Age of puberty and Sleep duration: Observational and Mendelian randomization study

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Earlier age of puberty has detrimental consequences for many aspects of health. Here, for the first time, we assessed the association of earlier puberty with sleep duration observationally and with validation using Mendelian Randomization. In the “Children of 1997” birth cohort (n = 8,327), we used adjusted multivariable logistic regression to assess the associations of each clinically assessed marker of earlier puberty with self-report sleep duration in adolescence. Using two-sample MR, we assessed the effect of earlier puberty timing based on 203 single nucleotide polymorphisms applied to genome wide association studies of sleep duration in adults (n = 335,410). In “Children of 1997”, cross-sectionally, older age of menarche was associated with longer (9+ hours) sleep duration [odds ratio (OR) 1.11, 95% confidence interval (CI) 1.01 to 1.21] at 13.5 years. The other earlier puberty markers were unrelated to sleep duration. Using inverse variance weighting, later of age at menarche increased adult sleep duration [0.020 per category, 95% CI 0.006 to 0.034]. This study demonstrated a causal effect of age at menarche on adult sleep duration, since age of menarche also affects obesity, our novel finding may be relevant to the observed relation of sleep duration with obesity and poor health.

Sleep serves an important restorative function in humans. However, sleep duration has decreased for children and adolescents in the last 20 years1,2. Short sleep duration is associated with a wide range of poor health outcomes including stress, headache, cardiovascular disease, diabetes mellitus and even cancer. Short sleep is also associated with screen time, low socioeconomic position (SEP) and sedentary time, making it difficult to ascertain whether these associations are causal or confounded. Sleep patterns change significantly at puberty with marked differences by sex11,12, suggesting puberty may be a critical stage for the development of sleep patterns. Earlier puberty is associated with many non-communicable diseases13. However a Mendelian Randomization study was not completely supportive of sleep as causing all these outcomes14. As such sleep may also be a consequence of other attributes, such as pubertal timing, which have causal effects on some non-communicable diseases. Notably, puberty affecting sleep duration falls within the emerging paradigm of evolutionary public health, i.e., considering health within the well-established paradigm of natural selection favoring reproductive success rather than wellbeing15. Laboratory studies suggested sex hormones affect sleep, potentially with sex-specific consequences. For instance, a randomized controlled trial (RCT) found testosterone shortened sleep duration in older men16, while estrogen plus progestin has been associated with better sleep in postmenopausal women17.

Observationally earlier menarche is associated with sleep disorders in adolescents from Western and Chinese settings18,19. However, observational studies are hard to interpret because they could be confounded by factors such as socio-economic position (SEP) and lifestyle. In this situation where experimental evidence is lacking Mendelian Randomization (MR), i.e., instrumental variable analysis with genetic instruments, may provide a way forward. Given genetic variants are allocated randomly at conception, MR, as a quasi-experimental study design, is less susceptible to confounding and so provides an alternative means of assessing the causal effect of puberty on sleep duration. Several studies using this approach have recently clarified the associations of age of menarche with adolescent depression20, time spent in education21 and adult body mass index13, but no MR study has assessed the causal effects of earlier puberty on adult sleep duration14.

To clarify the effect of pubertal timing on sleep duration, we first conducted an observational study to assess associations of clinically assessed age of puberty with self-reported longer (9+ hours) compared to shorter

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13.1 years. Later pubertal development was associated with higher family SEP, such as parents' birthplace, highest parental occupation, household income per head in quintiles and highest parental education level (Table 1).

Girls 2961 9.60 (1.28) 1736 10.56 (1.03) 3177 11.94 (1.08)

Parents’ birthplace

Both parents migrant 1156 10.00 (1.33) 716 10.86 (1.07) 1781 13.08 (1.18)

One parent migrant 980 10.03 (1.42) 638 10.90 (1.16) 1055 12.30 (1.23)

Both parents Hong Kong 2608 10.18 (1.34) 1717 11.06 (1.17) 2555 12.39 (1.25)

Highest parental occupation

<0.01
<0.01
<0.01

I (professional) 1046 10.2 (1.38) 691 11.06 (1.20) 1089 12.45 (1.26)

II (managerial) 625 10.05 (1.30) 403 11.00 (1.14) 639 12.33 (1.24)

III NM (nonmanual skilled) 1298 10.14 (1.39) 855 10.97 (1.16) 1262 12.34 (1.21)

III M (manual skilled) 700 10.03 (1.36) 454 10.94 (1.11) 748 12.35 (1.26)

IV (semi-skilled) 404 9.96 (1.28) 261 10.87 (1.17) 420 12.26 (1.22)

V (unskilled) 155 9.95 (1.30) 99 10.98 (1.08) 141 12.29 (1.30)

Household income per head in quintiles

<0.01
0.01
0.109

1st quintile (HK$ 1751 ± 413) 806 10.03 (1.38) 519 10.87 (1.11) 835 12.26 (1.23)

2nd quintile (HK$ 2856 ± 325) 899 10.09 (1.40) 570 10.92 (1.21) 868 12.34 (1.27)

3rd quintile (HK$ 4362 ± 556) 845 10.06 (1.33) 555 10.99 (1.12) 897 12.38 (1.22)

4th quintile (HK$ 5440 ± 886) 901 10.16 (1.35) 616 11.06 (1.14) 892 12.36 (1.24)

5th quintile (HK$ 14850 ± 16050) 900 10.21 (1.34) 564 11.07 (1.22) 908 12.45 (1.23)

Highest parental education level

<0.01
<0.01
<0.01

Grade 9 or below 1415 10.02 (1.37) 894 10.90 (1.12) 1457 12.25 (1.25)

Grade 10–11 2115 10.12 (1.35) 1397 10.96 (1.13) 2121 12.40 (1.25)

Grade 12 or above 1286 10.17 (1.37) 831 11.08 (1.21) 1333 12.41 (1.23)

1531 genome-wide significant (p-value < 5 × 10^{-8}) signals for age of voice breaking were obtained from summary genetic associations concerning 329,345 women of European ancestry. These summary genetic associations are based on the ReproGen consortium (N = 179,117 from 40 studies), in addition to 23andMe (N = 76,631) and the UK Biobank (N = 73,397)

R² = 0.001). Of the remaining 208 SNPs, 203 SNPs were available for sleep duration and no proxy was found for the 5 missing SNPs, giving 203 SNPs. Figure 1 shows the selection of SNPs related to age of menarche used as instruments. For age of voice breaking 11 genome-wide significant (p-value < 5 × 10^{-8}) signals for age of voice breaking were obtained from 55,871 European men from the 23andMe study

| Characteristic | Classification | n | Onset of Breast/Genitalia Development (Mean (SD), y) | P value | n | Onset of Pubic Hair Development (Mean (SD), y) | P value | n | Age of Menarche/ Voice breaking, (Mean (SD), y) | P value |
|----------------|----------------|---|-----------------------------------------------|---------|---|-----------------------------------------------|---------|---|-----------------------------------------------|---------|
| Gender         |                |   |                                               |         |   |                                               |         |   |                                               |         |
| Boys           |                | 1921| 10.88 (1.08)                                 | <0.01   | 1428| 11.48 (1.09)                                 | <0.01   | 1781| 13.08 (1.18)                                 | <0.01   |
| Girls          |                | 2961| 9.60 (1.28)                                  | <0.01   | 1736| 10.56 (1.03)                                 | <0.01   | 3177| 11.94 (1.08)                                 | <0.01   |
| Parents’ birthplace |              |    |                                               |         |   |                                               |         |   |                                               |         |
| Both parents migrant |            | 1156| 10.00 (1.33)                                 | <0.01   | 716 | 10.86 (1.07)                                 | <0.01   | 1244| 12.37 (1.26)                                 | 0.109   |
| One parent migrant |             | 980 | 10.03 (1.42)                                 | <0.01   | 638 | 10.90 (1.16)                                 | <0.01   | 1055| 12.30 (1.23)                                 |         |
| Both parents Hong Kong |         | 2608| 10.18 (1.34)                                 | <0.01   | 1717| 11.06 (1.17)                                 | <0.01   | 2555| 12.39 (1.25)                                 |         |

Table 1. Baseline Characteristics according to Age of Puberty from Hong Kong’s “Children of 1997” Birth Cohort. (Available case Analysis).

(<9 hours) sleep duration in the large population-representative Hong Kong Chinese “Children of 1997” birth cohort. Second, we used two-sample MR to validate the causal effect of later puberty on adult sleep duration.

Results

Observational study in the Chinese “Children of 1997” birth cohort. Among the original 8,327 “Children of 1997” participants22, at the time of survey I (2008–09), 26 participants had permanently withdrawn, and 365 were not contactable. Of the 7,936 potential respondents, 3,603 provided sleep duration in Survey I (37.8% <9 hours and 62.2% 9+ hours), 3,933 provided sleep duration in Survey II (65.0% <9 hours and 35% 9+ hours) and 3,142 in the “Children of 1997” Biobank Clinical follow-up (79.0% <9 hours and 21% 9+ hours). In total 4,958 had age of menarche or voice breaking. The participants with and without puberty status differed in sex, parents’ birthplace, highest parental occupation, household income per head in quintiles and highest parental education levels but the Cohen effect sizes indicated these differences were small (Appendix Table 1). Multicollinearity is also not a major issue in our study (Appendix Table 2). Mean age of onset of breast development was 9.6 years and 10.8 years for genitalia development. Girls (10.56 ± 1.03) had earlier age of onset of pubic hair than boys (11.48 ± 1.09). Mean age of menarche was 11.9 years and mean age of voice breaking was 13.1 years. Later pubertal development was associated with higher family SEP, such as parents’ birthplace, highest parental occupation, household income per head in quintiles and highest parental education level (Table 1).

Age of menarche was positively associated with sleep duration [odds ratio (OR) 1.11, 95% CI (1.01 to 1.21)] at age 13.5 years, but other measures of puberty timing were unrelated to sleep duration. (Table 2).

Mendelian randomization study. Genetic predictors of pubertal timing (exposure). In total 389 single nucleotide polymorphisms (SNPs) predicting age at menarche (per year) at genome-wide significance (p-value < 5 × 10^{-8}) were obtained from summary genetic associations concerning 329,345 women of European ancestry. These summary genetic associations are based on the ReproGen consortium (N = 179,117 from 40 studies), in addition to 23andMe (N = 76,631) and the UK Biobank (N = 73,397)

F statistics ranging from 29 to 953 (Appendix Table 3). The 389 loci explained 7.4% of the variance in age of menarche and the overall F-statistic was about 63. Of these 389 SNPs, 181 SNPs were excluded because of linkage disequilibrium (R² < 0.001). Of the remaining 208 SNPs, 203 SNPs were available for sleep duration and no proxy was found for the 5 missing SNPs, giving 203 SNPs. Figure 1 shows the selection of SNPs related to age of menarche used as instruments. For age of voice breaking 11 genome-wide significant (p-value < 5 × 10^{-8}) signals for age of voice breaking were obtained from 55,871 European men from the 23andMe study23 After excluding 7 correlated SNPs, 4 independent SNPs
Two independent SNPs (p-value \(< 5 \times 10^{-6}\)) predicting age at Tanner stage in girls and one in boys were obtained from 11,000 Europeans from the Early Growth Genetics (EGG) Consortium\(^25\). Appendix Table 3 summarizes the information extracted for each SNP.

Genetic associations with sleep duration (outcome).

Genetic associations with sleep duration were obtained from the UK Biobank\(^26\), which recruited more than 500,000 people of white British ancestry (intended age range 40–69 years) in Great Britain from 2006 to 2010.

Later age at menarche was associated with longer adult sleep duration using all methods except MR-Egger where the confidence interval included the null value. Heterogeneity was high (p \(< 0.0001\)), but the MR-Egger intercept did not indicate horizontal pleiotropy (p = 0.95). MR-PRESSO removed some SNPs as outliers (p \(< 0.0001\)), but the corrected estimates also showed a positive causal effect of age of menarche on sleep duration. The results were similar after removing 10 SNPs relevant to potential confounders and 7 potentially pleiotropic SNPs (Appendix Table 4). In boys, age of voice breaking was positively associated with sleep duration but the confidence interval included the null value. Tanner stage age in girls and boys was unrelated to sleep duration (Table 3).

| Age 11–12 OR' (95% CI) | Age 13–14 OR' (95% CI) | Age 17–18 OR' (95% CI) |
|------------------------|------------------------|------------------------|
| **Overall**            |                        |                        |
| Age of menarche/voice breaking | 1.05 (0.98, 1.11) | 1.01 (0.94, 1.09) |
| Onset of Breast/Genitalia | 1.02 (0.96, 1.08) | 1.02 (0.96, 1.09) | 1.05 (0.98, 1.12) |
| Onset of Pubic Hair Development | 1.03 (0.97, 1.10) | 1.04 (0.97, 1.12) | 1.02 (0.93, 1.11) |
| **Girls**              |                        |                        |
| Age of menarche/voice breaking | 1.11 (1.01, 1.21) | 1.06 (0.94, 1.18) |
| Onset of Breast/Genitalia | 1.04 (0.96, 1.13) | 1.02 (0.93, 1.11) | 1.08 (0.99, 1.19) |
| Onset of Pubic Hair Development | 1.06 (0.96, 1.17) | 1.09 (0.98, 1.22) | 1.04 (0.92, 1.18) |
| **Boys**               |                        |                        |
| Age of menarche/voice breaking | 1.00 (0.92, 1.10) | 0.97 (0.88, 1.08) |
| Onset of Breast/Genitalia | 0.995 (0.91, 1.09) | 1.03 (0.95, 1.12) | 0.99 (0.89, 1.11) |
| Onset of Pubic Hair Development | 0.996 (0.90, 1.10) | 1.01 (0.92, 1.10) | 0.98 (0.87, 1.11) |

Table 2. Adjusted\(^*\) Associations of Age of pubertal status (year) with sleep duration (9+ hours versus <9 hours) in Hong Kong’s “Children of 1997” Birth Cohort. \(^*\)Adjusted for parents’ place of birth, highest parental occupation, household income per head and highest parental education levels. \(^\#\)Odds ratio (OR) per 1-year older age of puberty; thus, a significant OR >1 indicates that older age of puberty is associated with higher odds of longer sleep duration (9+ hours). Bold font: Statistical significance.

Figure 1. Selection of SNPs for age of menarche related to sleep duration used in Mendelian Randomization.
Table 3. Mendelian Randomization Estimates of the Effect of puberty timing (year) on adult sleep duration (category). IVW: Inverse Variance Weighting WM: Weighted median method MR-PRESSO: Mendelian randomization pleiotropy residual sum and outlier Bold font: Statistical significance.

Discussion

In a population-representative Chinese birth cohort (“Children of 1997”)22 from an understudied non-Western population, age of menarche was positively associated with sleep duration at about 13.5 years. This finding was validated in a Mendelian Randomization study of the association of age of menarche with adult sleep duration. The evidence was not very supportive of pubertal markers causing sleep duration in boys or for other indicators of pubertal timing.

Our observational study extends previous studies by using separate indicators of the onset of puberty clinically assessed by a trained physician evaluation, rather than self-reports19,27. A previous genetic study using linkage disequilibrium (LD) score regression found genetic correlations between sleep duration and age of menarche26. Our two-sample MR study confirmed these findings with directional estimates using more genetic instruments and a larger sample. However, earlier age of voice breaking in boys was unrelated to sleep in both the observational and the MR study.

We used observational and two-sample MR designs to assess the association of earlier puberty with sleep duration, but limitations exist. First, child sleep duration was reported, which might be less accurate than actigraphy or polysomnography. However, self- and parentally-reported sleep duration has been widely used in previous studies28. Second, observationally sleep duration in children could only be analyzed in two groups, reducing discrimination. However, the National Sleep Foundation has suggested that 9–11 hours sleep is appropriate for 6–13 year olds29. Third, adjustment was not made for other factors such as diet and physical activity which may affect pubertal timing and sleep duration. However, we expected systematic rather than differential effects from residual or unmeasured confounding. Fourth, MR has three assumptions. First, strong associations of the genetic predictors with the exposure are required. In the current study, the SNPs for age of menarche and voice breaking all reached genome-wide significance with a high average F-statistic (73.2 for age of menarche and 84.5 for age of voice breaking). Some of these SNPs are in genes functionally relevant to puberty timing. For example, rare coding mutations in MKRN3 cause central precocious puberty31. The evidence was not very supportive of pubertal markers causing sleep duration in boys or for other indicators of pubertal timing.

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since only summary statistics, rather than individual level data, for the exposure and outcome from two different samples were used, we were unable to check for possible non-linear associations of earlier puberty with sleep duration. However, the risk of chance associations resulting from the underlying data structure in single sample is reduced when using separate sample instrumental variable analysis.33

Although the mechanisms underlying the causal effect of earlier puberty on sleep duration are unclear, several potential explanations exist for our findings. First, sex hormones may play a role, since dramatic changes in testosterone and estrogen occur at puberty. In an RCT, estrogen increased sleep duration in postmenopausal women,34 but the effect could differ by baseline levels. Animal studies found estrogen suppresses sleep in female rats.35,36 As such, the mechanisms underlying the effects on sleep duration in women remain to be clarified. In men testosterone shortens sleep duration.37 Second, earlier age of puberty was associated with higher risk of depressive symptoms only in girls in this cohort,37 which could account for the different estimates of earlier puberty with sleep duration by sex. Third, puberty-related sex differences in hypothalamic–pituitary–adrenal (HPA) axis activity may be another contributing factor, such as via corticosterone.8

Age of menarche causally affects obesity13 and cancer.38 Age of menarche also affecting sleep duration may explain the observed, but possibly non-causal relation of sleep duration with obesity.29 Sex-specific associations are consistent with previous observational studies showing the association of earlier puberty with sleep duration was less clear in boys28,27, which may partly be due to the lack of recordable and validated measures of pubertal timing compared with the more clear-cut milestone of menarche in girls. This means some estimates in boys could be biased towards the null by non-differential misclassification. For example, in the observational study, we had a larger sample with age of menarche (n = 3,177) than other pubertal markers. However, the same direction of effect estimates in women and men in the MR study suggests a role for pubertal timing in sleep duration in general rather than menarche specifically. Together with evidence of a similar causal effect of age of voice breaking on obesity and cardiometabolic traits,24 our results could indicate sleep duration is a marker, not a driver, of obesity40 and possibly other chronic diseases.

Methods
Observational study. First, we used the “Children of 1997” birth cohort to conduct the observational study, which is a population-representative Chinese birth cohort (n = 8,327) that covered 88% of all births in Hong Kong from April 1 to May 31, 1997, described in detail elsewhere.22 Baseline characteristics, including SEP (parental education and an indicator of parental migrant status) and birth characteristics were obtained from a self-administered questionnaire at recruitment.41 Passive follow-up via record linkage was instituted in 2005 to obtain pubertal stage from the Student Health Service (SHS), based on an internal reference number, Department of Health, which provides free annual check-ups for all school students. Active follow-up via direct contact was instituted in 2007, with surveys conducted in 2008/9 (Survey I), 2010/12 (Survey II), 2011/12 (Survey III) and a “Children of 1997” Biobank Clinical follow-up in 2013-6.

Exposure – Age of puberty. Markers of puberty including breast/genitalia, pubic hair development and age of menarche were the exposures. Pubertal status were visually assessed by physicians at the SHS according to the criteria of Marshall and Tanner in grades 1, 3, 5, and 7 (usually at 6–7 years, 8–9 years, 10–11 years, and 12–13 years, respectively).42,43 We defined the onset of puberty as onset of breast development for girls and pubic hair development for boys, as measured by a change from Tanner stage I to stage II. Since the exact age of pubertal onset could not be precisely observed, we assumed the onset occurred midway between the latest time point when Tanner stage I was observed and the earliest time point when Tanner stage II was observed, assuming equal intervals between Tanner stages.44,45 The age of onset of pubic hair development was estimated in the same way. Children with infeasible sequences of pubertal stages, such as Tanner stage II before stage I, were excluded (n = 87).37 Age of menarche was self-reported at SHS clinics, in Survey III and in the “Children 1997” Biobank Clinical follow-up (for the Chinese birth cohort). Age of voice breaking was self-reported in Survey III and in the “Children 1997” Biobank Clinical follow-up.

Outcome – Sleep duration. The main outcome was sleep duration, which was assessed at three time points: Survey I (11.5 years), Survey II (13.5 years) and at the Biobank Clinical follow-up (17.5 years) in the Chinese “Children of 1997” birth cohort. Sleep duration was obtained in Survey I and II using a parent-reported questionnaire. Sleep duration was asked as “≤ 1 hours”, “2–4 hours”, “5–8 hours”, “9–12 hours” and “≥ 13 hours”, but almost all reported “5–8 hours” or “9–12 hours” sleep, so sleep duration was classified as < 9 hours and 9+ hours. Sleep duration (in hours) was also obtained by self-report in the Biobank Clinical follow-up from the difference between bedtime and wake-up time, reported as the most common evening bedtime and wake-up time during the past month at about 17.5 years old. We also considered sleep duration as < 9 hours and 9+ hours for consistency.

Mendelian randomization. Second, in order to validate the observational results, we used summary genetic associations from 2 different genome-wide association studies (GWAS) to test each association. We obtained genetic predictors of age of menarche from a GWAS of a combined study including ReproGen, 23andMe and UK Biobank,35 of age of voice breaking from 23andMe,34 and of Tanner stage from EGG.25 We obtained genetic associations with sleep duration from UK Biobank,26 restricted to participants of European descent. We obtained SNPs strongly (p-value < 5 x 10-8) associated with age of puberty from the largest and most recent genome-wide association studies (GWAS)23,24. Linkage disequilibrium between these SNPs was identified using the “Clumping” function of MR-base.46 We used UK Biobank data to check for any associations at Bonferroni corrected significance of the selected SNPs with potential confounders, such as education, smoking, physical activity and alcohol use, using the UK Biobank, a large cohort study accessible to researchers worldwide.47 We repeated the
analysis after removing these SNPs in sensitivity analysis. Potentially pleiotropic effects (linked to the outcome other than via sleep) of the chosen SNPs were obtained from comprehensive curated genotype to phenotype cross-references, Ensembl and PhenoScanner. As sensitivity analysis we also repeated the analysis after excluding potentially pleiotropic SNPs. To identify any unknown pleiotropic effects, statistically, we used MR-Egger and the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test.

These genetic instruments were applied to the largest publicly available GWAS of sleep duration from the UK Biobank, as described above. The GWAS includes 335,410 unrelated individuals of white British ancestry and provides sex-specific and overall genetic associations, adjusted, where appropriate, for sex, age, age squared, the interaction of age and sex, of sex and age squared and the first 20 principal components. Sleep duration was considered in three ordered categories: <7 hours, ≥7 and <8 hours and ≥8 hour.

Statistical analysis. In the “Children of 1997” birth cohort we used chi-squared tests and Cohen effect sizes to compare confounders for children with and without information about pubertal status. The association of pubertal timing with sleep were assessed using multivariable linear regression. Confounders were selected as likely common causes of sleep duration and pubertal timing, including parents’ place of birth, highest parental occupation, household income per head, highest parental education level. Multicollinearity was assessed by variance inflation factor (VIF). A VIF of 10 or above suggests that interpretation of the relevant coefficients could be problematic.

To account for loss to follow-up, we used a combination of multiple imputation (MI) and IPW. First, we used multiple imputation to predict missing confounders and exposures. Second, we estimated IPW using logistic regression to retrieve the original sample. Third, Rubin’s Rules were used to combine each IPW effect estimator with its corresponding sandwich variance estimator.

In the two-sample MR study, the causal associations of age of menarche with sleep duration were obtained using instrumental variable analysis. The F statistic for each SNP and overall was calculated to evaluate the strength of the instrument. We pooled Wald estimates (SNP on outcome/SNP on exposure) for independent SNPs (R² < 0.01) using IVW meta-analysis with multiplicative random effects which assumes balanced pleiotropy. To assess heterogeneity, we used the I² statistic, where a higher value indicates more pleiotropy. However, given the possibility of unknown unbalanced pleiotropy, IVW could be invalid. When an exposure had at least 3 genetic predictors, we used the weighted median (WM) and MR-Egger with more relaxed assumptions. The WM gives robust estimates as long as valid SNPs contribute >50% of the information. MR-Egger with wide confidence intervals provides valid estimates and checks for potentially unknown horizontal pleiotropy (the SNPs affect the outcomes via mechanisms other than sleep duration) through non-null intercept, but it requires that the Instrument Strength Independent of Direct effect (INSIDE) assumption is satisfied. Since MR-Egger could not detect outliers and has limited statistical power, MR-PRESSO was used to identify horizontal pleiotropic outliers and if necessary to correct for pleiotropy via outlier removal. Using 100,000 simulations, we obtained the empirical p-value for the MR-PRESSO global test. The Mendelian randomization estimate is valid if the global test is non-significant (p > 0.05). We harmonized the effect allele for exposure and outcomes on the effect allele letter and if necessary to correct for pleiotropy via outlier removal. Using 100,000 simulations, we obtained the empirical p-value for the MR-PRESSO global test. The Mendelian randomization estimate is valid if the global test is non-significant (p > 0.05). We harmonized the effect allele for exposure and outcomes on the effect allele letter and if necessary to correct for pleiotropy via outlier removal.

To account for loss to follow-up, we used a combination of multiple imputation (MI) and IPW. First, we used multiple imputation to predict missing confounders and exposures. Second, we estimated IPW using logistic regression to retrieve the original sample. Third, Rubin’s Rules were used to combine each IPW effect estimator with its corresponding sandwich variance estimator.

All statistical analysis was performed using Stata version 13.1 (StataCorp LP, College Station, TX) and R version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria), and the “MendelianRandomization”, “TwoSampleMR” and “MRPRESSO” packages.

Ethics approval and consent to participate. Ethical approval for the study, including comprehensive health related analyses, was obtained from Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB). The Mendelian randomization study is an analysis of publicly available summary data that does not require ethical approval.

Data availability The datasets for MR analysis of this article are available from two published genome-wide association studies: https://www.nature.com/articles/ng.3841 and http://www.nealelab.is/uk-biobank. The “Children of 1997” data were available on request after approval. https://aprmay97.sph.hku.hk. The “Children of 1997” data and sample access committee is responsible for responding to birth cohort data access requests by bona fide researchers, and the relevant procedures involved (e.g., application and suggested revisions). The committee will also ensure access to the data is in line with the interests of the birth cohort participants, and access is in accordance with said protocol (i.e. measures to avoid confidentiality breaches, and notification of relevant publications arising from the requested data).

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Author contributions
J.W. conducted the analyses and wrote the first draft of the manuscript. C.M.S. revised drafts of the manuscript critically and supervised the study from conception to completion. M.K.K. and S.L.A.Y. helped to run the follow-up of the birth cohort and reviewed the manuscript. J.Z. checked the analysis and reviewed the manuscript. G.M.L., A.M.L. and H.S.L. provided content matter expertise and helped to collect the birth cohort data. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Competing interests
The authors declare no competing interests.

Additional information
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