Research Paper

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

Mingli Li[1,2,3,4,5,*], Jiali Lin[1,2,3,4,5,*], Shuang Liang[1,2,3,4,5,*], Zefeng Chen[1,2,3,4,5], Yulan Bai[1,2,3,4,5], Xinyang Long[1,2,3,4,5], Shengzhu Huang[1,2,3,4,5], Zengnan Mo[1,2,3,4,6]

[1]Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning 530021, Guangxi, China
[2]Guangxi Key Laboratory for Genomic and Personalized Medicine, Nanning 530021, Guangxi, China
[3]Guangxi Collaborative Innovation Center for Genomic and Personalized Medicine, Nanning 530021, Guangxi, China
[4]Guangxi Key Laboratory of Colleges and Universities, Nanning 530021, Guangxi, China
[5]School of Public Health of Guangxi Medical University, Nanning 530021, Guangxi, China
[6]Institute of Urology and Nephrology, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi, China
*Equal contribution

Correspondence to: Zengnan Mo; **email:** mozengnan@gxmu.edu.cn
Keywords: age at menarche, age at menopause, Alzheimer's disease, mendelian randomization study
Received: December 27, 2020 **Accepted:** May 31, 2021 **Published:** August 4, 2021

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 IVWOR = 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).

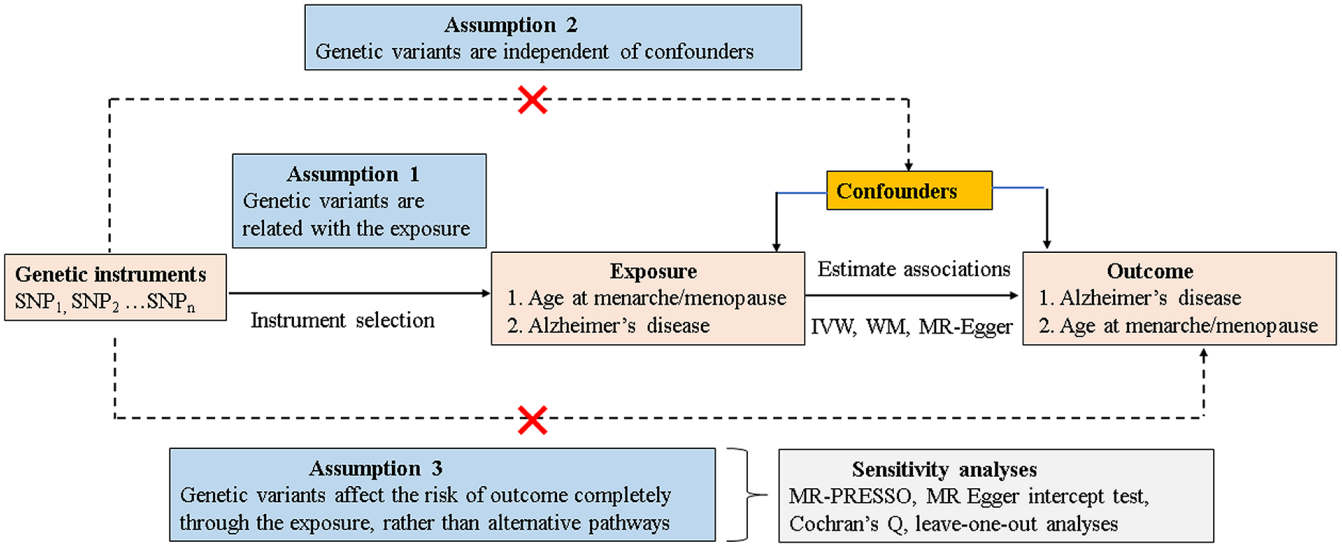


Figure 1. Schematic model of the MR study. SNP, single-nucleotide polymorphism; MR, mendelian randomization; IVW, inverse variance–weighted; WM, weighted median.

IV selection and validation

SNPs associated with exposure at genome-wide significance ($P < 5\times10^{-8}$) 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],

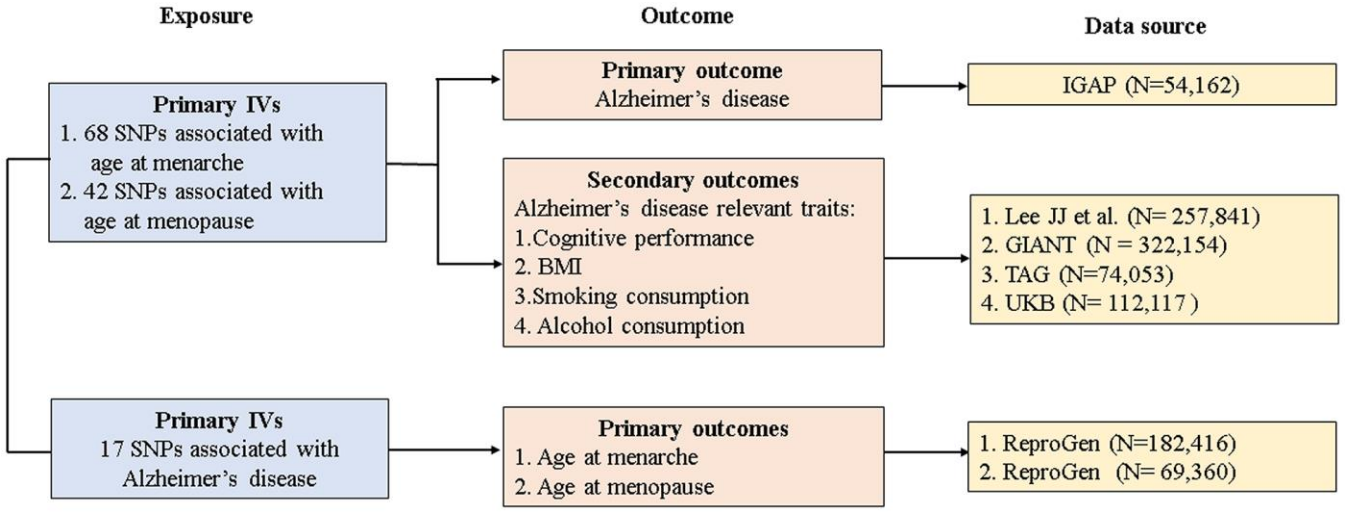


Figure 2. Study design and data sources. IGAP, International Genomics of Alzheimer's Project; ReproGen, Reproductive Genetics Consortium; GIANT, Genetic Investigation of Anthropometric Traits; TAG, Tobacco and Genetics Consortium; UKB, UK biobank IVs, instrument variables; SNP, single-nucleotide polymorphism; 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/cm^2) [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 (r^2 > 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; $Q_{MR\text{-}Egger}$ = 62.143, $P_{MR\text{-}Egger}$ = 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 (IVWβ = 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, IVWβ = -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 6, 7 and 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 β = -0.043; 95% CI, -0.077 to -0.009, P = 0.014; outlier-adjusted weighted median β = -0.048; 95% CI, -0.093 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)[a]	*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.

[a]Indicates odds ratio for AD per 1-SD increase in mean age at menarche/menopause.

[b]Indicates model removal of potential pleiotropic IVs.

[c]Indicates 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	54[a]	0.251	0.009	0.372	63.114	0.161	62.143	0.158
Age at menarche-AD	31[b]	0.824	-0.010	0.451	32.074	0.364	31.440	0.345
Age at menopause-AD	38[a]	0.052	0.008	0.300	54.193	0.034	52.581	0.037
Age at menopause-AD	23[b]	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.

[a]Indicates model removal of potential pleiotropic IVs.

[b]Indicates model without removal of potential pleiotropic IVs.

causation 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	B(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

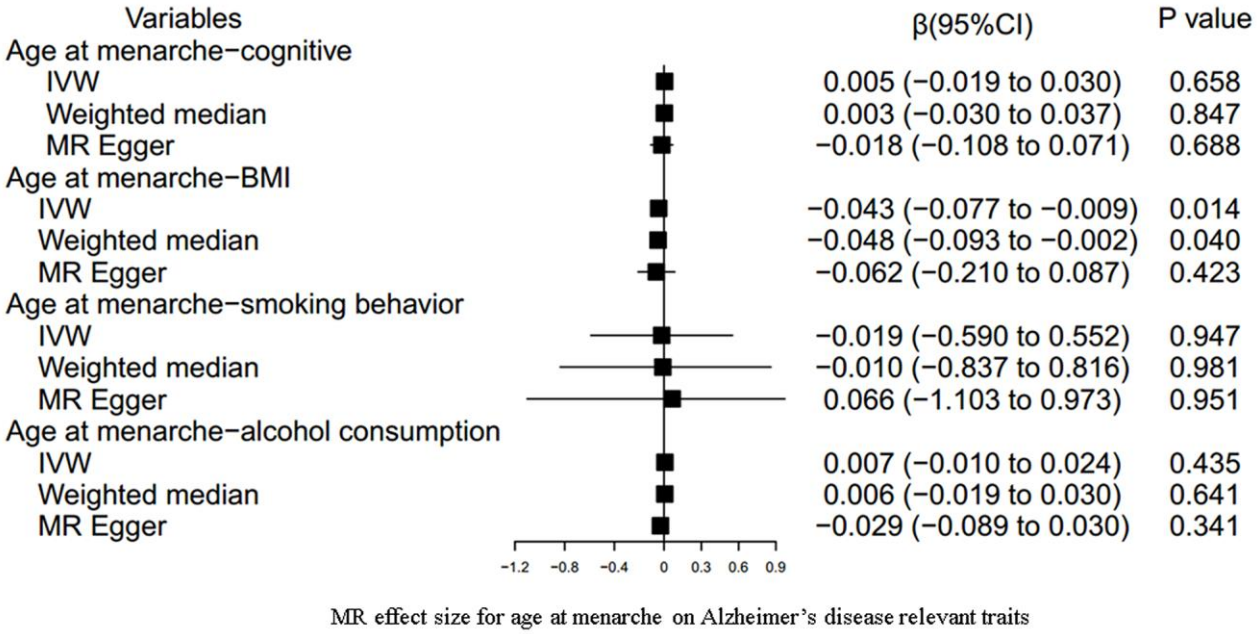


Figure 3. MR estimate plot for age at menarche on Alzheimer's disease relevant traits. IVW indicates inverse variance–weighted method.

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

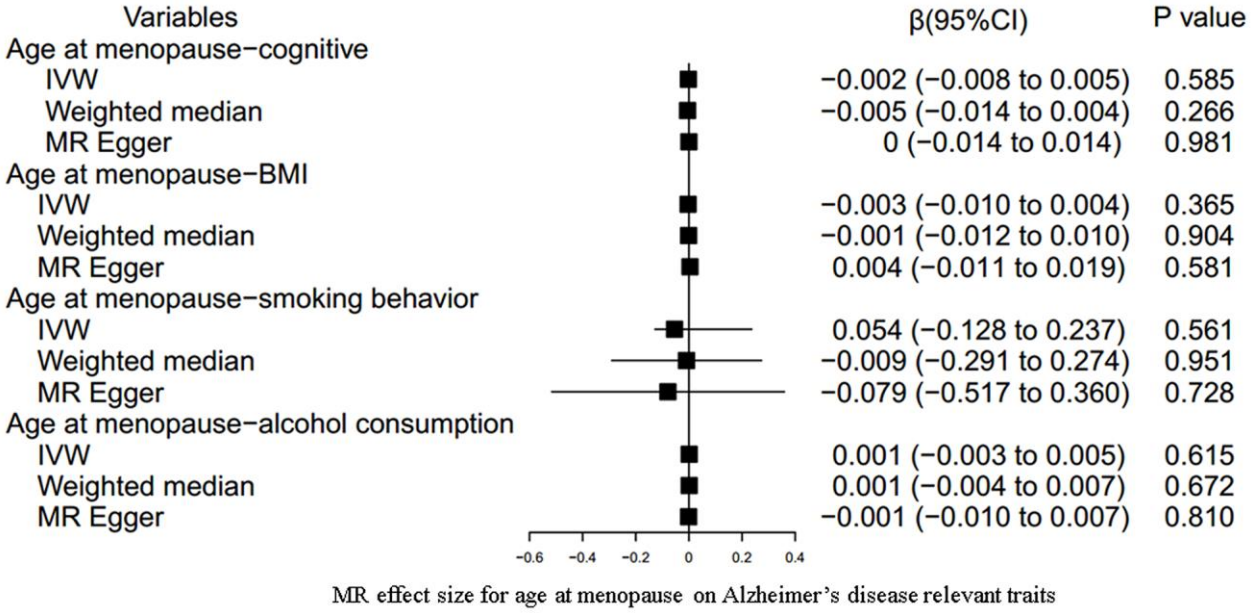


Figure 4. MR estimate plot for age at menopause on Alzheimer's disease relevant traits. IVW indicates inverse variance–weighted method.

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.

ACKNOWLEDGMENTS

We thank the following consortiums: the IGAP, the ReproGen, the GIANT, TAG, and UKB, as well as Lee JJ et al., for making their GWAS summary-level statistics publicly available.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING

This study was funded by National Key Research and Development Program of China (grant number 2017YFC0908000); the National Natural Science Foundation of China (grant number 81770759); the Major Project of Guangxi Innovation Driven (grant number AA18118016); Guangxi Key Laboratory for Genomic and Personalized Medicine (grant number 16-380-54, 17-259-45, 19-050-22, 19-185-33, 20-065-33).

REFERENCES

1. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 2013; 9:63–75.e2. https://doi.org/10.1016/j.jalz.2012.11.007 PMID:23305823

2. Chêne G, Beiser A, Au R, Preis SR, Wolf PA, Dufouil C, Seshadri S. Gender and incidence of dementia in the Framingham Heart Study from mid-adult life. Alzheimers Dement. 2015; 11:310–20. https://doi.org/10.1016/j.jalz.2013.10.005 PMID:24418058

3. Lee JH, Jiang Y, Han DH, Shin SK, Choi WH, Lee MJ. Targeting estrogen receptors for the treatment of Alzheimer's disease. Mol Neurobiol. 2014; 49:39–49. https://doi.org/10.1007/s12035-013-8484-9 PMID:23771838

4. Whitmer RA, Quesenberry CP, Zhou J, Yaffe K. Timing of hormone therapy and dementia: the critical window theory revisited. Ann Neurol. 2011; 69:163–69. https://doi.org/10.1002/ana.22239 PMID:21280086

5. Song YJ, Li SR, Li XW, Chen X, Wei ZX, Liu QS, Cheng Y. The Effect of Estrogen Replacement Therapy on Alzheimer's Disease and Parkinson's Disease in Postmenopausal Women: A Meta-Analysis. Front Neurosci. 2020; 14:157. https://doi.org/10.3389/fnins.2020.00157 PMID:32210745

6. Yoo JE, Shin DW, Han K, Kim D, Won HS, Lee J, Kim SY, Nam GE, Park HS. Female reproductive factors and the risk of dementia: a nationwide cohort study. Eur J Neurol. 2020; 27:1448–58. https://doi.org/10.1111/ene.14315 PMID:32396982

7. Geerlings MI, Ruitenberg A, Witteman JC, van Swieten JC, Hofman A, van Duijn CM, Breteler MM, Launer LJ.

Reproductive period and risk of dementia in postmenopausal women. JAMA. 2001; 285:1475–81. https://doi.org/10.1001/jama.285.11.1475 PMID:11255424

8. Prince MJ, Acosta D, Guerra M, Huang Y, Jimenez-Velazquez IZ, Llibre Rodriguez JJ, Salas A, Sosa AL, Chua KC, Dewey ME, Liu Z, Mayston R, Valhuerdi A. Reproductive period, endogenous estrogen exposure and dementia incidence among women in Latin America and China; A 10/66 population-based cohort study. PLoS One. 2018; 13:e0192889. https://doi.org/10.1371/journal.pone.0192889 PMID:29489847

9. Coppus AM, Evenhuis HM, Verberne GJ, Visser FE, Eikelenboom P, van Gool WA, Janssens AC, van Duijn CM. Early age at menopause is associated with increased risk of dementia and mortality in women with Down syndrome. J Alzheimers Dis. 2010; 19:545–50. https://doi.org/10.3233/JAD-2010-1247 PMID:20110600

10. Mosconi L, Rahman A, Diaz I, Wu X, Scheyer O, Hristov HW, Vallabhajosula S, Isaacson RS, de Leon MJ, Brinton RD. Increased Alzheimer's risk during the menopause transition: A 3-year longitudinal brain imaging study. PLoS One. 2018; 13:e0207885. https://doi.org/10.1371/journal.pone.0207885 PMID:30540774

11. Bove R, Secor E, Chibnik LB, Barnes LL, Schneider JA, Bennett DA, De Jager PL. Age at surgical menopause influences cognitive decline and Alzheimer pathology in older women. Neurology. 2014; 82:222–29. https://doi.org/10.1212/WNL.0000000000000033 PMID:24336141

12. Georgakis MK, Kalogirou EI, Diamantaras AA, Daskalopoulou SS, Munro CA, Lyketsos CG, Skalkidou A, Petridou ET. Age at menopause and duration of reproductive period in association with dementia and cognitive function: A systematic review and meta-analysis. Psychoneuroendocrinology. 2016; 73:224–43. https://doi.org/10.1016/j.psyneuen.2016.08.003 PMID:27543884

13. Evans DM, Davey Smith G. Mendelian Randomization: New Applications in the Coming Age of Hypothesis-Free Causality. Annu Rev Genomics Hum Genet. 2015; 16:327–50. https://doi.org/10.1146/annurev-genom-090314-050016 PMID:25939054

14. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol. 2003; 32:1–22. https://doi.org/10.1093/ije/dyg070 PMID:12689998

15. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol. 2016; 40:304–14. https://doi.org/10.1002/gepi.21965 PMID:27061298

16. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–75. https://doi.org/10.1086/519795 PMID:17701901

17. Palmer TM, Lawlor DA, Harbord RM, Sheehan NA, Tobias JH, Timpson NJ, Davey Smith G, Sterne JA. Using multiple genetic variants as instrumental variables for modifiable risk factors. Stat Methods Med Res. 2012; 21:223–42. https://doi.org/10.1177/0962280210394459 PMID:21216802

18. Perry JR, Day F, Elks CE, Sulem P, Thompson DJ, Ferreira T, He C, Chasman DI, Esko T, Thorleifsson G, Albrecht E, Ang WQ, Corre T, et al, and Australian Ovarian Cancer Study, and GENICA Network, and kConFab, and LifeLines Cohort Study, and InterAct Consortium, and Early Growth Genetics (EGG) Consortium. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. Nature. 2014; 514:92–97. https://doi.org/10.1038/nature13545 PMID:25231870

19. Day FR, Ruth KS, Thompson DJ, Lunetta KL, Pervjakova N, Chasman DI, Stolk L, Finucane HK, Sulem P, Bulik-Sullivan B, Esko T, Johnson AD, Elks CE, et al, and PRACTICAL consortium, and kConFab Investigators, and AOCS Investigators, and Generation Scotland, and EPIC-InterAct Consortium, and LifeLines Cohort Study. Large-scale genomic analyses link reproductive aging to hypothalamic signaling, breast cancer susceptibility and BRCA1-mediated DNA repair. Nat Genet. 2015; 47:1294–303. https://doi.org/10.1038/ng.3412 PMID:26414677

20. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B, Russo G, Thorton-Wells TA, Jones N, et al, and European Alzheimer's Disease Initiative (EADI), Genetic and Environmental Risk in Alzheimer's Disease, Alzheimer's Disease Genetic Consortium, and Cohorts for Heart and Aging Research in Genomic Epidemiology. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet. 2013; 45:1452–58. https://doi.org/10.1038/ng.2802 PMID:24162737

21. Lee JJ, Wedow R, Okbay A, Kong E, Maghzian O, Zacher M, Nguyen-Viet TA, Bowers P, Sidorenko J, Karlsson Linnér R, Fontana MA, Kundu T, Lee C, et al, and

23andMe Research Team, COGENT (Cognitive Genomics Consortium), and Social Science Genetic Association Consortium. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. Nat Genet. 2018; 50:1112–21. https://doi.org/10.1038/s41588-018-0147-3 PMID:30038396

22. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, Croteau-Chonka DC, Esko T, Fall T, et al, and LifeLines Cohort Study, and ADIPOGen Consortium, and AGEN-BMI Working Group, and CARDIOGRAMplusC4D Consortium, and CKDGen Consortium, and GLGC, and ICBP, and MAGIC Investigators, and MuTHER Consortium, and MIGen Consortium, and PAGE Consortium, and ReproGen Consortium, and GENIE Consortium, and International Endogene Consortium. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015; 518:197–206. https://doi.org/10.1038/nature14177 PMID:25673413

23. Tobacco and Genetics Consortium. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nat Genet. 2010; 42:441–47. https://doi.org/10.1038/ng.571 PMID:20418890

24. Clarke TK, Adams MJ, Davies G, Howard DM, Hall LS, Padmanabhan S, Murray AD, Smith BH, Campbell A, Hayward C, Porteous DJ, Deary IJ, McIntosh AM. Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N=112 117). Mol Psychiatry. 2017; 22:1376–84. https://doi.org/10.1038/mp.2017.153 PMID:28937693

25. Widén E, Silventoinen K, Sovio U, Ripatti S, Cousminer DL, Hartikainen AL, Laitinen J, Pouta A, Kaprio J, Järvelin MR, Peltonen L, Palotie A. Pubertal timing and growth influences cardiometabolic risk factors in adult males and females. Diabetes Care. 2012; 35:850–56. https://doi.org/10.2337/dc11-1365 PMID:22338106

26. Canoy D, Beral V, Balkwill A, Wright FL, Kroll ME, Reeves GK, Green J, Cairns BJ, and Million Women Study Collaborators*. Age at menarche and risks of coronary heart and other vascular diseases in a large UK cohort. Circulation. 2015; 131:237–44. https://doi.org/10.1161/CIRCULATIONAHA.114.010070 PMID:25512444

27. Day FR, Thompson DJ, Helgason H, Chasman DI, Finucane H, Sulem P, Ruth KS, Whalen S, Sarkar AK, Albrecht E, Altmaier E, Amini M, Barbieri CM, et al, and LifeLines Cohort Study, and InterAct Consortium, and kConFab/AOCS Investigators, and Endometrial Cancer Association Consortium, and Ovarian Cancer Association Consortium, and PRACTICAL consortium. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. Nat Genet. 2017; 49:834–41. https://doi.org/10.1038/ng.3841 PMID:28436984

28. Charalampopoulos D, McLoughlin A, Elks CE, Ong KK. Age at menarche and risks of all-cause and cardiovascular death: a systematic review and meta-analysis. Am J Epidemiol. 2014; 180:29–40. https://doi.org/10.1093/aje/kwu113 PMID:24920784

29. Najar J, Östling S, Waern M, Zettergren A, Kern S, Wetterberg H, Hällström T, Skoog I. Reproductive period and dementia: A 44-year longitudinal population study of Swedish women. Alzheimers Dement. 2020; 16:1153–63. https://doi.org/10.1002/alz.12118 PMID:32573980

30. Hong X, Zhang X, Li H. [A case-control study of endogenous estrogen and risk of Alzheimer's disease]. Zhonghua Liu Xing Bing Xue Za Zhi. 2001; 22:379–82. PMID:11769698

31. Gilsanz P, Lee C, Corrada MM, Kawas CH, Quesenberry CP Jr, Whitmer RA. Reproductive period and risk of dementia in a diverse cohort of health care members. Neurology. 2019; 92:e2005–14. https://doi.org/10.1212/WNL.0000000000007326 PMID:30923235

32. Davison KK, Susman EJ, Birch LL. Percent body fat at age 5 predicts earlier pubertal development among girls at age 9. Pediatrics. 2003; 111:815–21. https://doi.org/10.1542/peds.111.4.815 PMID:12671118

33. Beydoun MA, Beydoun HA, Wang Y. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. Obes Rev. 2008; 9:204–18. https://doi.org/10.1111/j.1467-789X.2008.00473.x PMID:18331422

34. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol. 2013; 37:658–65. https://doi.org/10.1002/gepi.21758 PMID:24114802

35. Bell JA, Carslake D, Wade KH, Richmond RC, Langdon RJ, Vincent EE, Holmes MV, Timpson NJ, Davey Smith G. Influence of puberty timing on adiposity and cardiometabolic traits: A Mendelian randomisation study. PLoS Med. 2018; 15:e1002641. https://doi.org/10.1371/journal.pmed.1002641 PMID:30153260

36. Gill D, Brewer CF, Del Greco MF, Sivakumaran P, Bowden J, Sheehan NA, Minelli C. Age at menarche and adult body mass index: a Mendelian randomization study. Int J Obes (Lond). 2018; 42:1574–81. https://doi.org/10.1038/s41366-018-0048-7 PMID:29549348

37. Ahmed ML, Ong KK, Dunger DB. Childhood obesity and the timing of puberty. Trends Endocrinol Metab. 2009; 20:237–42. https://doi.org/10.1016/j.tem.2009.02.004 PMID:19541497

38. Johnson W, Li L, Kuh D, Hardy R. How Has the Age-Related Process of Overweight or Obesity Development Changed over Time? Co-ordinated Analyses of Individual Participant Data from Five United Kingdom Birth Cohorts. PLoS Med. 2015; 12:e1001828. https://doi.org/10.1371/journal.pmed.1001828 PMID:25993005

39. Wade KH, Chiesa ST, Hughes AD, Chaturvedi N, Charakida M, Rapala A, Muthurangu V, Khan T, Finer N, Sattar N, Howe LD, Fraser A, Lawlor DA, et al. Assessing the causal role of body mass index on cardiovascular health in young adults: Mendelian randomization and recall-by-genotype analyses. Circulation. 2018; 138:2187–201. https://doi.org/10.1161/CIRCULATIONAHA.117.033278 PMID:30524135

40. Verhoef M, van den Eijnden RJ, Koning IM, Vollebergh WA. Age at menarche and adolescent alcohol use. J Youth Adolesc. 2014; 43:1333–45. https://doi.org/10.1007/s10964-013-0075-6 PMID:24327296

41. Chen Y, Liu Q, Li W, Deng X, Yang B, Huang X. Association of prenatal and childhood environment smoking exposure with puberty timing: a systematic review and meta-analysis. Environ Health Prev Med. 2018; 23:33. https://doi.org/10.1186/s12199-018-0722-3 PMID:30021511

42. Chou HT, Wu PY, Huang JC, Chen SC, Ho WY. Late Menarche, Not Reproductive Period, Is Associated with Poor Cognitive Function in Postmenopausal Women in Taiwan. Int J Environ Res Public Health. 2021; 18:2345. https://doi.org/10.3390/ijerph18052345 PMID:33673620

43. van Asselt KM, Kok HS, Pearson PL, Dubas JS, Peeters PH, Te Velde ER, van Noord PA. Heritability of menopausal age in mothers and daughters. Fertil Steril. 2004; 82:1348–51. https://doi.org/10.1016/j.fertnstert.2004.04.047 PMID:15533358

44. Murabito JM, Yang Q, Fox C, Wilson PW, Cupples LA. Heritability of age at natural menopause in the Framingham Heart Study. J Clin Endocrinol Metab. 2005; 90:3427–30. https://doi.org/10.1210/jc.2005-0181 PMID:15769979

45. van der Plaat DA, Pereira M, Pesce G, Potts JF, Amaral AF, Dharmage SC, Garcia-Aymerich JM, Thompson JR, Gómez Real F, Jarvis DL, Minelli C, Leynaert B, and ALEC Project. Age at menopause and lung function: a Mendelian randomisation study. Eur Respir J. 2019; 54:1802421. https://doi.org/10.1183/13993003.02421-2018 PMID:31439684

46. Hartge P. Genetics of reproductive lifespan. Nat Genet. 2009; 41:637–38. https://doi.org/10.1038/ng0609-637 PMID:19471299

47. Finch CE. The menopause and aging, a comparative perspective. J Steroid Biochem Mol Biol. 2014; 142:132–41. https://doi.org/10.1016/j.jsbmb.2013.03.010 PMID:23583565

48. Kuh D, Cooper R, Moore A, Richards M, Hardy R. Age at menopause and lifetime cognition: Findings from a British birth cohort study. Neurology. 2018; 90:e1673–81. https://doi.org/10.1212/WNL.0000000000005486 PMID:29643079

49. Song X, Wu J, Zhou Y, Feng L, Yuan JM, Pan A, Koh WP. Reproductive and hormonal factors and risk of cognitive impairment among Singapore Chinese women. Am J Obstet Gynecol. 2020; 223:410.e1–23. https://doi.org/10.1016/j.ajog.2020.02.032 PMID:32112728

50. Zhao L, Woody SK, Chhibber A. Estrogen receptor β in Alzheimer's disease: From mechanisms to therapeutics. Ageing Res Rev. 2015; 24:178–90. https://doi.org/10.1016/j.arr.2015.08.001 PMID:26307455

51. Genin E, Hannequin D, Wallon D, Sleegers K, Hiltunen M, Combarros O, Bullido MJ, Engelborghs S, De Deyn P, Berr C, Pasquier F, Dubois B, Tognoni G, et al. APOE and Alzheimer disease: a major gene with semi-dominant inheritance. Mol Psychiatry. 2011; 16:903–07. https://doi.org/10.1038/mp.2011.52 PMID:21556001

52. Choi JI, Han KD, Lee DW, Kim MJ, Shin YJ, Lee HN. Relationship between alcohol consumption and age at menopause: The Korea National Health and Nutrition Examination Survey. Taiwan J Obstet Gynecol. 2017; 56:482–86. https://doi.org/10.1016/j.tjog.2017.05.002 PMID:28805605

53. Zhu D, Chung HF, Pandeya N, Dobson AJ, Cade JE, Greenwood DC, Crawford SL, Avis NE, Gold EB, Mitchell ES, Woods NF, Anderson D, Brown DE, et al. Relationships between intensity, duration, cumulative dose, and timing of smoking with age at menopause: A pooled analysis of individual data from 17 observational studies. PLoS Med. 2018; 15:e1002704. https://doi.org/10.1371/journal.pmed.1002704 PMID:30481189

54. Ding X, Tang R, Zhu J, He M, Huang H, Lin Z, Zhu J. An Appraisal of the Role of Previously Reported Risk Factors in the Age at Menopause Using Mendelian Randomization. Front Genet. 2020; 11:507. https://doi.org/10.3389/fgene.2020.00507 PMID:32547598

55. Brinton RD, Chen S, Montoya M, Hsieh D, Minaya J. The estrogen replacement therapy of the Women's Health Initiative promotes the cellular mechanisms of memory and neuronal survival in neurons vulnerable to Alzheimer's disease. Maturitas. 2000 (Suppl 2); 34:S35–52. https://doi.org/10.1016/s0378-5122(00)00107-9 PMID:10915920

56. Wnuk A, Przepiórska K, Rzemieniec J, Pietrzak B, Kajta M. Selective Targeting of Non-nuclear Estrogen Receptors with PaPE-1 as a New Treatment Strategy for Alzheimer's Disease. Neurotox Res. 2020; 38:957–66. https://doi.org/10.1007/s12640-020-00289-8 PMID:33025361

57. Pompili A, Arnone B, Gasbarri A. Estrogens and memory in physiological and neuropathological conditions. Psychoneuroendocrinology. 2012; 37:1379–96. https://doi.org/10.1016/j.psyneuen.2012.01.007 PMID:22309827

58. Dratva J, Gómez Real F, Schindler C, Ackermann-Liebrich U, Gerbase MW, Probst-Hensch NM, Svanes C, Omenaas ER, Neukirch F, Wjst M, Morabia A, Jarvis D, Leynaert B, Zemp E. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. Menopause. 2009; 16:385–94. https://doi.org/10.1097/gme.0b013e31818aefef PMID:19034049

59. Brinton RD. Investigative models for determining hormone therapy-induced outcomes in brain: evidence in support of a healthy cell bias of estrogen action. Ann N Y Acad Sci. 2005; 1052:57–74. https://doi.org/10.1196/annals.1347.005 PMID:16024751

60. Shao H, Breitner JC, Whitmer RA, Wang J, Hayden K, Wengreen H, Corcoran C, Tschanz J, Norton M, Munger R, Welsh-Bohmer K, Zandi PP, and Cache County Investigators. Hormone therapy and Alzheimer disease dementia: new findings from the Cache County Study. Neurology. 2012; 79:1846–52. https://doi.org/10.1212/WNL.0b013e318271f823 PMID:23100399

SUPPLEMENTARY MATERIALS

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.

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

where β is the effect size (beta coefficient) for each SNP; MAF is the minimum allele frequency; SE(β) 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].

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

where β is the effect size (beta coefficient) for each SNP; SE(β) 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) neuro-psychological 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/cm^2) 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 ($P < 5\times10^{-8}$), 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 α 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.

Supplementary References

1. Shim H, Chasman DI, Smith JD, Mora S, Ridker PM, Nickerson DA, Krauss RM, Stephens M. A multivariate genome-wide association analysis of 10 LDL subfractions, and their response to statin treatment, in 1868 Caucasians. PLoS One. 2015; 10:e0120758. https://doi.org/10.1371/journal.pone.0120758 PMID:25898129

2. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. Int J Epidemiol. 2011; 40:740–52. https://doi.org/10.1093/ije/dyq151 PMID:20813862

3. Perry JR, Day F, Elks CE, Sulem P, Thompson DJ, Ferreira T, He C, Chasman DI, Esko T, Thorleifsson G, Albrecht E, Ang WQ, Corre T, et al, Australian Ovarian Cancer Study, GENICA Network, kConFab, LifeLines Cohort Study, InterAct Consortium, and Early Growth Genetics (EGG) Consortium. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. Nature. 2014; 514:92–97. https://doi.org/10.1038/nature13545 PMID:25231870

4. Day FR, Ruth KS, Thompson DJ, Lunetta KL, Pervjakova N, Chasman DI, Stolk L, Finucane HK, Sulem P, Bulik-Sullivan B, Esko T, Johnson AD, Elks CE, et al, PRACTICAL consortium, kConFab Investigators, AOCS Investigators, Generation Scotland, EPIC-InterAct Consortium, and LifeLines Cohort Study. Large-scale genomic analyses link reproductive aging to hypothalamic signaling, breast cancer susceptibility and BRCA1-mediated DNA repair. Nat Genet. 2015; 47:1294–303. https://doi.org/10.1038/ng.3412 PMID:26414677

5. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B, Russo G, Thorton-Wells TA, Jones N, et al, European Alzheimer's Disease Initiative (EADI), Genetic and Environmental Risk in Alzheimer's Disease, Alzheimer's Disease Genetic Consortium, and Cohorts for Heart and Aging Research in Genomic Epidemiology. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet. 2013; 45:1452–58. https://doi.org/10.1038/ng.2802 PMID:24162737

6. Lee JJ, Wedow R, Okbay A, Kong E, Maghzian O, Zacher M, Nguyen-Viet TA, Bowers P, Sidorenko J, Karlsson Linnér R, Fontana MA, Kundu T, Lee C, et al, 23andMe Research Team, COGENT (Cognitive Genomics Consortium), and Social Science Genetic Association Consortium. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. Nat Genet. 2018; 50:1112–21. https://doi.org/10.1038/s41588-018-0147-3 PMID:30038396

7. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015; 518:197–206. https://doi.org/10.1038/nature14177 PMID:25673413

8. Tobacco and Genetics Consortium. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nat Genet. 2010; 42:441–47. https://doi.org/10.1038/ng.571 PMID:20418890

9. Clarke TK, Adams MJ, Davies G, Howard DM, Hall LS, Padmanabhan S, Murray AD, Smith BH, Campbell A, Hayward C, Porteous DJ, Deary IJ, McIntosh AM. Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N=112 117). Mol Psychiatry. 2017; 22:1376–84. https://doi.org/10.1038/mp.2017.153 PMID:28937693

10. Gilsanz P, Lee C, Corrada MM, Kawas CH, Quesenberry CP Jr, Whitmer RA. Reproductive period and risk of dementia in a diverse cohort of health care members. Neurology. 2019; 92:e2005–14. https://doi.org/10.1212/WNL.0000000000007326 PMID:30923235

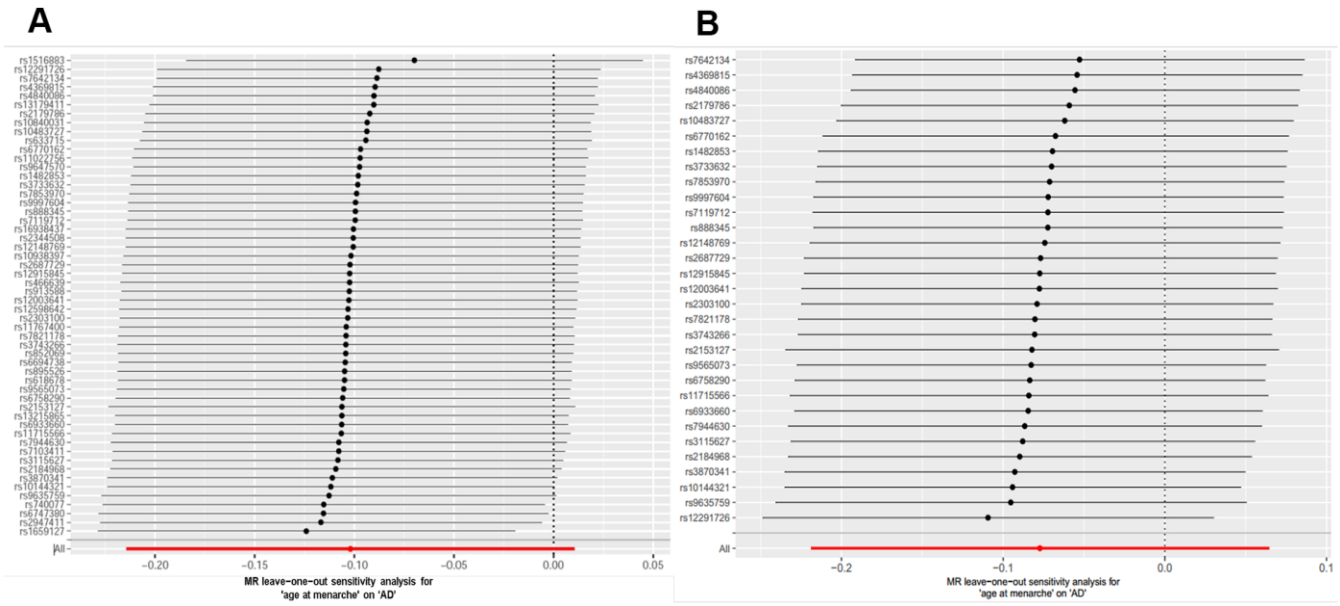
11. Najar J, Östling S, Waern M, Zettergren A, Kern S, Wetterberg H, Hällström T, Skoog I. Reproductive period and dementia: A 44-year longitudinal population study of Swedish women. Alzheimers Dement. 2020; 16:1153–63. https://doi.org/10.1002/alz.12118 PMID:32573980

12. Kamat MA, Blackshaw JA, Young R, Surendran P, Burgess S, Danesh J, Butterworth AS, Staley JR.

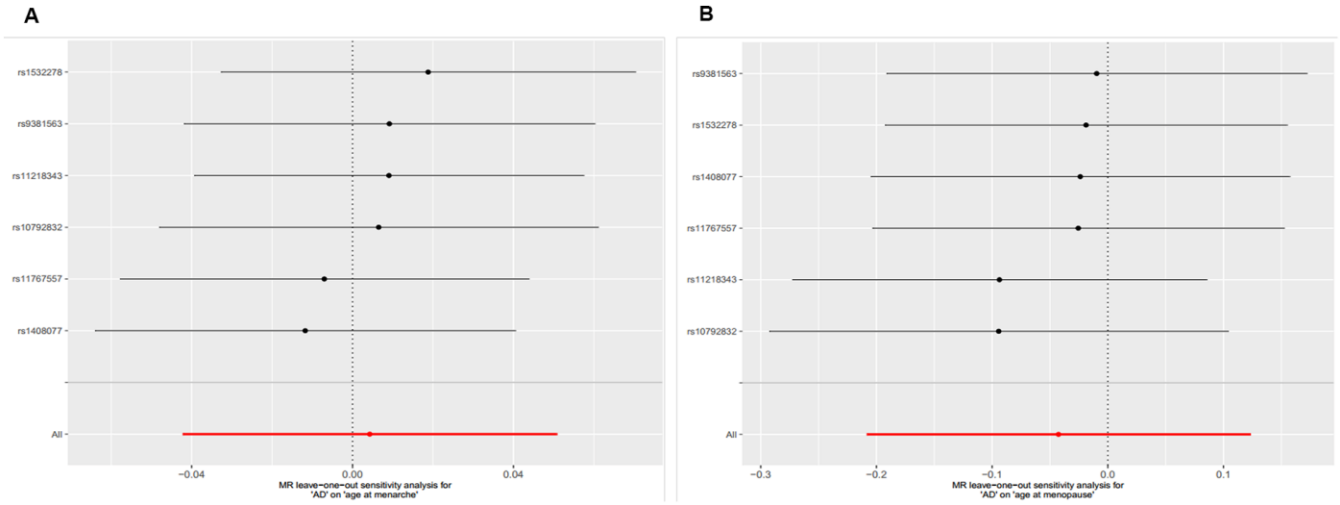
PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations. Bioinformatics. 2019; 35:4851–53. https://doi.org/10.1093/bioinformatics/btz469 PMID:31233103

13. Buniello A, MacArthur JA, Cerezo M, Harris LW, Hayhurst J, Malangone C, McMahon A, Morales J, Mountjoy E, Sollis E, Suveges D, Vrousgou O, Whetzel PL, et al. The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res. 2019; 47:D1005–12. https://doi.org/10.1093/nar/gky1120 PMID:30445434

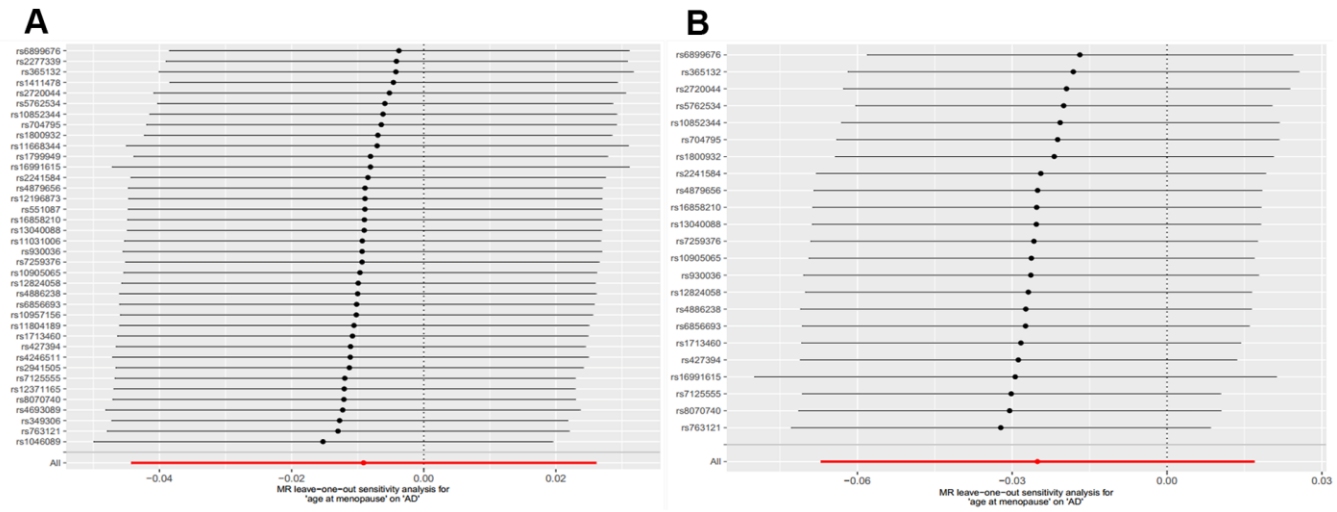
Supplementary Figures



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 α=0.05).

Age at menarche/menopause-AD (binary)						
Exposure-outcome	Actual N (outcome-GWAS)	Ratio of cases to controls (outcome-GWAS)	Observational HR	$R^2$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 β*	$R^2$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. *β equals to ln (HR).

Supplementary Table 2. Genome-wide significant SNPs (n = 68) for age at menarche ($P < 1\times10^{-8}$).

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

rs2836950	-	-	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
rs4801589	-	-	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.

[a]Fourteen 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.

[b]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 3. Genome-wide significant SNPs (n = 42) for age at menopause ($P < 1\times10^{-8}$).

SNP ID [a]	Proxy SNP [b]	r^2 for proxy	Effect allele (alternative)	Beta (SE) for age at menopause	Beta (SE) for AD	Variance explained (R^2)	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
rs10852344	-	-	T (C)	-0.160 (0.020)	0.025 (0.017)	0.0128	64
rs10905065	-	-	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
rs12371165	-	-	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
rs2236918	-	-	G (C)	0.150 (0.020)	0.009 (0.016)	0.0111	56
rs2241584	-	-	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
rs349306	-	-	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)	0.028 (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.

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

[b]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 4. Genome-wide significant SNPs (n = 17) for AD ($P < 1\times10^{-8}$).

SNP ID [a]	Proxy SNP [b]	r^2 for proxy	Effect allele (alternative)	AD (exposure)-age at menarche(outcome)		AD (exposure)-age at menopause (outcome)		Variance explained (R^2)	F statistic
				Beta (SE) for AD	Beta (SE) for age at menarche	Beta (SE) for AD	Beta (SE) for age at menopause		
rs10792832	-	-	G (A)	0.130 (0.016)	0.001 (0.007)	0.130 (0.016)	0.010 (0.020)	0.0079	65
rs10808026	rs11767557	0.97	C (T)	-0.129 (0.021)	0.008 (0.008)	-0.139 (0.021)	0.020 (0.030)	0.0058	39
rs11218343	-	-	C (T)	-0.270 (0.041)	-0.014 (0.023)	-0.270 (0.041)	-0.070 (0.060)	0.0060	43
rs118170342	-	-	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
rs1752684	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
rs41289512	-	-	G (C)	1.638 (0.059)	-	1.638 (0.059)	-	0.1602	760
rs41290100	-	-	T (C)	-0.570 (0.103)	-	-0.570 (0.103)	-	0.0163	31
rs41290120	-	-	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.

[a]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.

[b]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	Phenoscanner [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
rs10938397*	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

rs3733632	Impedance of arm left, height	TACR3	Heel bone mineral density, adolescent idiopathic scoliosis
rs3743266	Relative age voice broke, height	RORA/RORA-AS1	NA
rs3870341	Impedance of whole body	NA	NA
rs4369815	Age at menarche	NA	NA
rs466639*	Age at menarche	RXRG	Bipolar disorder, BMI-adjusted WC, AIDS
rs4840086	Age at menarche	NA	NA
rs618678*	Years of educational attainment, maternal smoking around birth	KDM4A	Educational attainment, squamous cell lung carcinoma, schizophrenia, smoking status
rs633715*	BMI, WC	NA	NA
rs6694738*	Age at menarche	AKT3	BMI, schizophrenia, educational attainment
rs6747380*	Age at menarche	CCDC85A	BMI, colorectal adenoma, self-reported educational attainment
rs6758290	Age at menarche	NA	NA
rs6770162	Age at menarche	NA	NA
rs6933660	Self-reported endometriosis	NA	NA
rs7103411*	BMI, WC	BDNF/BDNF-AS	BMI, coronary artery disease, smoking behavior
rs7119712	Acute sinusitis	TRPC6	Colorectal cancer or advanced adenoma, sleep duration, lung adenocarcinoma
rs740077*	Impedance of whole body, weight	KDM3B	Bipolar disorder, autism spectrum disorder or schizophrenia, sleep duration
rs7642134	Age at menarche	NA	NA
rs7821178	NA	NA	NA
rs7853970	Impedance of leg right	NA	NA
rs7944630	Relative age voice broke, height	NA	NA
rs852069*	BMI	LOC105372544	NA
rs888345	Age at menarche, height	KCNK9	parental longevity
rs895526*	Schizophrenia	SATB2	Intelligence, schizophrenia, educational attainment, general cognitive ability
rs913588*	Schizophrenia, relative age voice broke	KDM4C	Bipolar disorder and schizophrenia, BMI, educational attainment
rs9565073	Age at menarche	KLF12	Total PHF-tau, QRS duration, heel bone mineral density
rs9635759	Age at menarche	NA	NA
rs9647570*	Age at menarche	TENM2/LOC105377709	Smoking status, educational attainment, alcohol consumption, BMI, depression
rs9997604	Age at menarche	NA	NA

Abbreviations: GWAS, genome-wide association study; SNP, single-nucleotide polymorphism; BMI, body mass index; WC, waist circumference; AD, Alzheimer's disease; T2DM, Type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; MS, 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.

SNP ID	Phenoscanner [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; 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	PRRC2A	BMI, WC, schizophrenia, smoking status
rs10852344	Menopause age at onset	NA	NA
rs10905065	Age at menopause	TASOR2	Osteosarcoma, breast cance, cutaneous malignant melanoma
rs10957156*	Neutrophil percentage of granulocytes	CHD7	Smoking initiation, MDD
rs11031006*	Polycystic ovary syndrome	CHD7	Smoking initiation, MDD
rs11668344*	Ever used hormone-replacement therapy	CHD7	Smoking initiation, MDD
rs11804189*	Age at menopause	CHD7	Smoking initiation, MDD
rs12196873*	Age at menopause	MFSD4B	Smoking initiation, T2DM
rs12371165*	Age at menopause	GRIP1	Basophil percentage of white cells, PHF-tau measurement
rs12824058	Age at menopause	NA	NA
rs13040088	Age at menopause	DIDO1	Fat-free mass, monocyte count
rs1411478*	Ever used hormone-replacement therapy	STX6	Creutzfeldt-Jakob disease, progressive supranuclear palsy
rs16858210	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	Heel bone mineral density, tea consumption
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
rs551087*	Age at menopause	SPPL3	T2DM, depression, cognitive performance, educational attainment
rs5762534	Age at menopause	TTC28	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
rs763121	TG, mean corpuscular volume	DDX17/KDELR3	TPE interval, gallstone disease
rs8070740	Age at menopause	RPAIN	Neutrophil count, WBC
rs930036	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	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-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.