The role of age at menarche and age at menopause in Alzheimer’s disease: evidence from a bidirectional mendelian randomization study

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ABSTRACT

The association between endogenous estrogen exposure and Alzheimer’s disease (AD) remains inconclusive in previous observational studies, and few Mendelian randomization (MR) studies have focused on their causality thus far. We performed a bidirectional MR study to clarify the causality and causal direction of age at menarche and age at menopause, which are indicators of endogenous estrogen exposure, on AD risk. We obtained all genetic datasets for the MR analyses using publicly available summary statistics based on individuals of European ancestry from the IEU GWAS database. The MR analyses indicated no significant causal relationship between the genetically determined age at menarche (outlier-adjusted inverse variance weighted odds ratio [IVWOR] = 0.926; 95% confidence interval [CI], 0.803-1.066) or age at menopause (outlier-adjusted IWOR = 0.981; 95% CI, 0.941-1.022) and AD risk. Similarly, AD did not show any causal association with age at menarche or age at menopause. The sensitivity analyses yielded similar results. In contrast, an inverse association was detected between age at menarche and body mass index (BMI, outlier-adjusted IVW β = -0.043; 95% CI, -0.077 to -0.009). Our bidirectional MR study provides no evidence for a causal relationship between the genetically determined age at menarche or age at menopause and AD susceptibility, or vice versa. However, earlier menarche might be associated with higher adult BMI.

INTRODUCTION

As the population ages, almost 115.4 million people worldwide will have dementia by 2050, with the main cause being Alzheimer’s disease (AD) [1]. Notably, women have a more than 55% greater lifetime risk of AD at age 65 than men (24.6% vs. 15.5%) and constitute two-thirds of late-onset AD cases [2]. In recent years, sex hormones, especially estrogen [3], have gained increasing attention because accumulating population-based evidence has proposed a protective role for exogenous hormone replacement therapy (HRT) in cognitive decline.
and dementia progression in postmenopausal females [4, 5]. Because of the lifetime exposure of women to endogenous estrogens, understanding the impact of endocrine event signaling (such as age at menarche and age at menopause) on AD risk is imperative. However, observational findings to date show heterogeneity in the association between endocrine event signaling and the risk of dementia. For example, a large, diverse cohort study showed that delayed menarche increased dementia risk [6], but this association disappeared after adjusting for baseline risk factors of dementia in other studies [7, 8]. Similarly, inconsistent estimates ranged from a modest elevated dementia or AD risk with early onset of natural menopause [9] to an inverse association [6, 10] or an entire loss of statistical evidence [11, 12]. Thus, it is difficult to distinguish whether endogenous estrogen exposure indeed has a causal effect on AD susceptibility or whether this association is completely attributable to other unmeasured potential confounders, such as a high body fat mass or individual socioeconomic factors.

Mendelian randomization (MR) analysis, using genetic single-nucleotide polymorphisms (SNPs) that are known risk factors of interest as proxy instrument variables (IVs) [13, 14], has been widely established to estimate the causal inference of an exposure on an outcome. As genetic variants are allocated randomly at the time of conception and are relatively independent of environmental and lifestyle factors, the typical confounding factors or reverse causation limited from observational studies could be better mitigated. Nevertheless, MR analysis can provide indirect evidence for a causal association relying on the following three core assumptions [15] (Figure 1): 1) the IVs should be robustly correlated with exposure (assumption 1); 2) the IVs should be independent of any confounders of the exposure-outcome association (assumption 2); and 3) the IVs affect the risk of outcome only through the exposure, rather than any alternative pathways (assumption 3). The latter two assumptions are jointly known as independence from pleiotropy.

The effect of endogenous estrogen exposure on AD risk remains inconclusive in observational studies, and few MR studies have focused on their causal association thus far. Herein, we performed a bidirectional MR study to clarify the causality and causal direction between age at menarche and age at menopause and AD, using publicly available summary statistics from genome-wide association studies (GWAS) based on individuals of European ancestry.

**MATERIALS AND METHODS**

We obtained all genetic datasets for the MR analyses using publicly available summary statistics based on individuals of European ancestry from the IEU GWAS database (https://gwas.mrcieu.ac.uk/). Ethical review and informed consent were obtained from the original GWAS. Briefly, in the forward direction, we first analyzed whether genetically determined age at menarche/menopause (SNP exposure) causally affects AD and its relevant traits (SNP outcome), while in the reverse direction, we determined whether genetic predisposition to AD (SNP exposure) affects age at menarche/menopause (SNP outcome).

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**Figure 1. Schematic model of the MR study.** SNP, single-nucleotide polymorphism; MR, mendelian randomization; IVW, inverse variance–weighted; WM, weighted median.
IV selection and validation

SNPs associated with exposure at genome-wide significance ($P < 5 \times 10^{-8}$) in the GWAS datasets were selected as IVs. We clumped SNPs to achieve independent loci with a threshold of linkage disequilibrium (LD) $r^2 > 0.001$ and a distance of 10,000 kb in PLINK [16]. Then, we extracted the effect estimates of the selected IVs in each outcome GWAS dataset, where target IVs were not available in the outcome of interest. We replaced proxy SNPs in high LD ($r^2 > 0.80$) using the online platform LDlink (https://ldlink.nci.nih.gov/). Next, we harmonized the exposure and outcome data using the “TwoSample MR” package to ensure their effects on SNPs corresponding to the same allele or removed all palindromic SNPs from the analysis.

To satisfy the first MR assumption, we applied an F-statistic to evaluate the strength of each selected SNP, and an F-statistic > 10 suggests that the SNP is sufficiently strong to lessen any potential bias [17]. We also computed the variance ($R^2$) explained by each IV in the exposure (for F-statistic and $R^2$ calculations see Supplementary Methods 1). To address the second MR assumption, we further explored the associations between age at menarche/ menopause and the following AD-relevant traits: cognitive performance, body mass index (BMI), smoking behavior and alcohol consumption. To assess the third MR assumption, we performed additional heterogeneity and sensitivity tests to assess the horizontal pleiotropy of the selected SNPs (see section on heterogeneity and sensitivity tests). MR-PRESSO, which assumes that at least 50% of the selected IVs are valid, was further performed to detect and remove any potential pleiotropic outlier SNPs.

Data sources

The study design and data sources are presented in Figure 2. GWAS summary datasets for age at menarche ($n = 182,416$) and age at menopause ($n = 69,360$) were obtained from the Reproductive Genetics (ReproGen) consortium. Briefly, the ReproGen consortium included women with self-reported age at menarche of 9-17 years old and birth year as the only covariates to allow for secular trends in menarche timing [18]. Women with self-reported age at natural menopause of 40-60 years old were included, excluding those with menopause induced by bilateral ovariectomy, hysterectomy, radiation or chemotherapy and those using HRT before menopause [19]. GWAS summary datasets for AD were derived from the largest two-stage study performed by the International Genomics of Alzheimer’s Project (IGAP) [20]. In our MR study, we extracted individual SNPs associated with AD from stage 1 of the IGAP. In stage 1, the IGAP genotyped and imputed data on 7,055,881 SNPs consisting of 17,008 AD cases and 37,154 controls, and adjustments were made for age, sex, and principal components in genetic association analysis.

GWAS summary datasets for AD-relevant traits were selected from the following consortiums or studies: Lee JJ et al. for cognitive performance ($n = 257,841$) [21],

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Figure 2. Study design and data sources. IGAP, International Genomics of Alzheimer's Project; ReproGen, Reproductive Genetics Consortium; GIANT, Genetic Investigation of Anthropometric Traits; TAG, Tobacco and Genetics Consortium; UKB, UK biobank IVs, instrument variables; SNP, single-nucleotide polymorphism; BMI, body mass index.
the Genetic Investigation of Anthropometric Traits (GIANT) consortium for BMI (n = 322,154) [22], the Tobacco and Genetics (TAG) consortium for smoking behaviors (n = 74,053) [23], and the UK Biobank (UKB) for alcohol consumption (n = 112,117) [24]. Details of studies and participants are given in Supplementary Methods 2.

Definition of phenotypes

Menarche was defined as the onset of first menstruation in girls [18]. Menopause was defined as the onset of last naturally occurring menstrual period followed by at least 12 consecutive months of amenorrhea [19]. AD cases were confirmed by autopsy or clinical diagnosis according to the national criteria [20]. Cognitive performance was measured by the respondent’s score on a test of verbal cognition [21]. BMI was calculated as the weight to-squared-height ratio (kg/cm²) [22]. Smoking behavior was the average number of cigarettes smoked per day [23]. Alcohol consumption was the average intake in units per week [24].

Statistical analyses for MR estimates

We estimated an overall causal effect between exposure and outcome using the inverse variance-weighted (IVW) method. IVW is considered the most reliable MR method, assuming all SNPs are valid IVs with no evidence of directional pleiotropy. Given that the results could be biased by the horizontal pleiotropy of IVs, we compared IVW results with other MR methods (i.e., Egger regression and weighted median) whose estimates are more robust to horizontal pleiotropy, although at the expense of lowered statistical power. Egger regression allows for the slope representing the causal effect estimate and the intercept as an indicator of average pleiotropic bias. The weighted median method provides more robust MR estimates; even up to 50%, IVs are invalid. MR-PRESSO was further applied to provide outlier-adjusted estimates, with a significant global test P value < 0.05. Effect estimates are reported in β values where the outcome was continuous (i.e., age at menarche/menopause) and converted to an odds ratio (OR) where the outcome was dichotomous (i.e., AD status).

Heterogeneity and sensitivity assessment

To further assess the heterogeneities and pleiotropy between IVs, we conducted additional heterogeneity and sensitivity tests. Assuming that all valid IVs have an equivalent effect, Cochran’s Q test was used to estimate the heterogeneities between SNPs. MR Egger intercept regression, representing an indicator of average pleiotropic bias, was conducted to identify the directional pleiotropy between SNPs. Furthermore, “leave-one-out” analyses were performed to estimate the causal effect of outlying IVs by stepwise removing each IV from the MR analysis.

Finally, we searched the potential confounding traits for each IV and their proxies (r² > 0.80) in the PhenoScannerV2 database (http://www.phenoscanner.medschl.cam.ac.uk/) and GWAS catalog (https://www.ebi.ac.uk/gwas/) to stepwise remove the IV with possible pleiotropic effects until Cochran’s Q test made no difference from the null. In the context of age at menarche/menopause-AD, the potential confounders included cognitive performance, BMI, smoking behavior, and alcohol consumption, while in the context of AD age at menarche/menopause, BMI was most likely to be a major confounder.

All analyses were conducted using R statistical software (version 4.0.2) with the R packages “TwoSample MR” and “MR-PRESSO,” and P < 0.05 was considered statistically significant.

Sample size and power calculations

We estimated MR power for binary and continuous outcomes at a two-sided α of 0.05 using the mRnd power calculation tool (https://shiny.cnsgenomics.com/mRnd/). Both forward- and reverse-direction MR analyses had sufficient (> 80%) power to detect a statistically significant effect, suggesting that the associations did not arise from chance. Furthermore, all sample sizes of the corresponding GWAS summary datasets were much larger than the sample size required for 80% power. Sample size and power calculations are given in Supplementary Methods 3 and Supplementary Table 1.

RESULTS

IV selection

In the forward direction, 68 SNPs associated with age at menarche were included as IVs and together accounted for 3.55% of the total variance. Meanwhile, 42 SNPs associated with age at menopause were eligible as IVs and together accounted for 4.69% of the total variance. In the reverse direction, 17 SNPs associated with AD were selected as IVs and together accounted for 3.37% of the total variance. The F-statistic value for each selected IV was more than 10, suggesting that the selected SNPs were sufficiently strong and that the causal estimate was unlikely to be biased by weak IVs. The association between each SNP exposure and the corresponding SNP outcome is presented in Supplementary Tables 2–4.
MR estimates of age at menarche and AD

As presented in Table 1, after removing 14 SNPs for being palindromic, no causal association was observed between the genetically determined age at menarche and AD across the three MR methods for 54 SNPs (all $P > 0.05$). Meanwhile, MR-PRESSO did not detect any potential outliers (global $P = 0.251$); Cochran’s Q statistics showed no notable heterogeneities between IVs ($Q_{IVW} = 63.114, P_{IVW} = 0.161; Q_{MR-Egger} = 62.143, P_{MR-Egger} = 0.158$); and no horizontal pleiotropy (intercept = 0.009; $P = 0.372$) was observed in the MR Egger intercept test (Table 2). However, “leave-one-out” analyses indicated that the causal estimate of IVW was driven by four SNPs (i.e., rs1659127, rs2947411, rs740077 and rs6747380) (Supplementary Figure 1A). Therefore, we searched the PhenoScanner database and mapped SNPs to known genes implicated in the GWAS catalog to identify those nominally associated with AD or its relevant traits (Supplementary Table 5). Finally, 23 of the 54 SNPs were removed for being potentially pleiotropic, and the MR estimate remained null after removing the outliers (outlier-adjusted IVWOR, 0.926 for AD per 1-SD increase in mean age at menarche; 95% CI, 0.803-1.066; $P = 0.284$). The weighted median and MR Egger analysis yielded a similar pattern of effects (Table 1), with no single SNP driving the results (Supplementary Figure 1B).

In the reverse direction, only six of the 17 IVs were found in the age at menarche summary datasets and were included for MR analyses. We discovered no statistically significant association between genetic predisposition to AD and age at menarche (IVW $\beta = 0.006$ in mean age at menarche per AD vs. control status; 95% CI, -0.039 to 0.051, $P = 0.793$). The weighted median and MR Egger analyses yielded a similar pattern of effects, and no potential outliers, notable heterogeneities, or horizontal pleiotropy were detected (Tables 2, 3), without a single SNP driving the results (Supplementary Figure 2). Neither the PhenoScanner database nor the GWAS catalog detected the IVs associated with BMI (Supplementary Figure 2 and Supplementary Table 6).

MR estimates of age at menopause and AD

Similarly, we also found no evidence of a causal relationship between the genetically determined age at menopause and AD, regardless of whether pleiotropic SNPs were removed (for 24 SNPs, outlier-adjusted IVWOR, 0.981 for AD per 1-SD increase in mean age at menopause; 95% CI, 0.941-1.022; $P = 0.352$; for 38 SNPs, IVWOR, 0.991 for AD per 1-SD increase in mean age at menopause; 95% CI, 0.957-1.026; $P = 0.611$); or between genetic predisposition to AD and age at menopause (for six SNPs, IVWOR $\beta = -0.044$ in mean age at menopause per AD vs. control status; 95% CI, -0.206 to 0.119, $P=0.598$). A similar pattern of effects was also indicated in the weighted median and MR Egger analyses (Tables 1–3, Supplementary Tables 1–3, Supplementary Figures 2, 3).

MR estimates of age at menarche/ menopause and AD-relevant traits

Our results indicated an inverse association between age at menarche and BMI (for 31 SNPs, outlier-adjusted IVW $\beta = -0.043; 95\% \text{ CI}, -0.077 \text{ to } -0.009, P = 0.014$; outlier-adjusted weighted median $\beta = -0.048; 95\% \text{ CI}, -0.093 \text{ to } -0.002, P = 0.040$), although the MR Egger regression analysis suggested a null causal effect. However, neither age at menarche nor age at menopause had a significant association with the other remaining AD-relevant traits across the three MR methods (all $P > 0.05$) (Figures 3, 4 and Supplementary Table 8).

DISCUSSION

In this large bidirectional MR study, we did not discover a causal relationship between the genetically determined age at menarche or age at menopause on AD susceptibility, or vice versa. Additionally, multiple heterogeneity and sensitivity analyses have been performed to detect and remove any potential of pleiotropy (i.e., where the genetic IVs do not have direct effects on outcomes independent of exposures), making these results more reliable and transparent.

Age at menarche, as a high polygenetic childhood trait, is a prominent milestone of puberty timing in women [18]. MR evidence has suggested the detrimental effects of early menarche on diverse health outcomes including obesity [25], cardiovascular disease [26], cancer [27], and all-cause mortality [28]. Our MR findings corroborated the results from some prospective studies [8, 29] showing a null association between self-reported age at menarche and AD risk, although some studies [30, 31] found a positive association. For example, X Hong et al. [30] reported that increased AD risk paralleled an increased age at menarche (adjusted OR = 1.16 for each increased year, $P = 0.0342$). Gilsanz et al. [31] found a hazard ratio (HR) of 1.23 (95% CI, 1.01-1.50) for age at menarche (≥ 16 vs.13.0 years) in association with dementia, independent of demographics and life course health indicators. These conflicting findings observed in conventional observational studies are possibly due to reverse
Table 1. MR results for the relationships between age at menarche/menopause and AD.

| Exposure-outcome                | Method        | OR(95% CI)* | P value | No. of SNPs |
|---------------------------------|---------------|-------------|---------|-------------|
| Age at menarche-AD              | Main model b  | IVW         | 0.926 (0.803-1.066) | 0.284 | 31 |
|                                 |               | Weighted median | 0.972 (0.801-1.179) | 0.770 | 31 |
|                                 |               | MR Egger     | 1.160 (0.639-2.107) | 0.629 | 31 |
|                                 | With outliers c | IVW         | 0.903 (0.807-1.010) | 0.075 | 54 |
|                                 |               | Weighted median | 0.939 (0.800-1.102) | 0.444 | 54 |
|                                 |               | MR Egger     | 0.749 (0.491-1.142) | 0.185 | 54 |
| Age at menarche-AD              | Main model b  | IVW         | 0.975 (0.935-1.017) | 0.241 | 23 |
|                                 |               | Weighted median | 0.985 (0.931-1.043) | 0.612 | 23 |
|                                 |               | MR Egger     | 0.939 (0.860-1.025) | 0.172 | 23 |
|                                 | With outliers c | IVW         | 0.991 (0.957-1.026) | 0.611 | 38 |
|                                 |               | Weighted median | 0.985 (0.941-1.031) | 0.520 | 38 |
|                                 |               | MR Egger     | 0.954 (0.883-1.032) | 0.251 | 38 |

Abbreviations: MR, Mendelian randomization; AD, Alzheimer’s disease; IVW, inverse variance-weighted; OR, odds ratio; SNP, single-nucleotide polymorphism.
aIndicates odds ratio for AD per 1-SD increase in mean age at menarche/menopause.
bIndicates model removal of potential pleiotropic IVs.
cIndicates model without removal of potential pleiotropic IVs.

Table 2. The heterogeneity and sensitivity results of age at menarche/menopause and AD before and after removal of pleiotropic IVs.

| Exposure-outcome    | No. of SNPs | MR-PRESSO | MR Egger intercept | Cochran's heterogeneity test |
|---------------------|-------------|-----------|--------------------|-----------------------------|
|                     |             | Global P value | Intercept value | P value | IVW-Q value | IVW-P value | Egger-Q value | Egger-P value |
| Age at menarche-AD  | 54a         | 0.251     | -        | 0.009 | 0.372 | 63.114 | 0.161 | 62.143 | 0.158 |
| Age at menopause-AD | 31b         | 0.824     | -        | -0.010 | 0.451 | 32.074 | 0.364 | 31.440 | 0.345 |
| Age at menarche-AD  | 38a         | 0.052     | -        | 0.008 | 0.300 | 54.193 | 0.034 | 52.581 | 0.037 |
| Age at menopause-AD | 23b         | 0.226     | -        | 0.008 | 0.342 | 26.473 | 0.189 | 27.664 | 0.187 |
| AD-age at menarche  | 6           | 0.489     | -        | -0.003 | 0.850 | 4.319 | 0.504 | 4.276 | 0.370 |
| AD-age at menopause | 6           | 0.052     | -        | -0.058 | 0.266 | 4.222 | 0.518 | 2.549 | 0.636 |

Abbreviations: MR, Mendelian randomization; AD, Alzheimer’s disease; IVW, inverse variance-weighted; OR, odds ratio; SNP, single-nucleotide polymorphism.
aIndicates model removal of potential pleiotropic IVs.
bIndicates model without removal of potential pleiotropic IVs.

causal bias or improper adjustment for residual confounders that underlie the causal pathway, such as childhood or adult obesity.

Higher BMI in childhood is linked with earlier menarche [32], and increases AD risk [33]. In our MR study, both the IVW method and weighted median methods consistently demonstrated an inverse association between age at menarche and BMI after removing their high degree of genetic overlap SNPs or SNPs associated with childhood BMI, although MR Egger regression analysis yielded a null causal effect. In fact, the first two MR methods have better accuracy in the causal estimates [15], greater empirical power [34], and better finite-sample type I error rates [15] compared to MR Egger regression. Thus, it is reasonable to believe that earlier menarche could causally influence a higher risk for BMI in adulthood, in line with the results from previous MR studies [35, 36]. Namely, females who have earlier menarche onset are more likely to develop adiposity, implying that BMI might be a critical potential confounder of the age at menarche-AD association in observational studies. Nevertheless, some studies also argued that age at menarche has a limited influence on future adiposity because higher adiposity in childhood could induce earlier puberty [37] and then track forward into adulthood [38, 39]. Therefore, a more extensive sample size and more rigorously designed studies are necessary to resolve their causal direction. Contrary to other epidemiological evidence [40-42], our
Table 3. MR results for the relationships between AD and age at menarche/menopause.

| Exposure-outcome | Method          | β(95%CI)* | P value | No. of SNPs |
|------------------|-----------------|-----------|---------|------------|
| AD-age at menarche | IVW             | 0.006 (-0.039 to 0.051) | 0.793  | 6          |
|                   | Weighted median | 0.000 (-0.063 to 0.063) | 0.990  | 6          |
|                   | MR Egger        | 0.029 (-0.195 to 0.252) | 0.815  | 6          |
| AD-age at menopause | IVW             | -0.044 (-0.206 to 0.119) | 0.598  | 6          |
|                   | Weighted median | -0.114 (-0.332 to 0.104) | 0.306  | 6          |
|                   | MR Egger        | 0.374 (-0.280 to 1.028) | 0.325  | 6          |

Abbreviations: MR, mendelian randomization; AD, Alzheimer’s disease; IVW, inverse variance-weighted; OR, odds ratio; SNP, single-nucleotide polymorphism.

*Indicates change in mean year at menarche/menopause per AD vs control status.

Results indicate no causal associations between the age at menarche and other relevant traits, such as cognitive performance, smoking behavior, and alcohol consumption.

Menopause marks reproductive senescence and is highly heritable with estimates of 0.40-0.70 from twin and sibling studies [43, 44]. Menopausal women are susceptible to lung function [45], osteoporosis [46], cardiovascular disease [46], and age-related morbidity and mortality outcomes [47]. Evidence from accumulating neurobiological studies [48, 49] has declared that later natural menopause delays cognitive decline or AD risk after full adjustment, pointing out the early endocrine aging process as the optimal window for preventing or delaying progression of AD in women. Later natural menopause onset is likely to involve estrogen receptor β function, which regulates brain-derived neurotrophic factors and in turn solidifies memory formation and storage [50]. However, in a 44-year longitudinal population study of Swedish women with natural menopause, AD risk increased as the age at menopause increased (adjusted OR = 1.07, 95% CI, 1.02-1.12) [29]. Compared to women who experienced menopause at younger than 48 years of age, the adjusted rate ratio (RR) for women aged 50-52 years was 1.64 (95% CI, 1.05-2.56), but there was no association with women older than 52 years of age (adjusted RR = 1.47, 95% CI, 0.88-2.46) [7]. Interestingly, our MR study detected no significant causal relationship between the

Figure 3. MR estimate plot for age at menarche on Alzheimer’s disease relevant traits. IVW indicates inverse variance–weighted method.
genetically determined age at menopause and AD, consistent with a population-based cohort study [8]. The disparities in these observational findings could be explained by not fully ruling out some possible confounders. For example, HRT use, which has been proposed for cognitive improvement or AD treatment [5], or the APOE locus, which is a major genetic risk factor for AD [51], are possible confounders. In contrast, in our MR study, all the women included from the GWAS datasets associated with age at menopause had experienced natural menopause, excluding those induced by using HRT, surgery or radiation before menopause. Furthermore, the SNPs associated with age at menopause linked to the APOE locus were removed to minimize type I error. Thus, it is believed that the interpretation of our MR results may be more credible. In addition, our study also indicated that there were no causal effects of age at menopause on any AD-relevant trait, although some previous observational studies have supported possible links [48, 52–54].

In the reverse direction, our findings also suggested a nonsignificant association between genetic predisposition to AD and the age at menarche/ menopause. That is, neither of these two biological traits is a consequence nor the cause of AD, although the beneficial effects of estrogens on the central nervous system are biologically plausible. Underlying mechanisms decrease the toxicities of amyloid-beta (Aβ) and glutamate [55], diminish tau protein hyperphosphorylation [56], reduce inflammation and improve synaptic plasticity in the brain [57]. Menarche and menopause currently show considerable variability between women with a high prevalence of obesity, especially in the age of natural menopause onset [58]. In addition, the absolute amounts of estrogens and mechanisms of endogenous estrogens are different from those of exogenous estrogens. We thus need to better understand the impact of prolonged exposure to endogenous estrogens on dementia or AD risk, rather than blindly applying HRT. The “time hypothesis” theory suggests that estrogens exert dual effects of being neuroprotective for healthy cells but neurotoxic in diseased cells [59]. This theory could partially explain why earlier menarche does not affect cognition. Meanwhile, HRT has a limited positive influence on dementia risk when administered within five years of menopause but causes subsequent adverse effects [4, 60]. Overall, the implications of our findings are that the genetically determined onset of menarche and menopause has limited beneficial effects on AD risk. Efforts to supplement estrogens as an effective prevention measure for AD are worthy of further verification.

To our knowledge, this is the first bidirectional MR study focused on the causality and causal direction between age at menarche/menopause and AD. The strengths of this study include the large sample size from GWAS summary datasets, and the robustness of the inherent confounding factors or reverse causation from the observational studies. Our study also has some

![Figure 4. MR estimate plot for age at menopause on Alzheimer’s disease relevant traits. IVW indicates inverse variance-weighted method.](image-url)
limitations. First, MR is a reliable way to assess causality in the absence of pleiotropy. There is high risk of pleiotropy in MR analyses because many selected SNPs have diverse or uncertain biological functions. To address this, we attempted to perform multiple sensitivity tests to thoroughly examine pleiotropic effects. It is reassuring that the MR estimates were robust, indicating negligible bias from other apparent sources of pleiotropy. Second, to minimize population stratification bias, our analyses were restricted to individuals of European ancestry and might not be generalizable to non-Europeans. Thus, evidence on the shared genetic variants for the age at menarche/ menopause or AD across ethnicities needs to be further validated. Third, since the age at menarche and menopause are based on self-reported information, potential recall bias and measurement error may reduce statistical power to some extent. Last, our analyses only included genetic datasets for the target phenotypes because their individual epidemiological datasets were not publicly accessible. Hence, we could not explore the causal effect of the reproductive period (i.e., technically defined by the time from menarche to menopause) on AD risk. Fortunately, some important confounders, such as age and sex, were well adjusted in the corresponding original GWAS, which may partially lower confounding bias. Moreover, evidence for a high correlation coefficient of 0.93 between age at menopause and the reproductive period was supported by a recent MR study [45], which indirectly indicated a causal effect of the reproductive period on AD risk in our MR analyses.

In conclusion, our bidirectional MR study provided no evidence for a causal effect of the genetically determined age at menarche or age at menopause on AD susceptibility, or vice versa. In contrast, earlier menarche might be associated with higher adult BMI. Further studies combining individual epidemiological and genetic data are warranted to validate and replicate these findings.

**AUTHOR CONTRIBUTIONS**

ZNM, MLL conceived and designed the study. MLL, JLL, SL wrote the manuscript, analyzed and interpreted of data. All authors revised and approved the final manuscript; and ZNM was responsible for the final content.

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**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

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Supplementary Methods 1. The proportion of variance and F-statistic calculations

The proportion of variance

The proportion of variance (conceptually similar to the $R^2$) for each single-nucleotide polymorphism (SNP) was calculated using the formula below [1]. The pooled variance of the SNPs was calculated in an additive model assuming no interaction between the individual SNPs.

$$ R^2 = \frac{2 \times 2 \times \text{MAF} \times (1 - \text{MAF})}{2 \beta^2 \times \text{MAF} \times (1 - \text{MAF}) + (\text{SE}(\beta))^2 \times 2 \times \text{N} \times \text{MAF} \times (1 - \text{MAF})} $$

where $\beta$ is the effect size (beta coefficient) for each SNP; MAF is the minimum allele frequency; SE($\beta$) is the standard error of effect size, and $N$ is the sample size.

F-statistic

The F-statistic of instrument variable was calculated using the formula below [2].

$$ F = \frac{\beta^2}{\text{SE}(\beta)^2} $$

where $\beta$ is the effect size (beta coefficient) for each SNP; SE($\beta$) is the standard error of effect size.
Supplementary Methods 2. Details of studies and participants

The reproductive genetics (ReproGen) consortium

The ReproGen consortium is an international network of investigators interested in better understanding the genetic basis of reproductive aging. They use large-scale meta-analyses of Genome-wide Association Study (GWAS) data to highlight genetic variants and genes that impact reproductive timing in humans.

Age at menarche

We used the summary data for age at menarche HapMap 2 GWAS meta-analysis results from Perry et al. [3] released by the ReproGen consortium. They meta-analyzed for self-reported age at menarche in a total of 182,416 women of European ancestry from 58 GWAS datasets. Women with self-reported age at menarche of 9-17 years old were included in the analysis, and birth year as the only covariates to allow for the secular trends in menarche timing. The mean age of participants ranged from 15.8 to 79.08 years old, along with the self-reported mean age at menarche ranged from 12.4 to 13.7 years old. Genome-wide SNP array data were available on up to 132,989 women from 57 studies. Each study imputed genotype data based on HapMap Phase II CEU build 35 or 36. SNPs were excluded from individual study datasets if they were poorly imputed or were rare (minor allele frequency, MAF < 1%). Test statistics for each study were adjusted using study-specific genomic control inflation factors and where appropriate individual studies performed additional adjustments for relatedness. Association statistics for each of the 2,441,815 autosomal SNPs that passed quality control (QC) in at least half of the studies were combined across studies in a fixed effects inverse-variance meta-analysis implemented in METAL. On meta-analysis, 3,915 SNPs reached the genome-wide significance threshold \((P<5\times10^{-8})\) for association with age at menarche, and they identified 23 independent signals for age at menarche at 106 genomic loci, and including 11 loci containing multiple independent signals using GCTA. The overall GC inflation factor was 1.266, consistent with an expected high yield of true positive findings in large-scale GWAS meta-analysis of highly polygenic traits.

Age at menopause

We used the summary data for age at menopause HapMap 2 GWAS meta-analysis results from Day et al. [4] released by the ReproGen consortium. They meta-analyzed for self-reported age at natural (non-surgical) menopause (ANM) involving up to 69,360 women of European ancestry from 33 GWAS datasets. Age at menopause was defined as the age at last naturally occurring menstrual period followed by at least 12 consecutive months of amenorrhea. The women with age at natural menopause of 40-60 years old were included, excluding those with menopause induced by hysterectomy, bilateral ovariectomy, radiation or chemotherapy, and those using hormone replacement therapy (HRT) before menopause. Studies were asked to use the full imputed set of HapMap Phase 2 autosomal SNPs, and to run an additive model including top principal components and study specific covariates. SNPs were filtered out if the MAF was less than 1%, or if the imputation quality metrics were low (imputation quality < 0.4). Studies and SNPs passing QC were combined using an inverse-variance weighted meta-analysis, implemented using METAL. Again, this meta-analysis was run by two analysts independently, who then separately used PLINK clumping commands to identify the most significant SNPs in associated regions (termed “Index SNPs”), using only those SNPs which had data from more than 50% of the studies. Finally, they reported 1,208 SNPs reached the genome-wide significance threshold \((P < 5\times10^{-8})\) for association with ANM, and identified independent signals located in 44 genomic regions using approximate conditional analysis implemented in GCTA.

International genomics of Alzheimer’s project (IGAP)

We used the largest summary statistics from the 2013 meta-analysis of GWAS data in Alzheimer’s disease (AD) released by the IGAP [5]. Details on the design of the arrays, sample processing and QC have been previously described in the original studies. In brief, the IGAP is a large two-stage GWAS study based on individuals of European ancestry. AD cases were confirmed by autopsy- or clinical diagnosis according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria, and age, sex and principal components were adjusted for in genetic association analysis. In stage 1, IGAP genotyped and imputed data on 7,055,881 SNPs consisting of 17,008 AD cases and 37,154 controls from four GWAS datasets (the Alzheimer Disease Genetics Consortium [ADGC], the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium [CHARGE], the European Alzheimer’s disease Initiative [EADI], and the Genetic and Environmental Risk in AD consortium [GERAD]). The average age of participants was 71 years, with 58.4% were women. In stage 2, 11,632 SNPs were genotyped and tested.
for association in an independent set of 8,572 AD cases and 11,312 controls. In our MR study, we only extracted the AD GWAS summary datasets from stage 1 of the IGAP.

The investigators within the IGAP contributed to the design and implementation of IGAP and/or provided data. IGAP was made possible by the generous participation of the control subjects, the patients, and their families. The i–Select chips was funded by the French National Foundation on AD and related disorders. EADI was supported by the LABEX (laboratory of excellence program investment for the future) DISTALZ grant, Inserm, Institut Pasteur de Lille, Université de Lille 2 and the Lille University Hospital. GERAD was supported by the Medical Research Council (Grant n° 503480), Alzheimer's Research UK (Grant n° 503176), the Wellcome Trust (Grant n° 082604/ 2/07/Z) and German Federal Ministry of Education and Research (BMBF): Competence Network Dementia (CND) grant n° 01GI0102, 01GI0711, 01GI0420. CHARGE was partly supported by the NIH/NIA grant R01 AG033193 and the NIA AG081220 and AGES contract N01–AG–12100, the NHLBI grant R01 HL105756, the Icelandic Heart Association, and the Erasmus Medical Center and Erasmus University. ADGC was supported by the NIH/NIA grants: U01 AG032984, U24 AG021886, U01 AG016976, and the Alzheimer's Association grant ADGC–10–196728.

**AD-relevant traits**

**Cognitive performance**

We extracted the GWAS summary data of cognitive performance, measured by the respondent’s score on a test of verbal cognition, from a sample-size-weighted meta-analysis (N = 257,841) based on healthy individuals of European ancestry performed by Lee JJ et al. [6]. They combined a published study of general cognitive ability (N = 35,298) conducted by the Cognitive Genomics Consortium (COGENT) with new genome-wide association analyses of cognitive performance in the UKB (N = 222,543). The COGENT consortium meta-analyzed 24 cohort studies (comprised of 35 sub-studies) from the general population in North America, the United Kingdom and the European continent. Briefly, each COGENT sub-study administered an average of 8 (SD ± 4) neuropsychological tests. Participant included in COGENT at least had one neuropsychological measure across at least three domains of cognitive performance (for example, digit span for working memory; logical memory for verbal declarative memory; and digit symbol coding for processing speed), or the use of a validated g-sensitive measure was required. Finally, Lee JJ et al. identified 225 genome-wide significant SNPs for cognitive performance.

**Genetic investigation of anthropometric traits (GIANT) consortium**

We used the largest summary statistics from the 2015 meta-analysis of GWAS data in body mass index (BMI, kg/cm²) released by GIANT consortium [7]. Briefly, it is a large two-stage GWAS meta-analysis study based on individuals of European ancestry. In stage 1 they performed meta-analysis of 80 GWAS (N = 234,069); and stage 2 incorporated data from 34 additional studies (N = 88,137) genotyped using Metabochip, and adjusted for age, age squared, and any necessary study-specific covariates (for example, genotype-derived principal components) in a linear regression model. Details on the design of the arrays, sample processing and QC have been previously described in the original studies. Finally, this analysis identified 97 BMI-associated loci (P < 5x10⁻⁸), accounting for ~2.7% of BMI variation, and genome-wide estimates suggest that common variation accounts for >20% of BMI variation.

**The tobacco and genetics (TAG) consortium**

We used the largest summary statistics from the 2010 meta-analysis of GWAS data for smoking behavior within the cohorts of the TAG consortium, involving up to 74,053 individuals of European ancestry [8]. The TAG consortium conducted GWAS meta-analyses across 16 studies originally designed to evaluate other phenotypes (for example, cardiovascular disease and type 2 diabetes). The 16 TAG studies performed their own genotyping, quality control, and imputation, and study sample size ranged from 585 to 22,307, with the mean age varied from 39.6 to 70.5 years old. In this TAG meta-analysis, four smoking phenotypes-smoking initiation (ever versus never been a regular smoker), age of smoking initiation, smoking quantity (number of cigarettes smoked per day, CPD) and smoking cessation (former versus current smokers) were carefully examined and harmonized. Finally, they performed genotype imputation resulting in a common set of ~2.5 million SNPs, and identified three loci associated with CPD, eight SNPs exceeded genome-wide significance for smoking initiation, and one SNP significantly associated with smoking cessation.

**UK biobank (UKB)**

We extracted the summary data of self-reported alcohol consumption from a GWAS performed by UKB, comprising of 112,117 white British individuals [9]. UKB is a population-based sample involving 502,629 individuals age of 40 to 69 years resident in the United Kingdom. In this study, participants were asked to report their current drinking status (never, previous, current, prefer not to say) and average weekly and
monthly alcohol consumption of a range of drink types (red wine, white wine, champagne, spirits, beer/cider, fortified wine). After excluding all former drinkers from the analysis, alcohol consumption was derived an average intake of alcohol consumption in units per week (mean = 15.13, SD = 16.56), and was then log (units +1) transformed, this left 112 117 individuals with data on both alcohol consumption and genome-wide genotype data. Consideration of the mean alcohol intake in males was significantly higher than in females, they regressed age and weight in kg onto weekly units of alcohol consumed in males and females separately. Finally, the sample comprised 52.7% of females, with the SNP-based heritability of alcohol consumption in females was estimated to be 13%, and sex-specific analyses found largely overlapping GWAS loci and the genetic correlation between male and female alcohol consumption was 0.90.
Supplementary Methods 3. Sample size and power calculations

We estimated MR power for binary and continuous outcomes at a two-sided $\alpha$ of 0.05, using the mRnd power calculation tool (https://shiny.cnsgenomics.com/mRnd/). MR power calculation given a desired sample size (outcome) relies on the following parameters: the proportion of variance ($R^2$) explained by genetic instruments in the exposure; the causal effect of the exposure on the outcome, which can be projected across plausible values to investigate impact on statistical power; and the ratio of cases to controls (for binary outcome). While the required sample size for MR given a desired power also relies on several parameters mention above.

The sample size and power calculations for MR analyses are presented in Supplementary Table 1.

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Supplementary Figures

Supplementary Figure 1. MR leave-one-out analysis plots for the relationships of age at menarche with AD. (A) MR leave-one-out analysis plots before removing pleiotropic IVs. (B) MR leave-one-out analysis plots after removing pleiotropic IVs. Abbreviation: MR, mendelian randomization; AD, Alzheimer’s disease; IVs, instrument variables.

Supplementary Figure 2. MR leave-one-out analysis plots for the relationships of AD with age at menarche/menopause. (A) MR leave-one-out analysis plot for the relationships of AD with age at menarche. (B) MR leave-one-out analysis plot for the relationships of AD with age at menopause. Abbreviation: MR, mendelian randomization; AD, Alzheimer’s disease.
Supplementary Figure 3. MR leave-one-out analysis plots for the relationships of age at menopause with AD. (A) MR leave-one-out analysis plots before removing pleiotropic IVs. (B) MR leave-one-out analyses plots after removing pleiotropic IVs. Abbreviation: MR, mendelian randomization; AD, Alzheimer's disease; IVs, instrument variables.
### Supplementary Tables

#### Supplementary Table 1. The sample size and power calculations for MR analyses (two-sided $\alpha=0.05$).

| Exposure-outcome | Actual N (outcome-GWAS) | Ratio of cases to controls (outcome-GWAS) | Observational $\beta^*$ | $R^2$ of IVs $(\%)$ | N required for 80% power | Power at actual N $(\%)$ |
|------------------|-------------------------|------------------------------------------|--------------------------|----------------------|---------------------------|--------------------------|
| Age at menarche-AD | 54,162                  | 0.458                                    | 1.23$^{[10]}$            | 3.55                 | 24,000                    | 98.8                     |
| Age at menopause-AD | 54,162                  | 0.458                                    | 1.17$^{[11]}$            | 4.69                 | 31,600                    | 95.7                     |

#### AD-age at menarche/ menopause (continuous)

| Exposure-outcome | Actual N (outcome-GWAS) | Ratio of cases to controls (outcome-GWAS) | Observational $\beta^*$ | $R^2$ of IVs $(\%)$ | N required for 80% power | Power at actual N $(\%)$ |
|------------------|-------------------------|------------------------------------------|--------------------------|----------------------|---------------------------|--------------------------|
| AD-age at menarche | 182,416                 | /                                        | 0.21$^{[10]}$            | 3.37                 | 5,500                     | 100                      |
| AD-age at menopause | 69,360                 | /                                        | 0.16$^{[11]}$            | 3.37                 | 9,500                     | 100                      |

Abbreviations: AD, Alzheimer’s disease; GWAS, genome-wide association study; IVs, instrument variables; HR, hazard ratio.

$^*$ $\beta$ equals to ln (HR).

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#### Supplementary Table 2. Genome-wide significant SNPs (n = 68) for age at menarche ($P < 1 \times 10^{-8}$).

| SNP ID $^a$ | Proxy SNP $^b$ | $r^2$ for proxy | Effect allele (alternative) | Beta (SE) for age at menarche | Beta (SE) for AD | Variance explained ($R^2$) | F statistic |
|-------------|----------------|-----------------|----------------------------|-------------------------------|-----------------|---------------------------|-------------|
| rs10144321  | -              | -               | G (A)                      | -0.042 (0.007)                | -0.023 (0.018)  | 0.0006                    | 36          |
| rs10483727  | -              | -               | C (T)                      | -0.037 (0.006)                | 0.026 (0.017)   | 0.0006                    | 38          |
| rs1079866   | -              | -               | G (C)                      | 0.072 (0.008)                 | -0.009 (0.023)  | 0.0011                    | 81          |
| rs10840031  | -              | -               | A (G)                      | 0.038 (0.006)                 | -0.032 (0.019)  | 0.0005                    | 40          |
| rs10938397  | -              | -               | G (A)                      | -0.038 (0.006)                | 0.005 (0.016)   | 0.0007                    | 40          |
| rs11022756  | -              | -               | C (A)                      | -0.048 (0.006)                | 0.016 (0.017)   | 0.0009                    | 64          |
| rs11715566  | -              | -               | T (C)                      | 0.052 (0.006)                 | 0.002 (0.015)   | 0.0014                    | 75          |
| rs11756454  | rs2249703      | 0.83            | A (T)                      | 0.034 (0.006)                 | 0.001 (0.015)   | 0.0006                    | 32          |
| rs11767400  | -              | -               | A (C)                      | 0.035 (0.006)                 | 0.003 (0.017)   | 0.0005                    | 34          |
| rs12003641  | -              | -               | T (C)                      | 0.082 (0.011)                 | -0.006 (0.028)  | 0.0009                    | 56          |
| rs12148769  | -              | -               | A (G)                      | -0.055 (0.010)                | 0.012 (0.026)   | 0.0006                    | 30          |
| rs12291726  | -              | -               | G (A)                      | -0.057 (0.008)                | -0.046 (0.021)  | 0.0006                    | 51          |
| rs12598642  | -              | -               | G (A)                      | 0.044 (0.006)                 | -0.021 (0.016)  | 0.0010                    | 54          |
| rs12915845  | -              | -               | T (C)                      | -0.035 (0.006)                | 0.003 (0.016)   | 0.0006                    | 34          |
| rs13179411  | -              | -               | T (G)                      | 0.060 (0.008)                 | -0.038 (0.021)  | 0.0010                    | 56          |
| rs13215865  | -              | -               | T (C)                      | -0.042 (0.007)                | -0.010 (0.020)  | 0.0004                    | 36          |
| rs1398217   | -              | -               | C (G)                      | 0.046 (0.006)                 | 0.009 (0.016)   | 0.0010                    | 59          |
| rs1482853   | -              | -               | A (C)                      | -0.038 (0.006)                | 0.013 (0.016)   | 0.0007                    | 40          |
| rs1516883   | -              | -               | A (G)                      | -0.091 (0.002)                | 0.042 (0.017)   | 0.0035                    | 2070        |
| rs1518080   | -              | -               | G (C)                      | -0.051 (0.006)                | 0.045 (0.016)   | 0.0013                    | 72          |
| rs1659127   | -              | -               | A (G)                      | 0.044 (0.006)                 | 0.052 (0.018)   | 0.0008                    | 54          |
| rs16938437  | -              | -               | T (C)                      | -0.067 (0.010)                | 0.013 (0.028)   | 0.0004                    | 45          |
| rs17351680  | -              | -               | G (C)                      | 0.044 (0.008)                 | -0.004 (0.022)  | 0.0004                    | 30          |
| rs1874984   | -              | -               | C (G)                      | 0.037 (0.006)                 | 0.007 (0.018)   | 0.0007                    | 38          |
| rs2153127   | -              | -               | C (T)                      | -0.077 (0.002)                | 0.003 (0.016)   | 0.0029                    | 1482        |
| rs2179786   | -              | -               | T (G)                      | -0.039 (0.006)                | 0.026 (0.016)   | 0.0007                    | 42          |
| rs2184968   | -              | -               | C (T)                      | -0.036 (0.006)                | -0.014 (0.016)  | 0.0006                    | 36          |
| rs2303100   | -              | -               | T (C)                      | 0.038 (0.006)                 | -0.000 (0.017)  | 0.0007                    | 40          |
| rs2344508   | -              | -               | A (G)                      | 0.034 (0.006)                 | -0.007 (0.016)  | 0.0006                    | 32          |
| rs2617056   | -              | -               | T (A)                      | -0.036 (0.006)                | -0.003 (0.016)  | 0.0006                    | 36          |
| rs2687729   | -              | -               | G (A)                      | 0.044 (0.007)                 | -0.004 (0.017)  | 0.0007                    | 40          |

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| rs          | -    | -    | G (C) | -0.035 (0.006) | -0.018 (0.017) | 0.0005 | 34 |
| rs2947411  | -    | -    | G (A) | -0.052 (0.008) | -0.034 (0.020) | 0.0006 | 42 |
| rs3115627  | -    | -    | G (A) | 0.038 (0.006)  | 0.014 (0.018)  | 0.0007 | 40 |
| rs3733632  | -    | -    | G (A) | 0.049 (0.008)  | -0.017 (0.021) | 0.0009 | 38 |
| rs3743266  | -    | -    | C (T) | -0.045 (0.006) | -0.001 (0.017) | 0.0009 | 56 |
| rs3870341  | -    | -    | G (A) | -0.043 (0.006) | -0.019 (0.018) | 0.0008 | 51 |
| rs3914188  | -    | -    | C (G) | 0.044 (0.007)  | -0.013 (0.018) | 0.0007 | 40 |
| rs4242496  | -    | -    | A (T) | -0.033 (0.006) | 0.005 (0.016)  | 0.0005 | 30 |
| rs4369815  | -    | -    | G (T) | -0.080 (0.012) | 0.064 (0.032)  | 0.0006 | 44 |
| rs466639   | -    | -    | C (T) | 0.075 (0.008)  | -0.007 (0.025) | 0.0013 | 88 |
| rs48801589 | -    | -    | G (C) | 0.032 (0.006)  | -0.004 (0.017) | 0.0005 | 28 |
| rs4840086  | rs2894891 | 1.00 | G (A) | -0.036 (0.006) | 0.034 (0.016)  | 0.0006 | 36 |
| rs618678   | -    | -    | T (C) | -0.034 (0.006) | -0.006 (0.017) | 0.0005 | 32 |
| rs633715   | -    | -    | C (T) | -0.051 (0.007) | 0.028 (0.021)  | 0.0008 | 53 |
| rs6694738  | rs7522883 | 0.98 | A (C) | -0.044 (0.008) | 0.011 (0.027)  | 0.0006 | 30 |
| rs6747380  | -    | -    | A (G) | 0.065 (0.008)  | 0.025 (0.021)  | 0.0012 | 66 |
| rs6758290  | -    | -    | C (T) | -0.040 (0.006) | -0.006 (0.017) | 0.0008 | 44 |
| rs6770162  | -    | -    | A (G) | 0.036 (0.006)  | -0.017 (0.016) | 0.0006 | 36 |
| rs6933660  | -    | -    | A (C) | -0.036 (0.006) | -0.008 (0.017) | 0.0005 | 36 |
| rs7103411  | -    | -    | T (C) | -0.043 (0.007) | -0.012 (0.019) | 0.0006 | 38 |
| rs7119712  | -    | -    | A (G) | -0.041 (0.006) | 0.011 (0.018)  | 0.0005 | 47 |
| rs740077   | -    | -    | C (A) | -0.046 (0.007) | -0.031 (0.019) | 0.0007 | 43 |
| rs7642134  | -    | -    | G (A) | 0.038 (0.006)  | -0.036 (0.016) | 0.0007 | 40 |
| rs7821178  | -    | -    | A (C) | -0.045 (0.006) | -0.000 (0.017) | 0.0010 | 56 |
| rs7853970  | -    | -    | C (T) | -0.037 (0.006) | 0.013 (0.018)  | 0.0007 | 38 |
| rs7944630  | -    | -    | A (G) | 0.047 (0.006)  | 0.006 (0.016)  | 0.0011 | 61 |
| rs852069   | -    | -    | G (A) | 0.036 (0.006)  | 0.002 (0.016)  | 0.0006 | 36 |
| rs888345   | -    | -    | A (G) | -0.044 (0.007) | 0.014 (0.022)  | 0.0006 | 40 |
| rs895526   | -    | -    | C (T) | 0.044 (0.008)  | 0.006 (0.021)  | 0.0006 | 30 |
| rs913588   | -    | -    | A (G) | -0.034 (0.006) | 0.002 (0.015)  | 0.0006 | 32 |
| rs9373571  | -    | -    | A (T) | 0.034 (0.006)  | 0.009 (0.016)  | 0.0006 | 32 |
| rs9555810  | -    | -    | G (C) | 0.047 (0.006)  | -0.013 (0.018) | 0.0009 | 61 |
| rs9565073  | -    | -    | C (T) | 0.034 (0.006)  | 0.006 (0.016)  | 0.0006 | 32 |
| rs9635759  | -    | -    | A (G) | 0.058 (0.006)  | 0.013 (0.017)  | 0.0015 | 93 |
| rs9647570  | -    | -    | G (T) | 0.046 (0.008)  | -0.023 (0.023) | 0.0004 | 33 |
| rs9939609  | -    | -    | A (T) | -0.042 (0.005) | 0.006 (0.016)  | 0.0009 | 71 |
| rs9997604  | -    | -    | C (A) | 0.039 (0.007)  | -0.011 (0.017) | 0.0006 | 31 |

Abbreviations: AD, Alzheimer’s disease; SNP, single-nucleotide polymorphism; SE, Standard error.

*aFourteen SNPs (rs1079866, rs11756454, rs1398217, rs1518080, rs17351680, rs1874984, rs2617056, rs2836950, rs3914188, rs4242496, rs4801589, rs9373571, rs9555810, rs9939609) being palindromic were removed, and 54 SNPs were included for MR analyses.

bProxy SNP reported where the targeted SNP was not available in the outcome datasets, and the effect allele and beta (SE) reported for proxy SNP.
Supplementary Table 3. Genome-wide significant SNPs (n = 42) for age at menopause (P < 1×10⁻⁸).

| SNP ID a | Proxy SNP b | rs² for proxy | Effect allele (alternative) | Beta (SE) for age at menopause | Beta (SE) for AD | Variance explained (R²) | F statistic |
|---------|-------------|---------------|-----------------------------|-------------------------------|-----------------|------------------------|------------|
| rs1046089 | - | - | A (G) | -0.220 (0.020) | -0.034 (0.017) | 0.0211 | 121 |
| rs1054875 | - | - | T (A) | -0.190 (0.020) | -0.009 (0.016) | 0.0170 | 90 |
| rs10852444 | - | - | T (C) | -0.160 (0.020) | 0.025 (0.017) | 0.0128 | 64 |
| rs1095065 | - | - | A (G) | -0.110 (0.020) | -0.005 (0.016) | 0.0058 | 30 |
| rs10957156 | - | - | A (G) | -0.140 (0.020) | -0.010 (0.018) | 0.0067 | 49 |
| rs11031006 | - | - | A (G) | 0.220 (0.030) | 0.000 (0.022) | 0.0095 | 54 |
| rs11668344 | - | - | G (A) | -0.410 (0.020) | 0.010 (0.017) | 0.0765 | 420 |
| rs11804189 | - | - | A (G) | 0.110 (0.020) | 0.015 (0.016) | 0.0059 | 30 |
| rs12196873 | - | - | C (A) | 0.160 (0.030) | -0.005 (0.023) | 0.0050 | 28 |
| rs1237165 | - | - | T (C) | 0.180 (0.030) | 0.038 (0.023) | 0.0091 | 36 |
| rs12599106 | - | - | A (T) | -0.120 (0.020) | -0.022 (0.022) | 0.0072 | 36 |
| rs12824058 | - | - | G (A) | -0.140 (0.020) | -0.006 (0.017) | 0.0095 | 49 |
| rs13040088 | - | - | G (A) | -0.160 (0.020) | 0.003 (0.020) | 0.0069 | 64 |
| rs1411478 | - | - | G (A) | 0.130 (0.020) | -0.039 (0.016) | 0.0082 | 42 |
| rs16858210 | - | - | A (G) | 0.140 (0.020) | -0.003 (0.018) | 0.0067 | 49 |
| rs16991615 | - | - | A (G) | 0.880 (0.040) | -0.013 (0.032) | 0.1140 | 484 |
| rs1713460 | - | - | G (A) | -0.140 (0.020) | -0.015 (0.017) | 0.0069 | 49 |
| rs1799949 | - | - | A (G) | 0.140 (0.020) | -0.010 (0.016) | 0.0089 | 49 |
| rs1800932 | - | - | G (A) | 0.170 (0.030) | -0.028 (0.022) | 0.0102 | 32 |
| rs2136918 | - | - | G (C) | 0.150 (0.020) | 0.009 (0.016) | 0.0111 | 56 |
| rs2215184 | - | - | A (G) | -0.140 (0.020) | 0.007 (0.016) | 0.0095 | 49 |
| rs2277339 | - | - | G (T) | -0.310 (0.030) | 0.054 (0.027) | 0.0188 | 107 |
| rs2720044 | - | - | C (A) | 0.290 (0.030) | -0.032 (0.022) | 0.0237 | 93 |
| rs2941505 | - | - | G (A) | 0.130 (0.020) | 0.019 (0.017) | 0.0070 | 42 |
| rs349206 | - | - | A (G) | 0.230 (0.040) | 0.055 (0.029) | 0.0112 | 33 |
| rs365132 | - | - | T (G) | 0.240 (0.020) | -0.024 (0.016) | 0.0122 | 144 |
| rs4246511 | - | - | C (T) | -0.220 (0.020) | -0.012 (0.019) | 0.0199 | 121 |
| rs427394 | - | - | G (A) | -0.130 (0.020) | -0.016 (0.016) | 0.0082 | 42 |
| rs4693089 | - | - | G (A) | 0.200 (0.020) | 0.015 (0.016) | 0.0199 | 100 |
| rs4879656 | - | - | A (C) | -0.120 (0.020) | 0.003 (0.016) | 0.0072 | 36 |
| rs4886238 | - | - | A (G) | 0.180 (0.020) | 0.004 (0.016) | 0.0151 | 81 |
| rs551087 | - | - | A (G) | 0.130 (0.020) | -0.003 (0.017) | 0.0054 | 42 |
| rs5762534 | - | - | C (T) | 0.160 (0.030) | -0.047 (0.022) | 0.0079 | 28 |
| rs6856693 | - | - | G (A) | 0.160 (0.020) | 0.007 (0.017) | 0.0127 | 64 |
| rs6899676 | - | - | G (A) | 0.230 (0.030) | -0.041 (0.020) | 0.0149 | 59 |
| rs704795 | - | - | A (G) | -0.160 (0.020) | 0.021 (0.016) | 0.0127 | 64 |
| rs7125555 | - | - | T (C) | -0.120 (0.020) | -0.023 (0.016) | 0.0072 | 36 |
| rs7259376 | - | - | G (A) | 0.110 (0.020) | 0.001 (0.016) | 0.0060 | 30 |
| rs763121 | - | - | G (A) | -0.160 (0.020) | -0.027 (0.016) | 0.0118 | 64 |
| rs8070740 | - | - | G (A) | 0.150 (0.020) | 0.026 (0.018) | 0.0054 | 56 |
| rs930036 | - | - | A (G) | -0.190 (0.020) | 0.001 (0.016) | 0.0170 | 90 |
| rs9796 | - | - | T (A) | -0.130 (0.020) | -0.002 (0.016) | 0.0079 | 42 |

Abbreviations: AD, Alzheimer’s disease; SNP, single-nucleotide polymorphism; SE, Standard error.

aFour SNPs (rs1054875, rs12599106, rs2236918, rs9796) being palindromic were removed, and 38 instrument SNPs were included for MR analyses.

bProxy SNP reported where the targeted SNP was not available in the outcome datasets, and the effect allele and beta (SE) reported for proxy SNP.
### Supplementary Table 4. Genome-wide significant SNPs (n = 17) for AD (P < 1×10^-8).

| SNP ID * | Proxy SNP b | r² for proxy | Effect allele (alternative) | AD (exposure)-age at menarche(outcome) | AD (exposure)-age at menopause | Variance explained (R²) | F statistic |
|----------|-------------|--------------|-----------------|--------------------------------------|-------------------------------|------------------------|------------|
| rs10792832 | - | - | G (A) | 0.130 (0.016) | 0.001 (0.007) | 0.130 (0.016) | 0.010 (0.020) | 0.0079 | 65 |
| rs10080026 | rs11767557 | 0.97 | C (T) | -0.129 (0.021) | 0.008 (0.008) | -0.139 (0.021) | 0.020 (0.030) | 0.0058 | 39 |
| rs1218343 | - | - | C (T) | -0.270 (0.041) | -0.014 (0.023) | -0.270 (0.041) | -0.070 (0.060) | 0.0060 | 43 |
| rs18170342 | - | - | C (T) | 0.871 (0.057) | - | 0.871 (0.057) | - | 0.0594 | 233 |
| rs12590654 | - | - | A (G) | -0.097 (0.018) | - | -0.097 (0.018) | - | 0.0042 | 30 |
| rs1752884 | rs1408077 | 0.9335 | A (C) | -0.143 (0.020) | 0.009 (0.007) | -0.154 (0.020) | 0.020 (0.030) | 0.0060 | 53 |
| rs346771 | - | - | C (T) | 0.303 (0.040) | - | 0.303 (0.040) | - | 0.0134 | 58 |
| rs41298512 | - | - | G (A) | 1.638 (0.059) | - | 1.638 (0.059) | - | 0.1602 | 760 |
| rs12490100 | - | - | T (C) | -0.570 (0.103) | - | -0.570 (0.103) | - | 0.0163 | 31 |
| rs14290120 | - | - | A (G) | -0.608 (0.050) | - | -0.608 (0.050) | - | 0.0214 | 146 |
| rs4147929 | - | - | G (A) | -0.135 (0.022) | - | -0.135 (0.022) | - | 0.0055 | 36 |
| rs4663105 | - | - | C (A) | 0.184 (0.017) | - | 0.184 (0.017) | - | 0.0163 | 114 |
| rs72924659 | - | - | T (C) | -0.141 (0.020) | - | -0.141 (0.020) | - | 0.0083 | 52 |
| rs7982 | rs1532278 | 0.98 | T (C) | 0.143 (0.017) | 0.009 (0.008) | 0.140 (0.017) | -0.040 (0.040) | 0.0097 | 75 |
| rs8093731 | - | - | T (C) | -0.614 (0.112) | - | -0.614 (0.112) | - | 0.0089 | 30 |
| rs9272561 | - | - | A (G) | -0.136 (0.023) | - | -0.136 (0.023) | - | 0.0091 | 35 |
| rs9381563 | - | - | T (C) | -0.097 (0.017) | 0.002 (0.006) | -0.097 (0.017) | -0.020 (0.020) | 0.0041 | 34 |

Abbreviations: AD, Alzheimer’s disease; SNP, single-nucleotide polymorphism; SE, Standard error.

*None of SNP was removed for being palindromic, but only 6 IVs were found in outcome (age at menarche/ menopause) datasets and included for MR analyses.

**Proxy SNP reported where the targeted SNP was not available in the outcome datasets, and the effect allele and beta (SE) reported for proxy SNP.

### Supplementary Table 5. GWAS linked traits of 54 instrument SNPs of age at menarche.

| SNP ID | Phenoscaner [12] | dbSNP genes | GWAS catalog [13] traits linked to this gene |
|--------|-------------------|-------------|---------------------------------------------|
| rs10144321 | Age at menarche | WDR25 | Age at menarche, height |
| rs10483727 | Height, arm fat-free mass right | NA | NA |
| rs10840031* | BMI | STK33 | BMI |
| rs10933897* | BMI, obesity | NA | NA |
| rs11022756* | BMI, coronary artery disease | NA | NA |
| rs11715566 | Relative age voice broke | LOC107986022 | NA |
| rs11767400* | Height | CADPS2 | BMI |
| rs12003641 | Height, Relative age voice broke | NA | NA |
| rs12148769 | Age at menarche | NA | NA |
| rs12291726 | Impedance of arm left | GAB2 | eGFR, AD, TG, TC |
| rs12598642* | BMI, trunk fat mass, self-reported diabetes | WWP2 | IgE levels, smoking behavior |
| rs12915845 | Creatinine in urine, relative age voice broke | NA | NA |
| rs13179411* | BMI, relative age voice broke | JADE2 | BMI, T2DM, mental health |
| rs13215865* | Age at menarche | JADE2 | BMI, T2DM, mental health |
| rs1482853 | Birth weight, WC | LINC02029 | NA |
| rs1516883* | BMI, WC adjusted for smoking | NA | NA |
| rs1659127* | Leg fat-free mass right | NA | NA |
| rs16938437* | Arm fat-free mass left, weight | PHF21A | BMI, educational attainment, smoking initiation |
| rs2153127 | Relative age voice broke | NA | NA |
| rs2179786 | Age at menarche | FAM83B | Wellbeing, sleep duration, colorectal cancer |
| rs2184968 | Arm predicted mass right, T2DM, neutrophil count | CENPW | Brain volume measurement, cortical surface area measurement |
| rs2303100 | Sleep duration | OLFM2 | Waist-hip ratio, sleep duration |
| rs2344508* | BMI, hip circumference | TNNI3K | BMI, obesity, smoking initiation |
| rs2687729 | Asthma | EEFSEC | prostate carcinoma |
| rs2947411* | BMI, WC, leg fat mass right | NA | NA |
| rs3115627 | Rheumatoid arthritis, MS, myeloid white cell count | LOC105375010 | NA |
| SNP ID    | Phenoscaner [12]             | dbSNP genes | GWAS catalog [13] traits linked to this gene                                      |
|-----------|------------------------------|-------------|----------------------------------------------------------------------------------|
| rs10792832| AD in APOE e4 carriers       | NA          | NA                                                                               |
| rs10808026| AD in APOE e5 carriers       | EPHA1       | AD, blood protein levels                                                        |
| rs11218343| AD in APOE e6 carriers       | SORL1       | AD, alcohol consumption, insomnia                                               |
| rs1752684 | AD in APOE e7 carriers       | CR1         | AD, inflammatory biomarkers                                                        |
| rs7982    | AD in APOE e8 carriers       | CLU         | AD, panic disorder, refractive error                                              |
| rs9381563 | Height, reticulocyte count   | NA          | NA                                                                               |

Abbreviations: GWAS, genome-wide association study; SNP, single-nucleotide polymorphism; AD, Alzheimer’s disease; T2DM, Type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; M5, multiple sclerosis; eGFR, glomerular filtration rate; AIDS, acquired immune deficiency syndrome.

*Indicates instrument SNP with potential pleiotropic and was removed in the final MR analyses.

Supplementary Table 6. GWAS linked traits of 6 instrument SNPs of AD.

Abbreviations: GWAS, genome-wide association study; AD, Alzheimer’s disease; SNP, single-nucleotide polymorphism.
Supplementary Table 7. GWAS linked traits of 38 instrument SNPs of age at menopause.

| SNP ID     | Phenoscanner [12]                                      | dbSNP genes | GWAS catalog [13] traits linked to this gene                  |
|------------|--------------------------------------------------------|-------------|---------------------------------------------------------------|
| rs1046089* | T1DM, white blood cell count, schizophrenia            | PRR2C2A     | BMI, WC, schizophrenia, smoking status                        |
| rs10852344 | Menopause age at onset                                  | NA          | NA                                                            |
| rs10905065 | Age at menopause                                       | TASS2       | Osteosarcoma, breast cancer, cutaneous malignant melanoma     |
| rs10957156*| Neutrophil percentage of granulocytes                  | CHD7        | Smoking initiation, MDD                                       |
| rs11031006*| Polycystic ovary syndrome                              | CHD7        | Smoking initiation, MDD                                       |
| rs11608344*| Ever used hormone-replacement therapy                  | CHD7        | Smoking initiation, MDD                                       |
| rs11804189*| Age at menopause                                       | CHD7        | Basophil percentage of white cells, PHF-tau measurement      |
| rs12196873*| Age at menopause                                       | MFS2D4B     | BMI, WC, schizophrenia, smoking status                        |
| rs12371165*| Age at menopause                                       | GRIP1       | Osteosarcoma, breast cancer, cutaneous malignant melanoma     |
| rs12824058 | Age at menopause                                       | NA          | NA                                                            |
| rs13040088 | Age at menopause                                       | DIDO1       | Fat-free mass, monocyte count                                 |
| rs1311478* | Ever used hormone-replacement therapy                  | STX6        | Creutzfeldt-Jakob disease, progressive supranuclear palsy     |
| rs16588210 | Pulse rate                                             | NA          | NA                                                            |
| rs16991615 | Age at menopause                                       | MCM8        | Uterine fibroids, breast cancer                               |
| rs1713460  | Age at menopause                                       | NA          | NA                                                            |
| rs1799949* | Age at menopause                                       | BRCA1       | Ovarian cancer, BMI                                           |
| rs1800932  | Ever smoked                                            | MSH6        | Basophil percentage of white cells, PHF-tau measurement      |
| rs2241584  | Age at menopause                                       | RNF44       | Venous thromboembolism                                       |
| rs2277339* | Platelet crit, height                                  | PRIM1/HSD17B6| Smoking initiation, Mean corpuscular volume, T2DM            |
| rs2720044  | Age at menopause                                       | ASH2L       | Menopause (age at onset)                                     |
| rs2941505* | Asthma, HDL, sum basophil neutrophil counts           | PGAP3       | Lifetime smoking index, TG, bipolar disorder                 |
| rs349306*  | Age at menopause                                       | ARID3A      | Vertical cup-disc ratio, systemic lupus erythematosus         |
| rs365132   | Leiomyoma of uterus                                    | UIMC1       | Educational attainment, WC adjusted BMI                       |
| rs4246511* | Age at menopause                                       | RHBDL2/LOC105378662| TG, MDD, alcohol dependence                                |
| rs427394   | Age at menopause                                       | TENT4A      | MS, Coronary artery disease                                   |
| rs4693089* | Age at menopause                                       | HELQ        | Age-related cognitive decline, oral cavity and pharyngeal cancer |
| rs4879656  | Age at menopause                                       | APTX        | Vitamin B12 levels, IgG glycosylation, amyotrophic lateral sclerosis |
| rs4886238  | Age at menopause                                       | TDRD3       | Metabolite levels                                             |
| rs511087*  | Age at menopause                                       | SPPL3       | T2DM, depression, cognitive performance, educational attainment |
| rs5762534  | Age at menopause                                       | TCC28       | Breast cancer, epithelial ovarian cancer, prostate cancer     |
| rs6856693  | Age at menopause                                       | ACSL1/LOC105377587| T2DM, fulminant T1DM                                       |
| rs6899676  | Age at menopause                                       | SYCP2L      | COPD                                                          |
| rs704795   | TG, serum urate, platelet count, TC                    | FNDC4       | Age at menopause                                             |
| rs7125555  | Age at menopause                                       | NA          | NA                                                            |
| rs7259376  | Age at menopause                                       | NA          | NA                                                            |
| rs765121   | TG, mean corpuscular volume                            | DD1X17/KDEL3| TPE interval, gallstone disease                               |
| rs8077040  | Age at menopause                                       | RPAIN       | Neutrophil count, WBC                                        |
| rs900306   | Basal metabolic rate                                   | TLK1        | Height, platelet count, self-reported math ability            |

Abbreviations: GWAS, genome-wide association study; SNP, single-nucleotide polymorphism; BMI, body mass index; WC, waist circumference; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; MS, multiple sclerosis; MDD, major depressive disorder; COPD, chronic obstructive pulmonary disease; WBC, white blood cell count.

*Indicates instrument SNP with potential pleiotropic and was removed in the final MR analyses.
Supplementary Table 8. The heterogeneity and sensitivity results of age at menarche/menopause and AD relevant traits.

| Exposure-outcome                  | No. of SNPs* | MR-PRESSO Global P value | MR Egger intercept Intercept value | Cochran's heterogeneity test IVW-Q value | IVW-P value | Egger-Q value | Egger-P value |
|-----------------------------------|--------------|--------------------------|-----------------------------------|-----------------------------------------|-------------|---------------|---------------|
| Age at menarche-cognitive performance | 41           | 0.081                    | 0.001                             | 0.588                                   | 53.595      | 0.074         | 53.189        | 0.064 |
| Age at menarche-BMI               | 31           | 0.306                    | 0.001                             | 0.801                                   | 33.567      | 0.299         | 33.491        | 0.258 |
| Age at menarche-smoking behavior  | 48           | 0.709                    | -0.004                            | 0.934                                   | 40.716      | 0.729         | 40.709        | 0.693 |
| Age at menarche-alcohol consumption | 50           | 0.451                    | 0.002                             | 0.224                                   | 48.867      | 0.478         | 47.347        | 0.499 |
| Age at menopause-cognitive performance | 29           | 0.155                    | 0.000                             | 0.751                                   | 35.817      | 0.147         | 35.681        | 0.122 |
| Age at menopause-BMI              | 36           | 0.401                    | -0.002                            | 0.273                                   | 36.693      | 0.390         | 35.403        | 0.402 |
| Age at menopause-smoking behavior  | 30           | 0.754                    | 0.027                             | 0.519                                   | 23.734      | 0.742         | 23.306        | 0.718 |
| Age at menopause-alcohol consumption | 31           | 0.988                    | 0.000                             | 0.784                                   | 15.607      | 0.986         | 15.530        | 0.980 |

Abbreviations: MR, mendelian randomization; AD, Alzheimer’s disease; IVW, inverse variance-weighted; BMI, body mass index; SNP, single-nucleotide polymorphism.
*Indicates model removal of potential pleiotropic instrument SNPs.