic variants and adiposity measures (Table S1), and genetic variants and numeric confounders (Table S2). A logistic regression model was used to explore the relationships between genetic variants and binary confounders (Table S2), and genetic variants and binary outcomes (Table S1). A GEE approach taking into account intra-subject correlation of responses was used to estimate the relationship between repeated measures (adiposity measures) and menarcheal outcome (early menarche) (Table 3).

The two-stage least squares (2SLS) method is widely used in MR analysis, and is applicable for binary exposures and outcomes [26]. We performed this method to estimate the causal direction between adiposity exposures and early menarche (Fig. 1 and Tables 2 and 3). Further, sensitivity analyses (Fig. S3 and Table S4) and meta-analyses (Fig. S2) were implemented for supporting the robustness of the causal conclusions from the MR analysis (see supplemental information).

One-mediation and multi-mediation analyses were used to further understand the causal pathways leading to a risk of early menarche (Figs. 2). To compare the effect of a birthweight and

Table 1
Characteristics of all fifth- and sixth-grade girls in Taiwan Children Health Study.

Characteristics	Number at 11 years/12 years	Girls at 11 years (n = 1003)		Girls at 12 years (n = 938)	
		N or mean	% or SD	N or mean	% or SD
Menarcheal outcome					
Early menarche	1003/938	228	22.7	300	32.0
Prenatal exposure					
Birthweight (g)	707/693	3083.8	454.4	3080.9	471.3
Prepubertal exposure					
Time-varying BMI (kg/m^2)[a]	1003/938	17.8	3.0	17.8	3.0
Single BMI value (kg/m^2)[b]	1003/938	18.9	3.6	20.0	3.7
Total body fat (kg)	988/772	8.8	4.8	10.8	5.1
Fat-free mass (kg)	988/772	31.9	5.4	34.2	5.0
Sum of skinfolds (mm)	990/930	36.6	11.0	34.5	9.7
Hip (inch)	990/930	79.9	8.1	84.4	7.8
Waist/hip ratio (%)	990/928	80.0	5.7	74.9	6.0
Waist/height ratio (%)	990/928	43.7	5.6	42.3	5.6
Causal variables					
All genes (21 SNPs)	1003/938	34.3	8.1	34.4	7.9
FTO gene (2 SNPs)	1003/938	24.4	40.1	24.3	40.2
Non-FTO genes (19 SNPs)	1003/938	35.3	7.8	35.4	7.5
Confounders					
Age (yr)	1003/938	10.6	0.5	11.7	0.5
Maternal gestational age (wk)	707/693	38.6	1.9	38.6	3.1
Maternal gestational weight gain (kg)	945/880				
≤15		668	70.7	622	70.7
16–25		243	25.7	232	26.4
≥26		34	3.6	26	3.0
Maternal gestational diabetes	1003/938	16	1.6	14	1.5
Breastfeeding	687/660	350	50.9	332	50.3
Parental education	993/914				
High school or below		437	46.8	440	48.1
College or university		411	44.1	397	43.4
Post-graduate school		85	9.1	77	8.4
Household cigarette smoke	993/914	363	38.9	378	41.4

All data are presented as numbers (%) or means (SD).
[a] Time varying z-BMI is an approximation of the integral by a Riemann sum.
[b] The single BMI value was measured at 11–12 years.

Table 2
Summary of coefficients used for Mendelian randomisation analysis.

Outcome definition (E)	Genetic variants (G)	z-BMI exposure (A)	Genetic score (G) with intermediate trait (A)			Genetic score (G) with outcomes (E)			Two-stage IV model (G → A → E)		
			β_{XG}	(95% CI)	p	β_{YG}	(95% CI)	p	β_{IV}	(95% CI)	p
Early menarche	All genes	Time-varying[a]	1.93	(1.29, 2.58)	<0.001	0.31	(0.06, 0.55)	0.02	0.16	(0.03, 0.29)	0.01
		Single point[b]	1.75	(1.21, 2.30)	<0.001	0.31	(0.06, 0.55)	0.02	0.17	(0.03, 0.32)	0.02
	FTO genes	Time-varying[a]	0.14	(0.01, 0.27)	0.03	0.02	(−0.03, 0.07)	0.40	0.15	(−0.20, 0.50)	0.40
		Single point[b]	0.10	(−0.01, 0.21)	0.08	0.02	(−0.03, 0.07)	0.40	0.21	(−0.30, 0.72)	0.41
	Non-FTO genes	Time-varying[a]	1.93	(1.25, 2.60)	<0.001	0.31	(0.05, 0.57)	0.02	0.16	(0.03, 0.29)	0.02
		Single point[b]	1.83	(1.26, 2.40)	<0.001	0.31	(0.05, 0.57)	0.02	0.17	(0.03, 0.31)	0.02

[a] Time varying z-BMI Growth is an approximation of the integral by a Riemann sum.
[b] Single z-BMI value was measured at 11–12 years.

Table 3
Effects of various adiposity measures on early menarche in girls using generalised estimating equation (GEE) and two-stage least squares (2SLS) estimator.

Outcome definition	Adiposity measures (z-score)[b]	GEE[a] (A with E)			2SLS (G → A → E)		
		RR	(95% CI)	*p*	RR	(95% CI)	*P*
Early menarche	BMI	1.11	(1.08, 1.14)	<0.001	1.19	(1.03, 1.37)	0.02
	Total body fat	1.12	(1.09, 1.16)	<0.001	1.20	(1.05, 1.38)	0.01
	Fat free mass	1.16	(1.12, 1.19)	<0.001	1.33	(1.07, 1.65)	0.01
	Sum of skinfolds	1.03	(1.01, 1.06)	0.02	1.24	(1.03, 1.48)	0.02
	Hip	1.01	(1.57, 2.72)	<0.001	1.01	(1.01, 1.02)	0.02
	Waist/hip ratio	0.99	(0.96, 1.02)	0.43	2.48	(0.63, 9.74)	0.19
	Waist/height ratio	1.05	(1.02, 1.09)	<0.001	1.27	(1.03, 1.56)	0.03

G represents BMI genetic score; A represents the adiposity measures; E represents the potential outcome (early menarche).
[a] Models were adjusted for age, maternal gestational age, gestational weight gain, gestational diabetes, birthweight, breastfeeding, parental education and household cigarette smoke.
[b] Single values were measured at 11–12 years.

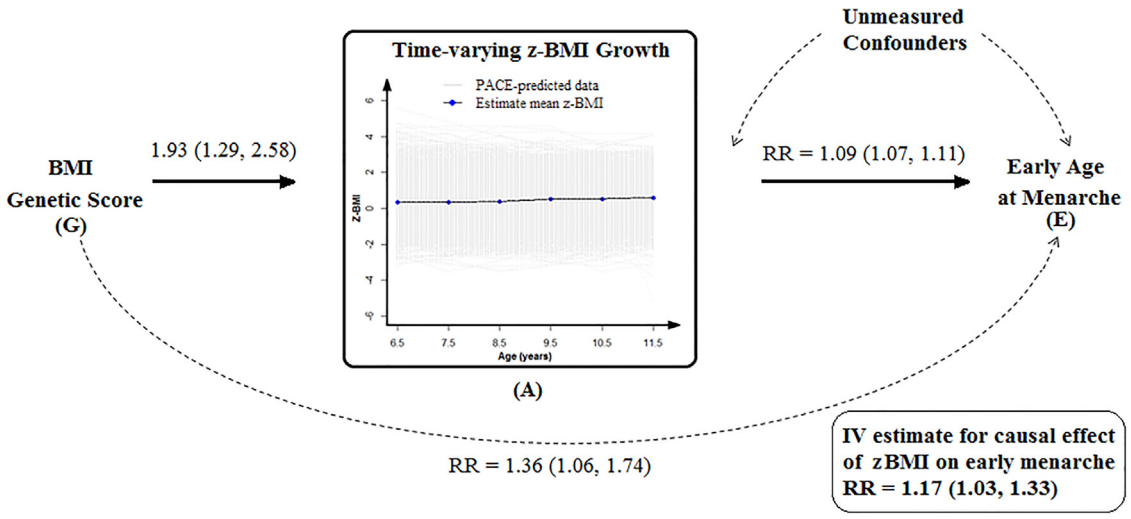
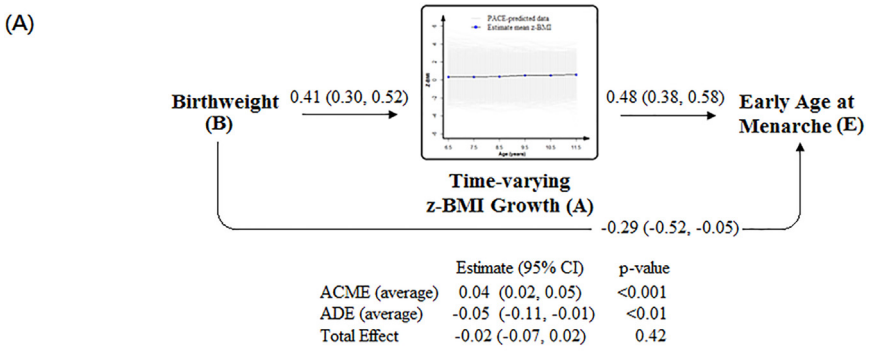
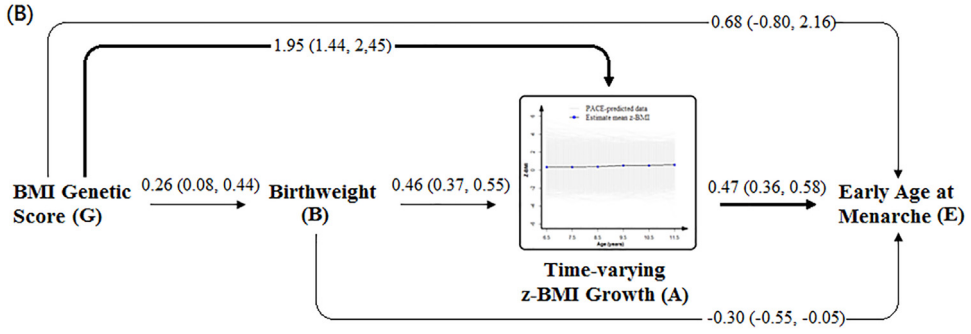


Fig. 1. Main Mendelian randomisation analysis with a time-varying z-BMI growth and results of the study.



	Estimate (95% CI)	p-value
ACME (average)	0.04 (0.02, 0.05)	<0.001
ADE (average)	-0.05 (-0.11, -0.01)	<0.01
Total Effect	-0.02 (-0.07, 0.02)	0.42



Pathway		Point estimate	(95% CI)	p-value
Risk Differences	G->E	0.12	(-0.10, 0.34)	0.294
	G->A->E	0.20	(0.08, 0.32)	0.001
	G->BE	-0.003	(-0.01, 0.01)	0.604
	Total Effect	0.31		
Risk Ratio	G->E	2.44	(0.63, 9.35)	0.195
	G->A->E	1.99	(1.26, 3.13)	0.003
	G->BE	0.99	(0.97, 1.02)	0.608
	Total Effect	4.92		

Fig. 2. Mediation pathways from BMI genetic score to early menarche. (A) The one-mediation analysis result: Mediation pathways from birthweight to early menarche. (B) The multiple-mediation analysis result: Mediation pathways from BMI genetic score to early menarche through birthweight and time-varying z-BMI growth.

a time-varying z-BMI growth on early menarche, one-mediation analysis was performed. A multi-mediation analysis [27] was used to compare birthweight and time-varying z-BMI growth as mediators of the pathway from genetic variants to early menarche (see Supplemental information).

Results

The study population comprised 1003 girls at age 11 years in 2011 and 938 girls at age 12 years in 2012. The prevalence rate of early menarche was 22.7% among these girls at age 11 years. 32% had an early menarche before or during age 12 years. The average BMI at age 11 years was 18.9 kg/m^2; and 20 kg/m^2 at age 12 years (Table 1).

Genetic associations

The 21 candidate SNPs, their allele frequencies, and their association with z-BMI and early menarche at age 11–12 years are listed in Table S1.

Table S1 lists associations of individual BMI-related SNP with z-BMI and early menarche at 11–12 years. Our supplemental references reported important evidence associating each SNP with adiposity. Little evidence of association between individual SNPs and early menarche were noted. The weighted genetic scores of the 21 SNPs were robustly correlated with z-BMI, and also correlated early menarche (Table 2). Genetic scores of the 21 SNPs served as strong instruments with F-statistics of over 10 (F statistics: 39.85).

Associations of genetic scores with single versus time-varying adiposity growth

Linear regression models were used to separately model genetic scores on single point z-BMI and time-varying z-BMI growth. The coefficients of genetic scores in relation to time-varying z-BMI growth were higher than the coefficients relating genetic score to single point z-BMI (Table 2). As a rule of thumb [28], values of DW between 1.5 and 2.5 show that the residuals of the two regressions are not linearly auto-correlated (Table S3 and Fig. S1). Regression models for time-varying z-BMI growth are homoscedastic and normally distributed ($p > 0.05$); while regression models for single point z-BMI showed a violation of the assumption of homoscedasticity and normality. Therefore, the PACE method which incorporated longitudinal data of a time-varying exposure could improve the accuracy of the first stage regression from 2SLS model.

Single versus time-varying adiposity cause early menarche

Fig. 1 depicts the main MR results of the study. Table 2 presents the coefficients of the MR analysis. All genetic scores had a stronger association with the time-varying z-BMI growth ($\beta = 1.93$, 95% CI: 1.29–2.58) than with the single point z-BMI ($\beta = 1.75$, 95% CI: 1.21–2.30). Even after the deletion of the well-known obesity gene FTO, the remaining 19 SNPs were more strongly associated with the time-varying z-BMI growth ($\beta = 1.93$, 95% CI: 1.25–2.60) than with the single point z-BMI value ($\beta = 1.83$, 95% CI: 1.26–2.40). The genetic scores of the 21 SNPs and the remaining 19 SNPs were significantly associated with early menarche.

In the MR analysis of the causal effect of z-BMI on early menarche using the genetic score of 21SNPs, the results based on the time-varying z-BMI growth (p-value of 0.01) were more significant than on the single point z-BMI (p-value of 0.02). This indicated that the time-varying z-BMI growth, a substantial adiposity measure, may avoid inflating a Type I error rate in second stage testing due to stable estimation in first stage.

MR sensitivity analysis

A sensitivity analysis was conducted to ascertain the robustness of the causal estimates from the MR analysis. MR with multiple-genetic instruments was used as a meta-analysis (Fig. S2), which showed little evidence of heterogeneity in BMI-SNPs (I-squared = 0, p-value of 0.948 and 0./928). The causal effect summary RR of the 21 SNPs on early menarche using the time varying z-BMI exposure was identical to the weighted genetic score 2SLS RRs (Fig. 1 and Table 2). The additional approaches of sensitivity analysis provided more evidence of a causal effect of the time-varying z-BMI exposure on early menarche (Table S4). The causal effect RRs of early menarche per unit increase in time varying z-BMI exposure were 1.17 and 1.18 for the inverse-variance weighted and Egger regression (slope) methods, respectively; both of which were consistent with the previous MR estimate of 1.17 (95% CI: 1.03–1.33, p = 0.01). Moreover, Scatter plots using genetic variants (both left and right) for early menarche are depicted in Fig. S3, and demonstrate the absence of any notable genetic pleiotropic effects.

Effects of different adiposity measures on early menarche

All of the adiposity exposures except waist/hip ratio were found to be significantly associated with early menarche, after adjustment for confounders (Table 3). In the MR analyses, the fat free mass was the strongest predictor of early menarche (RR: 1.33, 95% CI: 1.07–1.65). The waist/height ratio was also a strong predictor early menarche (RR: 1.27, 95% CI: 1.03–1.56). Based on the IV estimates, corresponding RRs for early menarche are 1.20 (95% CI: 1.05–1.38) for total body fat and 1.24 (95% CI: 1.03–1.48) for sum of skinfolds.

Effect of birthweight, time-varying adiposity growth on early menarche

The time-varying z-BMI growth acted as a mediator of the relationship between birthweight and early menarche (Fig. 2(A)). We found a positive relationship between the birthweight and time-varying z-BMI growth (B → A), and a positive association between time-varying z-BMI growth and early menarche (A → E). When the mediator (the time-varying z-BMI growth) was included in the model, the association between birthweight and early menarche was negative (B → E). From birthweight to early menarche, the total effect was −0.02 ($p = 0.42$). We discovered a significant average causal mediation effect (ACME = 0.04, $p < 0.001$) and a significant average direct effect (ADE = −0.05, $p < 0.01$), indicating the critical role of the time-varying z-BMI growth in the causal pathways from birthweight to early menarche.

Pathways from Genes to early menarche via birthweight and time-varying adiposity

Path coefficient from genetic score, birthweight, and time-varying z-BMI growth to early menarche was all significant, indicating birthweight and time-varying z-BMI growth were both possible mediators from genes to early menarche (Fig. 2(B)). Based on the closed form solution [29], we derived the path specific risk difference (RD) and risk ratio (RR) using the total effect from genes to early menarche (G → E) as the reference path. The RD and RR for the path 1 effect (G → E) were 0.12 (95% CI: −0.10 to 0.34) and 2.44 (95% CI: 0.63–9.35). The RD and RR of the path 2 effects (G → A → E) as compared to total effect (G → E) were significantly higher (p = 0.001, RD: 0.20 (95% CI: 0.08–0.32) and RR: 1.99 (95% CI: 1.26–3.13). The risk difference and risk ratio of the RD and RR of

path 3 effects (G → B → E or G → B → A → E) were −0.003 (95% CI: −0.01 to 0.01) and 0.99 (95% CI: 0.97–1.02). Time-varying z-BMI growth acted as a crucial mediator of the pathway from the genetic variants to early menarche.

Discussion

To the best of our knowledge, this study is the first to use time-varying z-BMI growth to conduct MR and mediation analyses for investigating the causal pathway from genes to early sexual maturation. In MR analyses, the results based on the time-varying z-BMI growth show more statistical power and capture more information of adiposity growth than on the single point z-BMI. The causal estimates from MR analyses have been confirmed by MR sensitivity analyses. Among adiposity markers, the strongest predictors of early menarche are fat free mass and waist/height ratio. In the one-mediation analysis, the time-varying z-BMI growth acted as a main mediator from birthweight to early menarche. In multi-mediation analysis, the time-varying z-BMI growth still plays a crucial mediator in the pathway from the genes to early menarche.

We discover that the prepubertal adiposity growth is a critical predictor for menarcheal timing. In addition to BMI, body composition might also play a role in menarcheal onset. Previous studies suggest that body fat is significantly associated with early menarche [7], a finding confirmed by our MR analysis. One possible explanation is that levels of the adipocyte-derived protein hormone leptin are higher in people with high BMI [30]. Leptin may accelerate the central activation of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which stimulates the anterior portion of the pituitary gland to produce and secrete luteinising hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH may accelerate the gonads, contributing to early estrogenisation in girls. In this study, we also discovered that the strongest effect of fat free mass on early menarche were slightly higher than total body fat, which is consistent with a previous study [7]. One possible explanation is that the increase in intra-myocellular lipid within lean tissue may exert sex hormone regulating effects. Interestingly, waist/height ratio, an indicator of central obesity, exerts a significantly higher effect on early menarche than waist/hip ratio. During pubertal development, most girls experienced fat accumulation in the hip region (gynecoid obesity), rather than the waist. This is why the waist/hip ratio shows an inverse relationship with early menarche (Table 1). One possible explanation is that the increase in abdominal fat may parallel the change in abdominal hormone level (such as estradiol and testosterone), and leading to early menarche [31]. For example, abdominal fat is strongly associated with both insulin and insulin resistance [32], which may lead to early menarche [33]. Future obesity interventions are suggested to aim at reducing central obesity, which will further reduce risk of early menarche. Based on an obese intervention study [34] and our IV estimates in Table 1, corresponding RR for early menarche is $1.3^{-2} = 0.59$ for waits/height ratio (41% risk reduction).

Many paediatric studies suggest that girls with a lower birthweight may experience early menarche [8,14], but girls with a lower body weight as both infants and in early childhood experience later onset of menarche [8,9]. Most such studies are limited in that they did not measure body weight in the prepubertal years (late childhood), or use mediation analysis to find crucial mediators for the path from gene to early menarche [29]. Thus, our study used one-mediation and multiple-mediation analyses to discover that the prepubertal adiposity growth is a more critical mediator compared with birthweight for the path from gene to early menarche. Although birthweight is directly but inversely related to menarcheal age, birthweight indirectly leading to menarcheal age via the time-varying z-BMI growth is a positive relationship. The possible hypothesised mechanism is that puberty hormone levels such as FSH and LH concentrations are normally low in infant and early childhood [35]. As the prepubertal years (usually between 10 and 14) approach, puberty hormones prompt the ovaries to being producing estrogen, which causes menarche [35]. Therefore, prepubertal years are crucial periods for girls whose body weight parallelling puberty hormone levels in that period, determining menarcheal age.

The major strengths of our study are that it includes a population-based cohort of children and multiple adiposity measurements. The multiple genetic IVs were derived from DNA samples and the accurate genotyping of each SNP. Robust IV estimates were consistently confirmed by inverse-variance weighted and MR-Egger regression. Furthermore, multiple-mediation analysis was able to unravel crucial mediators. Our sample size is a bit small; however, the repeated measurements at 11–12 years were used to enhance the validity and accuracy of the measurements. A limitation of this work is the lack of a time-varying assessment of accumulation of body composition. Future studies to address these issues are recommended.

Our genetic MR analysis using the recovered curves infers a robust causal effect of higher time-varying z-BMI growth on the risk of early menarche. The present study supports the hypothesis that greater prepubertal adiposity growth is a crucial mediator in the pathway from genes to early menarcheal development. For girls with strong obesity genes and lower birthweight, control of prepubertal adiposity growth would be a good strategy to prevent early menarcheal development.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

We are grateful to Stephannie Shih, Yen-Tsung Huang, Simin Liu (School of Public Health, Brown University, Providence, RI) who provided R packages for conducting the multi-mediation analysis in our research. This study was supported by grants (101-2314-B-532-002-MY3 and 104-2314-B-532-002-MY3) from the Ministry of Science and Technology in Taiwan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.orcp.2018.07.008.

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