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Age at Menarche and BMI at Early Adulthood : Mendelian Randomization

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서울대학교 보건대학원
보건학과 역학전공
김 하 경

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지도교수 성 주 헌

이 논문을 보건학 석사학위논문으로 제출함

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서울대학교 보건대학원
보건학과 역학전공
김 하 경

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위 원 장 조 성 일 (인)
부 위 원 장 원 성 호 (인)
위 원 성 주 헌 (인)

<Contents>

<Table>

<Figure>

Abstract

Background: Earlier menarche has been known to be a risk factor for adolescent obesity and vice versa. We assessed a causal relationship between higher adolescent body mass index (BMI) and early menarche using Mendelian randomization method.

Method: Total 10,327 individuals from Korean Genome and Epidemiology study (KoGES) with adolescent BMI (recalled BMI at the age of 16-18) data were included. Age at menarche and demographic information was obtained from self-administered questionnaires. Given that general decrease in age at menarche, premature menarche (PM) score and cohort-specific cut-off for earlier menarche is constructed. Adolescent BMI was used as a proxy of childhood BMI. As an instrumental variable, 4 markers [rs2033195 (MFAP3, GALNT 10), rs925946 (BNDF), rs7138803 (FAIM2), rs1421085 (FTO)] were selected among previously reported markers. Generic risk score (GRS) was calculated by summing the number of risk allele in each single nucleotide polymorphism (SNP).

Results: Among the controls, BMI increased by 0.21 for unit-increase of GRS score (trend $P < 0.01$). GRS score was not associated with potential confounders. PM score increased with $1kg/m^2$increase in BMI after adjusting for age group and educational level. Odds ratio of early menarche increased by 120% per $1kg/m^2$ increase of BMI. Adolescent BMI was associated with age at menarche, PM score, early menarche (both 12 year cut-off andc ohort-specific cut-off) inconventional epidemiologic analysis, although the causal relationship

between adolescent BMI and early menarche defined by conventional cut-off was not evident in MR analysis.

Conclusion: The finding suggests that adolescenthood overweight or obesity may contribute to earlier menarche within each birth cohort, but it does not explain the rapid declining trend of menarche age in general.

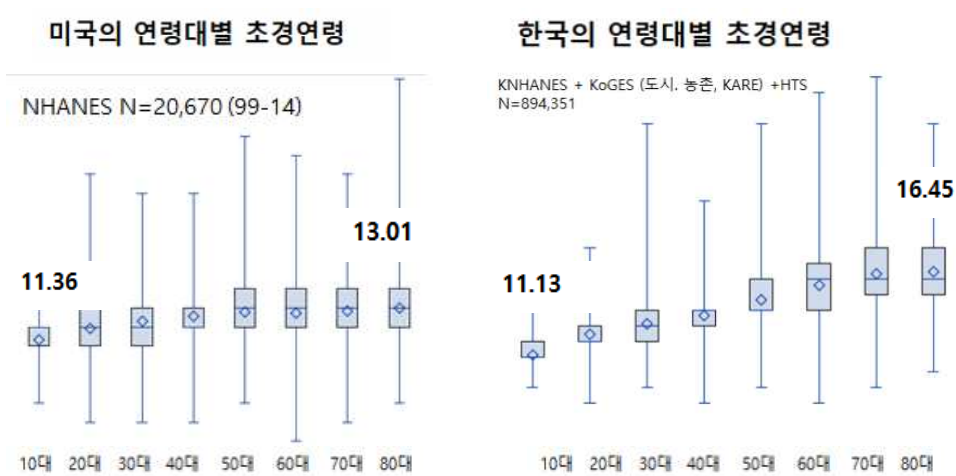
Student number : 2015-24075

1. Introduction

The menarche age ,the onset of menstrual period of girls, is considered as the central event of female puberty, meaning the possibility of fertility. Although, specific mechanism for menarche is not discovered, early menarche age is known as a risk factor of increasing health problems such as breast[1], ovarian cancer[2], diabetes[3], hypertension, cardiovascular disease[4], depression, eating disorder[5], and earlier sexual debut[6].

Descending trend of the average age of menarche has observed in developed country like USA, Western Europe (German, Sweden, UK, Finland, Denmark), South Africa, East Asia Japan and Korea. (Figure 1) [7,8,9,10,11] As follows, the determinants of menarche age and the reason of descending trend have become important issue in worldwide, because of its adverse effect.

Figure 1 Observed secular trend of menarche age in USA and KOREA



Despite of risk of early menarche, determinants of menarche age is not established. BMI of childhood and related factor[12,13] such as size of birth[14], growth rate in childhood[15] is suggested to be associated with menarche timing in, but not consistently. Maternal nutrition status at pregnant period, birth order, number of siblings, twin's zygosity[16] is also reported to influence the menarche age.

The mechanism is unclear, although, the effect of childhood obesity on menarche age has consistently reported through longitudinal study.[17,18,19] However, the limitation of observation study such as confounding, reverse causation, and bias, may have influence on results.

Because randomized controlled design (RCT) is not possible for this examination, we used Mendelian randomization (MR) analysis which design is similar to RCT. MR uses genetic marker related to exposure as an instrument variable so that other confounding has no chance to effect the results[20]. The main underlying assumption of MR is 1) The IV has a significant association with the exposure 2) The IV is not related with other confounding 3) The IV is only related to the phenotype through the exposure[21].

In this study, we used Mendelian randomization approach to estimate causal effect of higher BMI on menarche age and risk of early menarche. We specifically to 1) examine the instrument variable of childhood BMI 2) examine the causality of young-adult BMI on menarche age 3) compare results of conventional epi study. and MR study 4) examine the effect size of young-adult BMI on secular menarche age trend.

2. Method and Materials

1) Study Participants

Data of 118,569 individuals participated in the Korean Genome and Epidemiology study (KoGES) and Healthy Twin Study (HTS) was used. KoGES is a large community-based cohort study started in 2001. KoGES was comprised of 3 sub-cohort (Rural , Urban, Ansang-Ansung (KARE) cohort) which differ in resident area of participants. HTS is a nation-wide twin-family cohort study started in 2005. Genotype data was available for 10,835 individuals who have information of age at menarche. Of these, 376 with missing genotype of 5 candidate single nucleotide polymorphism (SNP)s was excluded. Among 10,327 individuals (3,044 of Rural cohort, 1,903 of Urban cohort, 3,829 of KARE cohort and 1,561 of HTS), adolescent BMI (recalled BMI of age 18-20) was measured only in 4,144 individuals of HTS, Rural, Urban cohort.

2) Measurements

Menarche age : Age at menarche was measured by the year of onset age. For inter-generational comparisons, we formulated a "premature menarche cutoff (PM) score), because the mean age at menarche differ substantially by birth-cohort. For example, menarche age of 12 falls into 2.6% for 70s group, whereas the same menarche age represented 34.6% for 30s.

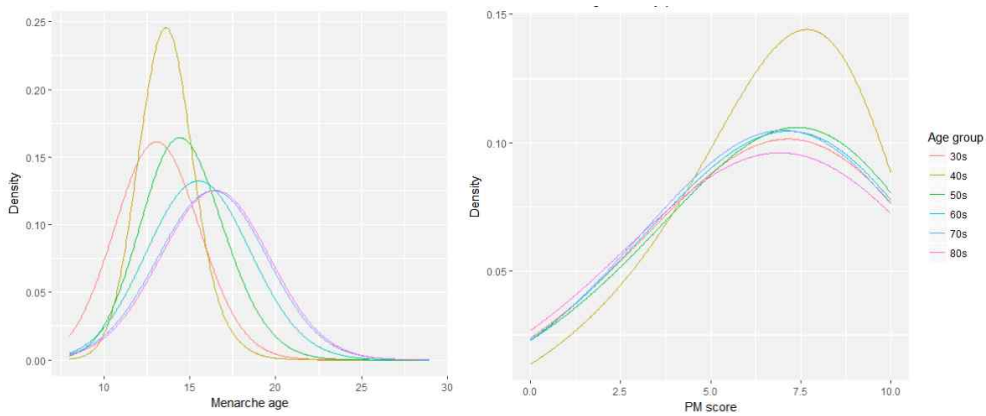
Among the same age group (10 year scale) using a large population data in Korea (KNHANES, the Korean Genome and Epidemiology

Figure 2 Equation of PM score

$$\text{PM score} = \sqrt{(percentile\ of\ individual\ at\ age\ group)}$$

Study, n= 897,323) as reference, we calculated the percentile value in the quartile menarche age as follows (Figure 2) After PM score applied, distribution of each age group seem to be almost same. (Figure 3)

Figure 3 Density plot of menarche age and PM score



Diagnostic age of premature menarche was selected in range of 5 to 11 percentile of PM score at each age group (Figure 4, Table 1). Two group was pooled together when calculating the diagnostic age of premature menarche, because the number of 30&40's was much smaller than other age group,

We applied different range of percentile as a diagnostic standard of early menarche for reduce the effect of the difference of each age group. For example, the young-adulthood BMI of 80's was 2.73 higher than that of 30&40's. In case of setting same percentile diagnostic to all age group, the results would be biased as the larger number of higher BMI case is included.

Figure 4 Compare early menarche composition of traditional cut-off (<12y) and age-specific cut-off

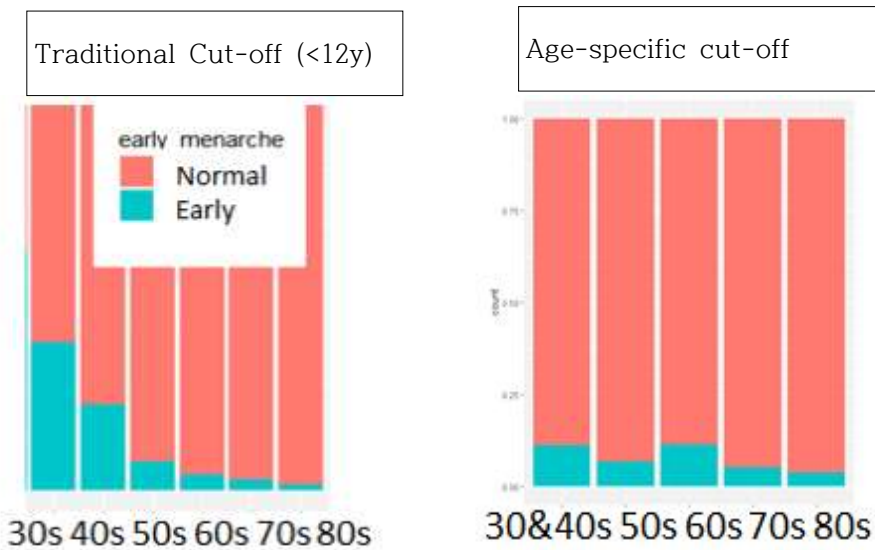


Table 1 Percent of early menarche by age group

Age group	30&40s	50s	60s	70s	80s
Cutoff	12	13	14	14	14
0 : Normal (N)	563	2480	2773	2787	998
1 : Early (N)	72	178	354	155	39
Early menarche (%)	0.11	0.07	0.11	0.05	0.04
Early menarche (N*%)	8.16	12.31	40.50	8.27	1.54

Young-adult BMI : It had been reported in many studies that

young-adult obesity has strong correlation with childhood obesity[2223]. Considering information about childhood weight and height is rare, young-adult BMI used as a proxy of childhood BMI.

Young-adult BMI calculated by using recalled weight of age 18-20 and height of examination point.

Other Covariates : Age was included as a form of dummy variable (10 year scale group). Criteria of education level was applied differently for each age group because composition of educational level was diverse by age group. (Table 2)

Table 2 Criteria of higher education by each age group

	30&40s	50s	60s	70s	80s
Criteria	University Registration	College graduate	High school graduate	Middle school graduate	Elementary graduate
N: Lower education	411	1946	1986	2255	602
N: Higher education	224	692	1121	674	416
% (higher education)	0.35	0.26	0.36	0.23	0.32

3) Genetic instrument variable

A recent GWAS study found about 66 markers associated with childhood BMI. (Table 3)

Table 3 Studies of childhood obesity gene

Author	Study	Journal (year)	Method
SällmanAlmén M, Rask-Andersen M, Jacobsson JA, et al.	Determination of the obesity-associated gene variants within the entire FTO gene by ultra deep targeted sequencing in obese and lean children.	Int J Obes (2013)	Gwas
Lasky-Su J, Lyon HN, Emilsson V, Heid IM, Molony C, Raby BA et al.	On the replication of genetic associations: timing can be everything!.	Am J Hum Genet (2008)	replicate study
Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM et al.	A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity.	Science (2007)	meta analysis
Wang, J., Mei, H., Chen, W., Jiang, Y., Sun, W., Li, F., ... &Jiang, F.	Study of eight GWAS-identified common variants for association with obesity-related indices in Chinese children at puberty.	International Journal of Obesity (2012)	replicate study
Xi B, Shen Y, Zhang M, et al.	The common rs9939609 variant of the fat mass and obesity-associated gene is associated with obesity risk in children and adolescents of Beijing, China.	BMC Med Genet. (2010)	replicate study

Table 3 Studies of childhood obesity gene (continued)

Elks, C. E., Loos, R. J., Sharp, S. J., Langenberg, C., Ring, S. M., Timpson, N. J., ... &Ong, K. K.	Genetic markers of adult obesity risk are associated with greater early infancy weight gain and growth.	PLoS Med (2010)	replicate study
Xi,B.,Cheng,H.,Shen,Y., Chandak,G.R.,Zhao,X.,Hou,D.,...&Mi,J.	Study of 11 BMI-associated loci identified in GWAS for associations with central obesity in the Chinese children.	PLoS One (2013)	Gwas
Warrington,N.M.,Howe, L.D.,Paternoster,L.,Kaakinen,M.,Herrala,S.,Huikari,V.,...&DaveySmith, G.	A genome-wide association study of body mass index across early life and childhood .	International journal of epidemiology (2015)	Gwas

8 SNPs were replicated in 0.05 significant level for a young-adult related SNPs.

Table 5 Results of GWAS replication on young-adult BMI related SNPs

CHR	SNP	Reported beta	Reported p-val	Replicated p-val
5	rs2033195	0.094	5.57E-06	0.0006105
5	rs4569924	0.032	3.48E-06	0.001885
11	rs925946	0.024255	8.5E-10	0.01587
11	rs6265	0.033434	5.1E-10	0.02695
11	rs10501087	0.038577	8.7E-11	0.02182
12	rs7138803	0.00656	1.2E-07	0.04313
16	rs9939609	0.81	0.00007	0.01386
16	rs1421085	0.059	4.53E-16	0.01744
16	rs1121980	0.56	0.00001	0.0235

4 SNPs was removed after LD score considered. (SNPs that of R2 above 0.3 when compared included SNPs were removed) (Table 5 and Figure 5,6)

Table 6 LD score calculation of near by SNPs

CHR	SNP1	SNP2	D′	LOD	R2
5	rs2033195	rs815611	0.995	396.12	0.986
	rs2033195	rs4569924	0.986	384.13	0.968
	rs815611	rs4569924	0.995	394.2	0.982
16	rs1421085	rs1121980	0.996	920.1	0.713
	rs1421085	rs1121980	0.996	920.1	0.713
	rs1121980	rs9939609	0.992	913.95	0.713

Figure 5 LD score plot of Chromosome 5

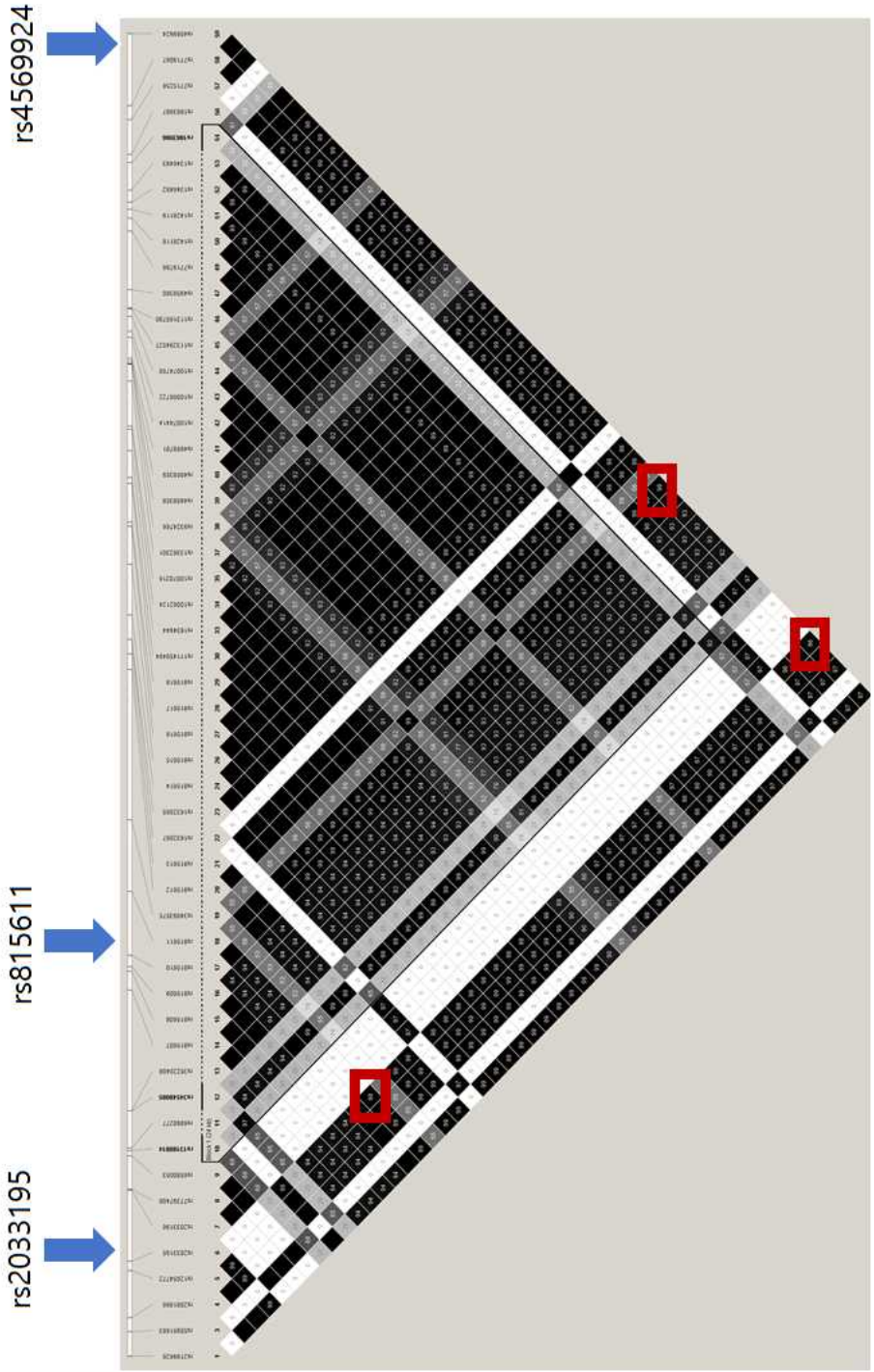
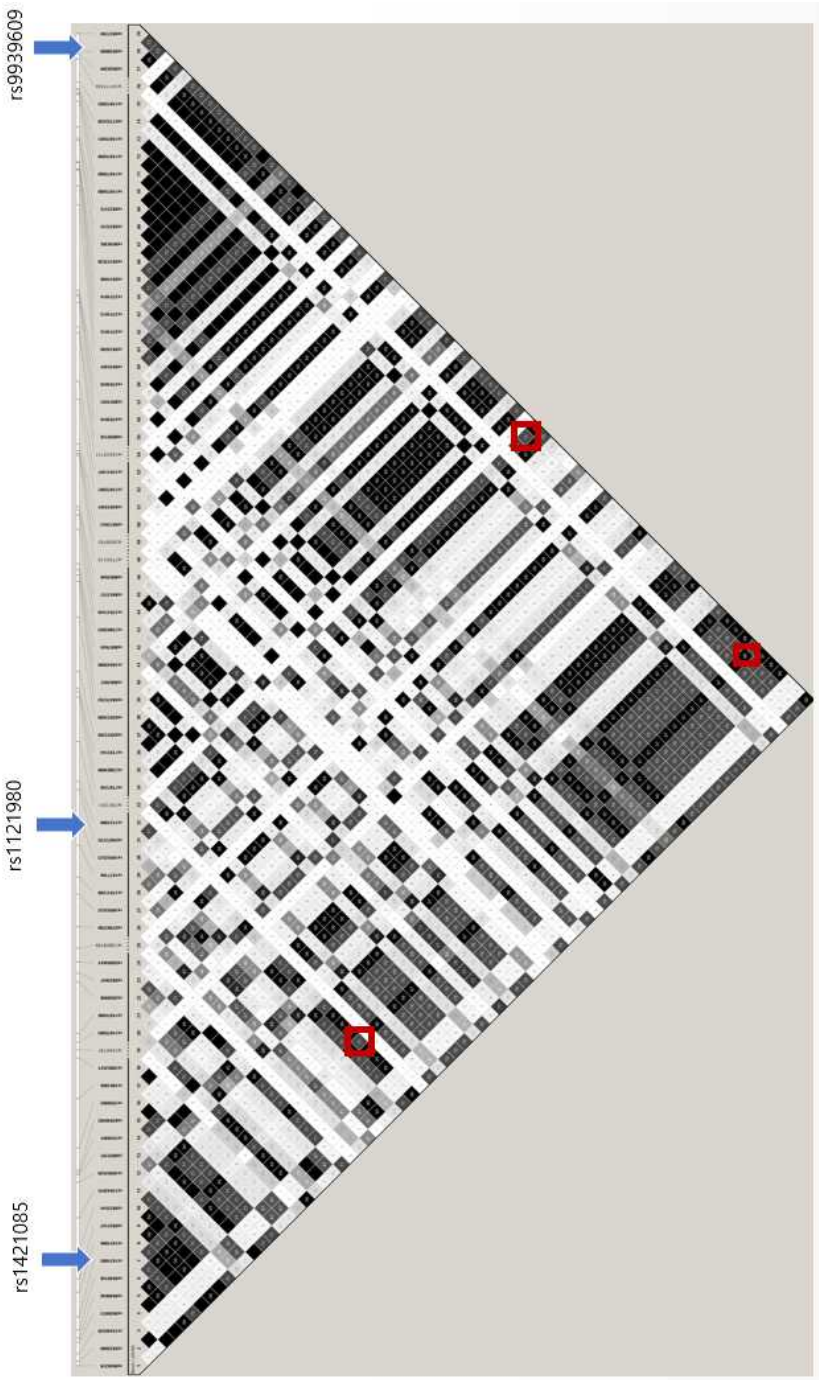


Figure 6 LD score plot of Chromosome 16



Finally, 4 markers including rs2033195 (MFAP3, GALNT 10), rs925946 (BNDF), rs7138803 (FAIM2), rs1421085 (FTO) were selected. (Table 6)

Table 7 Characteristic of selected markers for IV

CHR	SNP	Gene	BP	reported RA/nRA	MAF
5	rs2033195	MFAP3, GALNT10	153509596	T/C	0.021106
11	rs925946	BNDF	27667202	T/G	0.040803
12	rs7138803	FAIM2	50247468	A/G	0.277165
16	rs1421085	FTO	53820527	C/T	0.120807

Then, we created the IV score by summing the risk allele count of each SNPs. We created a genetic risk score (GRS) from 4 SNPs as instrumental variable for young-adult BMI. For each SNP, participants got from 0 to 2 value by counted number of risk allele. GRS was calculated by summing each allele count value across all SNPs.

Trend of confounders per risk allele increase was tested to examine all IV are randomized to potential confounders.(Table 7) The allele frequency of each SNPs has no differences between cohort and age group. (Table 8, 9) This indicates there is genetic differences between examined groups.

Table 8 Association of IV with potential confounders

	Genotype			P for trend
rs2033195	C/C	T/C	T/T	
Young adult BMI (sd)	20.92 (2.52)	21.47 (2.59)	23.97 (-)	0.01
age (sd)	65.35 (10.99)	65.54 (10.90)	63.80 (13.57)	0.53
High Education (N)	2526	96	0	0.13
rs925946	G/G	T/G	T/T	P for trend
Young adult BMI (sd)	20.91 (2.51)	21.34 (2.65)	21.64 (2.08)	0.01
age (sd)	65.32 (10.98)	65.74 (10.98)	68.88 (10.28)	0.17
High Education (N)	2439	176	4	0.06
rs7138803	G/G	A/G	A/A	P for trend
Young adult BMI (sd)	20.87 (2.54)	20.98 (2.52)	21.15 (2.43)	0.05
age (sd)	65.09 (11.36)	65.46 (11.11)	65.31 (10.95)	0.63
High Education (N)	1414	1010	199	0.14
rs1421085	T/T	C/T	C/C	P for trend
Young adult BMI (sd)	20.88 (2.50)	21.13 (2.59)	21.11 (2.70)	0.02
age (sd)	64.09 (11.36)	65.37 (11.11)	65.37 (10.95)	0.41
High Education (N)	2017	562	37	0.71

Table 9 Allele frequency difference by each cohort

		Allele frequency by Cohort				
		Twin	Nong	Urban	KARE	p for difference
	C/C	0.96	0.96	0.96	0.95	0.20
rs2033195	T/C	0.04	0.04	0.04	0.05	0.16
	T/T	0.00	0.00	0.00	0.00	0.25
	G/G	0.91	0.92	0.93	0.92	0.44
rs925946	T/G	0.09	0.08	0.07	0.08	0.40
	T/T	0.00	0.00	0.00	0.00	0.68
	G/G	0.53	0.51	0.51	0.53	0.37
rs7138803	A/G	0.40	0.40	0.41	0.39	0.63
	A/A	0.07	0.08	0.08	0.07	0.41
	C/C	0.79	0.78	0.77	0.76	0.05
rs1421085	C/T	0.20	0.21	0.22	0.22	0.08
	T/T	0.02	0.01	0.01	0.02	0.45

Table 10 Allele frequency difference by age group

Allele frequency by age group								
SNP	allele	30s	40s	50s	60s	70s	80s	p for difference
rs2033195	C/C	0.97	0.95	0.96	0.96	0.95	0.96	0.89
	T/C	0.03	0.05	0.04	0.04	0.05	0.04	0.89
	T/T	0.00	0.00	0.00	0.00	0.00	0.00	-
rs925946	G/G	0.91	0.93	0.93	0.92	0.92	0.92	0.26
	T/G	0.09	0.07	0.07	0.07	0.08	0.08	0.35
	T/T	0.00	0.00	0.00	0.00	0.00	0.00	0.14
rs7138803	G/G	0.59	0.58	0.50	0.53	0.52	0.51	0.60
	A/G	0.34	0.35	0.42	0.39	0.40	0.41	0.54
	A/A	0.07	0.07	0.08	0.08	0.08	0.08	0.89
rs1421085	C/C	0.70	0.81	0.77	0.78	0.77	0.77	0.63
	C/T	0.26	0.18	0.22	0.21	0.22	0.22	0.95
	T/T	0.05	0.01	0.02	0.01	0.01	0.01	0.05

4) Statistical analysis

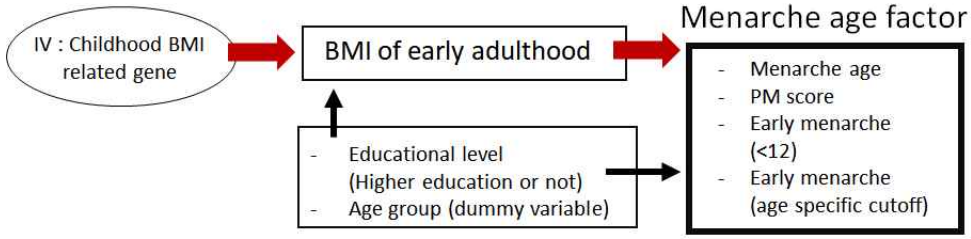
(1) Descriptive and observational analysis

Descriptive analysis were implemented in R. For continuous variable, we calculated means and standard errors, and for categorical variable, we explored the composition. Also, the difference in mean or percent between cohort groups and age group was examined. A trend test of all covariates was done by dividing of quartile of young-adult BMI to find that confounding effect is not removed in observation study.

(2) Mendelian randomization analysis

The analysis was carried out in Stata 14. In conventional epidemiology method, we used “regress” and “logistic” command for each of continuous and binary variable. Age group as a dummy variable and higher educational level was included as a covariates. In MR analysis, we implemented instrumental variable analysis of young-adulthood BMI and menarche age related phenotypes (menarche age, PM score, early menarche (traditional cut-off, age-specific cutoff) (Figure 7). First, statistics of F-statistic and R-square were calculated to ensure that genetic marker is strongly associated with exposure. Then, we used 2 stage-least-sqaure method to examine the estimates of causal risk difference in outcome (menarche age related phenotypes) per unit change in the exposure (young-adult BMI). All covariates were adjusted again in second stage.

Figure 7 Diagram of Mendelian randomization analysis



3. Results

1) Descriptive analysis

General characteristics by each cohort are shown in Table 10. Mean age of Rural cohort was 69.97 which is much higher than that of HTS (55.56) and Urban cohort (61.76), although mean age of KARE cohort was similar (68.06) with Rural's. Percent of higher education was much low in Rural cohort compared to others even though higher education was defined differently by age group. Young-adult BMI was little higher in Rural cohort, but not significantly. Menarche age was earlier in Twin (14.43) compared to other cohorts. Percent of traditional early menarche (<12y) was higher about from 500% to 3000%. This is because menarche age is substantially differ by age group. But, PM score and age specific cut-off applied early menarche show no difference by cohort. (p=0.22, 0.2)

Table 11 General characteristic by each cohort

Mean (sd)	HTS (N=1,561)	Rural (N=3,044)	Urban (N=1,903)	KARE (N=3,829)	p for difference
Age	55.56 (13.35)	69.67 (8.99)	61.76 (7.55)	68.06 (8.95)	<0.01
Higher Education (N, (%))	772 (0.49)	399 (0.13)	827 (0.43)	1139 (0.32)	0.06
Young-adult BMI	20.55 (2.33)	21.80 (2.85)	20.48 (2.15)	-	0.16
Menarche age	14.43 (2.05)	16.4 (1.95)	15.2 (1.81)	15.84 (1.89)	<0.01
PM score	6.46 (2.34)	5.52 (2.44)	6.19 (2.34)	6.10 (2.41)	0.22
N of Early Menarche - traditional (<12) (N, (%))	83 (0.05)	6 (0.002)	23 (0.012)	18 (0.005)	<0.01
Early Menarche - age specific (N, (%))	227 (0.14)	256 (0.08)	222 (0.114)	449 (0.117)	0.2

Table 12 General characteristic by age group

	Age group					p-val
	30&40s (N=635)	50s (N=2,638)	60s (N=3,107)	70s (N=2,929)	80s (N=1,028)	
age (sd)	42.48 (4.93)	55.87 (2.45)	64.05 (2.87)	74.54 (2.87)	82.39 (2.08)	<0.01
Higher Education (%)	224 (0.35)	692 (0.26)	1121 (0.36)	674 (0.23)	426 (0.41)	0.24
Young adult BMI (sd)	20.34 (2.37)	20.46 (2.13)	21.04 (2.42)	21.95 (2.78)	23.07 (3.48)	<0.01
Menarche age (sd)	13.19 (1.47)	14.85 (1.70)	15.75 (1.88)	16.56 (1.90)	16.57 (1.82)	<0.01
NPM score (sd)	6.51 (2.27)	5.80 (2.44)	5.87 (2.43)	5.82 (2.37)	7.15 (2.18)	<0.01
Early Menarche - traditional (<12)	72 (0.11)	36 (0.01)	16 (0.005)	6 (0.002)	0	<0.01
Early Menarche -age specific	72 (0.11)	181 (0.07)	356 (0.11)	156 (0.05)	40 (0.04)	<0.01

Various Characteristic by age group are described at table 11. All variable was differ by age group except for higher education. There was still age group effect remaining in PM score and age specific cut-off applied early menarche, even though the PM score was created to avoid age cohort effect.

Association between young-adult BMI and other covariates examined. By quartile of young-adult BMI, increasing trend of age and percent of higher education was observed. (Table 12)

Table 13 Mean values of various risk factors by quantile of BMI in controls

Quartile of young-adult BMI	Q1	Q2	Q3	Q4	P-val for trend
	≤19.23	>19.23,≤ 20.72	>22.37, ≤ 20.72	>22.37	
Young -adult BMI (sd)	18.08 (0.94)	19.96 (0.43)	21.48 (0.48)	24.35 (1.94)	<0.01
	0.94	0.43	0.48	1.94	
age (sd)	57.01 (10.66)	58.87 (10.41)	60.63 (10.26)	63.80 (11.58)	<0.01
	10.66	10.41	10.26	11.58	
Higher education	406 (44.03)	382 (42.12)	309 (33.08)	209 (22.50)	<0.01
	696 (44.03	625	550	315	

2) Mendelian randomization analysis

(1) Test of IV validation

The F-statistic of each SNPs range from 5.25 to 5.45. rs2033195 show the highest F-statistic among the replicated SNPs. The F-statistics of GRS score which was calculated by summing the number of risk allele of each SNP was 22.83, indicating that created IV is strongly associated with exposure. (Table 13) Boxplot of young-adult BMI by quintile of GRS score was a supportive evidence that IV is strongly related to young-adult BMI. (Figrue 8) Only one SNP (rs1421085) was strongly related to adulthood BMI. (Table 14)

Figure 8 Box-plot of Young-adulthood BMI by quintile of GRS

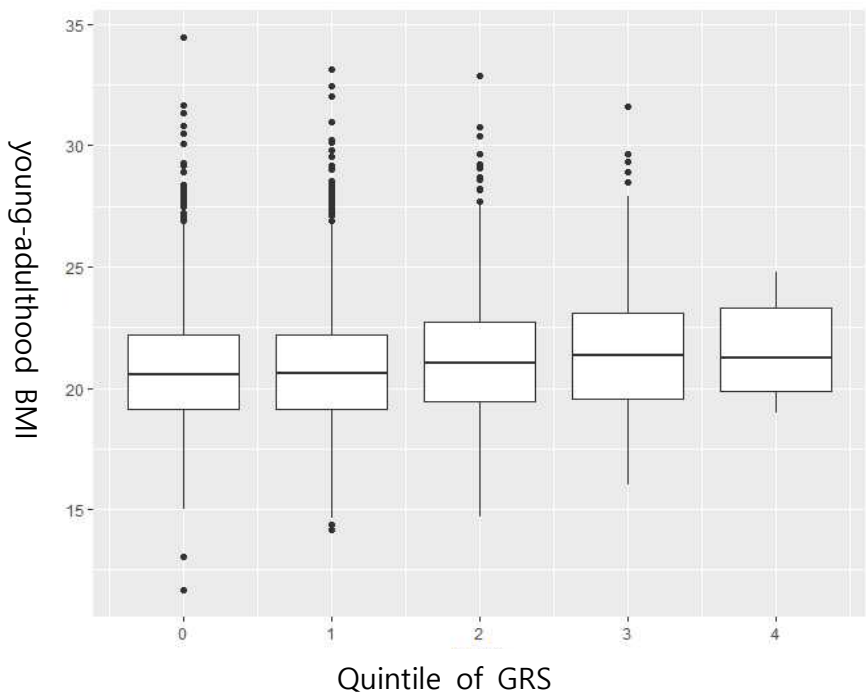


Table 14 Test of Instrument variable association with young-adulthood BMI

IV	coefficeint	p-val	F-statistic	R-square
rs2033195	0.032	<0.01	10.87	0.0001
rs925946	0.018	<0.01	6.92	0.0013
rs7138803	0.007	0.01	6.51	0.0013
rs1421085	0.010	0.02	5.45	0.0014
GRS score	0.010	<0.01	**22.83**	0.0054

Table 15 Test of Instrument variable association with Adulthood BMI

IV	coefficient	p-val	F-statistic	R-square
rs2033195	-8.70E-07	1	10.87	0
rs925946	-0.010	0.07	3.3	0.0003
rs7138803	-0.001	0.52	0.41	0
rs1421085	-0.014	<0.01	20.53	0.002

(2) MR analysis

Young-adult BMI was statistically significantly associated with menarche age, PM score, early menarche (both in 12 year cutoff and age-specific cutoff) in conventional epidemiology analysis after adjusted for age group and education, although menarche age and early menarche risk by traditional cut-off was not significant in MR analysis.

Young-adult BMI was effective for both menarche age in conventional epidemiology analysis. In these results, menarche age decreases 0.02 year while young-adult BMI increases 1kg/m2 (Table 16) Comparing MR results with conventional epidemiology results, higher young-adult BMI showed consistent decreasing effect on menarche age only for PM score. MR Results of simple menarche age showed no significance of young-adult BMI (p=0.08). PM score was increased 0.02 by 1kg/m2 Young-adult BMI increase after adjusting for age group and educational level. (Table 16)

For endogenous test, we performed Durbin-Wu-hausman test. Results of Durbin-Wu-Hausman(DWH) test were both significant of menarche age and PM score analysis (<0.01), suggesting there is endogenous variable in conventional epi. analysis and need for IV analysis. Also, p-values of DWH test indicated that significant effect difference between conventional epi. and MR results.

Saragon's test was performed to test overidentifying restrictions. P-value of Saragon's test was indicating that IV used in analysis is valid. (menarche age : 0.5, PM score : 0.06 , not shown).

Table 16 Comparison results of conventional Epi. and IV analysis of menarche age and PM score

Young-adult BMI	Conventional Epi.		MR		DWH p-val
	coefficient (P)	R-square	coefficient (P)	R-square	
Menarche age	**-0.02 (0.046)**	0.30	-0.20 (0.08)	0.16	<0.01
PM score	**0.03 (0.02)**	0.07	**0.02 (<0.01)**	0.08	<0.01

Higher young-adult BMI associated with increasing early menarche risk in conventional epidemiology analysis of both traditional and age-specific early menarche. However, in MR analysis of traditional early menarche, young-adult BMI had no longer significant effect for increasing early menarche risk (p=0.77) On the other hand, early menarche risk of age-specific cut-off still increased as young-adult BMI increases. Odds ratio of age specific early menarche increased by 123% per 1kg/m2 increase in young-adult BMI (P<0.01). (Table 17)

Table 17 Comparison results of conventional Epi. and IV analysis of traditional and age specific early menarche

Young-adult BMI	Conventional Epi.		MR	
	OR (P)	R-square	OR (P)	R-square
Traditional cutoff	**1.14 (<0.01)**	0.20	0.96 (0.77)	0.15
Age specific cutoff	**1.06 (<0.01)**	0.03	**1.23 (<0.01)**	0.03

(3) Differential analysis

After we demonstrated that the effect size of young-adult BMI on menarche age at each age group was 0.02, we used differential coefficient to determine whether the effect of young-adult BMI on

menarche age affects secular trends. (Figure 9)

In previous MR results, we found that the differential coefficient of $\frac{dy}{dz}$ is 1.38. So we differentiate both sides by z to know $\frac{dx}{dz}$.

Because $\frac{e^{\frac{(quantile(1-(\frac{y}{100})^2)^2)}{2}}}{y}$ is a standardized value of individual regardless of age group, $\frac{dx}{dz}$ is only dependent on σ of each age group.

Because p-value in the Leven's test for homogeneity of variance in each age group was under 0.001, we concluded that there is no variance difference between age group.

Therefore, the difference in menarche age per young-adult BMI difference is constant regardless of age group.

In conclusion, young-adult BMI have no effect on secular menarche age trend.

Figure 9 Differential equation to estimate the effect size of young-adult BMI on menarche age trend

y : PM score , x : menarche age, z : young − adult BMI,

m : mean menarche age at each age group, σ : standard deviataion of menarche age at each age group

$$y = 10\sqrt{1 - \int \left(\frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(x-m)^2}{2\sigma^2}}\right) dx}$$

$$\frac{dy}{dx} = \frac{-10\left(\frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(x-m)^2}{2\sigma^2}}\right)}{2\sqrt{1 - \int \left(\frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(x-m)^2}{2\sigma^2}}\right) dx}}$$

$$\frac{dx}{dz} = \frac{\frac{dy}{dz}}{\frac{dy}{dx}} = 0.02 \div \frac{-10\left(\frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(x-m)^2}{2\sigma^2}}\right)}{2\sqrt{1 - \int \left(\frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(x-m)^2}{2\sigma^2}}\right) dx}}$$

$$\frac{dx}{dz} = \frac{\frac{dy}{dz}}{\frac{dy}{dx}} = 0.02 \div \frac{-10\left(\frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{-(x-m)^2}{2\sigma^2}}\right)}{2y} \sim -\sqrt{\sigma^2} \times \frac{e^{-\frac{\left(quantile\left(1-\left(\frac{y}{100}\right)^2\right)\right)^2}{2}}}{y} \sim -\sigma$$

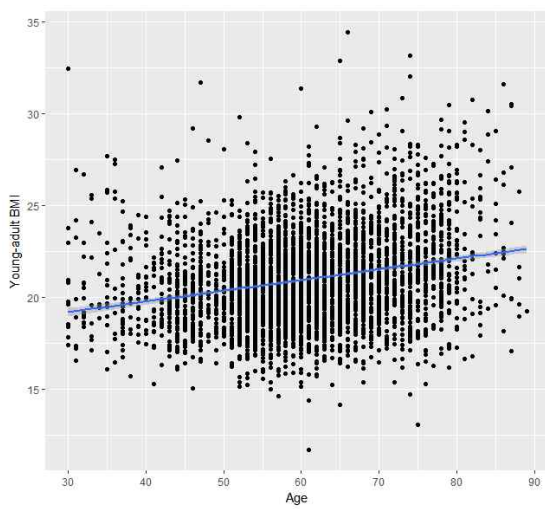
$$\frac{dx}{dz} \sim (Constant\ number\ regardless\ of\ age\ group)$$

4. Discussion

Young-adult obesity, proxy of childhood obesity, is one of the most likely related factor with menarche age. In this study, we provide the evidence for signifiant association on the young-adult BMI with menarche age. Unlike the results of conventional epidemiology study, young-adult BMI was significant only in PM score and age-specific early menarche analysis. In other words, young-adult BMI is associated with menarche age only when age-cohort effect was removed. This results indicate that young-adult BMI has causal effect on menarche age within same age group, but does not explain the secular decreasing trend of menarche age.

There are a few limitation in this study. The first is that the observed young-adult BMI trend of age group is different from the reported one. Globally, the prevalence of childhood obesity has

Figure 10 Scatter plot and regression line of young-adult BMI and current age



increased over time in developed countries[24], including Korea[2526]. Contrary to the worldwide trend, we can observe the little decreasing trend in our data ($p<0.01$) (Figure 9). For the reason of inconsistency in two trends, there may be possibility of recall biases. The person who are obese now and can not recall the exact weight in childhood, may think that she had have high weight in the past.

Another one could be pleiotropy effect on menarche age and young-adult BMI. Matkovic[27] reported serum leptin level inversely correlated with menarche age. Also, there are studies suggesting that age at menarche have shared genetic effect with adult obesity[28], peripubertal BMI[29], bone mineral density[30], height[31], bone metabolism, energy homeostasis and hormonal regulation[32]. In MR analysis, because we only considered the genetic variants only strongly associated with young-adult BMI, possibility remains that pleiotropic gene affecting both young-adult BMI and menarche age ignored.

Despite of limitation, this study give a explanation of role of young-adult BMI on menarche age. Young-adult BMI significantly decrease menarche age in same age group, but has proven to be not the reason for declining menarche age trend. To explain the decreasing menarche age trend, other factor should be considered.

5. Conclusion

Menarche age is related to multiple adverse health outcomes. Nevertheless, the determinants of age at menarche is not established yet. We explored the causal effect of young-adult BMI, one of the suggested determinants in observational study, on menarche age.

Young-adulthood BMI seems to have a causal association with menarche age in genetic instrumental analysis result, which is consistent with result of observed longitudinal analysis.

Our analysis using Mendelian randomization approach is free from possible limitation of conventional epidemiology study such as reverse causation and confounding effect. Though the mechanism is still unknown, the results demonstrated young-adult BMI play a significant role on decreasing menarche age and increasing early menarche risk. However, it was also revealed that young-adult BMI is not the major factor of worldwide secular trend of menarche age. Exploring mechanism of young-adult BMI on menarche age and possible reason for secular trend of menarche age should be an area of further research.

<국문 초록>

배경 및 목적

초경 연령은 유방, 난소 암, 조기 성관계 및 조기 폐경과 같은 건강 문제의 위험 요소로 알려져 있다. 선진국에서는 초경 평균 연령의 하강 추세가 관찰되어왔다. 초경 연령의 결정 요인은 현재 확립되지는 않았지만, 어린 시절의 비만은 초경시기와 관련이 있다고 제시되어왔다. 본 연구에서는 멘델리안 무작위 분석법을 이용하여 어린 시절의 높은 체질량지수가 초경나이와 조기초경의 위험도에 미치는 영향을 추정 하였다.

방법

총 10,327 명 (쌍둥이 및 가족 코호트 1,561 명, 농촌 코호트 3,044 명, 도시 코호트 1,903 명, 지역사회 코호트 3,829 명)이 연구에 포함되었다. 젊은 성인기의 체질량지수 (18 ~ 20 세의 체질량지수)는 쌍둥이 및 가족 코호트, 농촌코호트, 도시코호트의 4,144 명에서만 측정되었다. 초경 연령은 연단위로 측정되었다. 세대 간 비교를 위해 조기초경점수와 연령집단별 기준이 다른 이른 초경 변수를 새롭게 정립하였다. 젊은 성인기의 체질량지수는 아동기 체질량지수의 추정치로 사용되었다. 공변량의 경우, 연령집단과 고등 교육이 포함되었다. 도구변수로는 보고 된 66 개의 유전체마커 중에서 rs2033195 (MFAP3, GALNT 10), rs925946 (BNDF), rs7138803 (FAIM2), rs1421085 (FTO)를 포함하는 4 개의 마커가 선택되었다. GRS 점수는 각 SNP의 위험 대립 유전자의 수를 합산하여 계산되었다.

결과

대조군(이른 초경이 아닌 사람) 중 GRS 점수 당 0.21의 BMI가 증가했으며 ($p < 0.01$), GRS 점수는 잠재적인 교란변수와 연관성을 보이지 않

았다. 조기초경 점수는 젊은 성인기 체질량지수가 1kg/m2 증가 할 때마다 0.201 증가했다. 조기 초경의 확률은 젊은 성인기 체질량지수가 1kg/m2 증가 할 때 120 % 증가했다. 전통적인 역학분석의 결과에서는 초경연령, 조기초경점수, 이른 초경의 위험도 (12세 기준, 연령집단 별 기준)와 젊은 성인기 체질량지수가 연관성을 보였다. 하지만 멘델리안 무작위 분석법 결과 초경 연령과 12세를 기준으로 잡은 이른 초경의 위험도에 미치는 젊은 성인기의 체질량지수의 영향은 유의하지 않았고, 조기초경점수와 연령집단별 기준이 다른 이른 초경 위험도에서는 유의하게 나타났다.

결론

멘델리안 무작위 분석법의 결과 젊은 성인기의 체질량지수가 조기초경점수와 조기초경의 위험도를 높이는 것을 보여주었다. 이는 젊은 성인기의 체질량 지수는 연령그룹 안에서 초경나이를 감소시키는데 일조하지만, 연령그룹 별로 급격히 감소하는 양상을 설명하지는 않는다.

주요어: 초경 나이, 이른 초경, 체질량지수, 아동기 비만, 젊은 성인기 비만, 멘델리안 무작위분석법, 도구변수, 유전위험점수

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