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Evaluating the causal effect of tobacco smoking on white matter brain aging: a two-sample Mendelian randomization analysis in UK Biobank

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Abstract

Background and Aims: Tobacco smoking is a risk factor for impaired brain function, but its causal effect on white matter brain aging remains unclear. This study aimed to measure the causal effect of tobacco smoking on white matter brain aging.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

None.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Design: Mendelian randomization (MR) analysis using two non-overlapping data sets (with and without neuroimaging data) from UK Biobank (UKB). The group exposed to smoking and control group consisted of current smokers and never smokers, respectively. Our main method was generalized weighted linear regression with other methods also included as sensitivity analysis.

Setting: United Kingdom.

Participants: The study cohort included 23 624 subjects [10 665 males and 12 959 females with a mean age of 54.18 years, 95% confidence interval (CI) = 54.08, 54.28].

Measurements: Genetic variants were selected as instrumental variables under the MR analysis assumptions: (1) associated with the exposure; (2) influenced outcome only via exposure; and (3) not associated with confounders. The exposure smoking status (current versus never smokers) was measured by questionnaires at the initial visit (2006–10). The other exposure, cigarettes per day (CPD), measured the average number of cigarettes smoked per day for current tobacco users over the life-time. The outcome was the 'brain age gap' (BAG), the difference between predicted brain age and chronological age, computed by training machine learning model on a non-overlapping set of never smokers.

Findings: The estimated BAG had a mean of 0.10 (95% CI = 0.06, 0.14) years. The MR analysis showed evidence of positive causal effect of smoking behaviors on BAG: the effect of smoking is 0.21 (in years, 95% CI = 6.5×10^{-3}, 0.41; *P*-value = 0.04), and the effect of CPD is 0.16 year/cigarette (UKB: 95% CI = 0.06, 0.26; *P*-value = 1.3×10^{-3}; GSCAN: 95% CI = 0.02, 0.31; *P*-value = 0.03). The sensitivity analyses showed consistent results.

Conclusions: There appears to be a significant causal effect of smoking on the brain age gap, which suggests that smoking prevention can be an effective intervention for accelerated brain aging and the age-related decline in cognitive function.

Keywords

Brain aging; causal inference; cigarette per day; Mendelian randomization; smoking behaviors; smoking status; white matter fractional anisotropy

INTRODUCTION

Smoking behaviors are among the most studied modifiable risk factors related to accelerated brain atrophy and cognitive impairment [1–4]. This discovery has led to prosperous studies of the adverse impact of smoking behaviors on neuroimaging [1, 3, 5–8]. Substantial research has found that chronic smoking strongly impacts neurocognition and brain neurobiology [9–14]. For example, previous studies have reported that accelerated brain aging is associated with the production of oxidative stress and alteration of synaptic protein expressions from smoking behaviors [15, 16]. Also, the atherosclerotic processes of smoking had potentially harmful effects on brain aging [11, 17]. The microstructure of the human brain is constantly changing over the life-span with normal aging, reflecting brain shrinks, memory decline and increases in the development of severe chronic diseases such as vasculature diseases [18]. Therefore, it is critical to recognize the causal effect of smoking on brain aging and thus quantitatively understand the potential benefits of smoking intervention in reducing the acceleration of brain aging.

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'Brain age' is a metric for brain aging status related to cognitive functions [19–21], calculated based on structural or functional neuroimaging data using machine learning (ML) algorithms [22–24]. In the current research, we adopt a commonly used chronological age-adjusted brain age metric, brain age gap (BAG), as the outcome variable that quantifies the difference between brain and chronological age [22–25]. BAG is a scalar metric that avoids the challenges of multiplicity, and dependence adjustment as in multivariate imaging models thus often yields more robust and interpretable results [22, 23, 25]. The recent literature showed that applying advanced ML methods to neuroimaging data can provide a reliable estimate for BAG with small predictive biases [25–34].

Various factors can influence BAG, including genetic, environmental, biological and behavioral factors [19, 35, 36]. Among these, smoking behavior is a well-studied factor that was adversely related to accelerated brain age in many previous studies [1, 37], and its causal effect on neuroimaging features was jointly examined with other risk factors, including diastolic blood pressure, systolic blood pressure, pulse pressure, low-density lipoprotein, high-density lipoprotein, triglycerides, total cholesterol, body mass index, Type 2 diabetes and alcoholic drinks consumed per week [38]. However, it is challenging to establish the causal relationship solely between smoking behavior and BAG in traditional observational studies because smoking often co-occurs with other health risk factors also linking to abnormal brain aging, including alcohol use, mental disorders (e.g. schizophrenia) and chronic diseases (e.g. hypertension, dyslipidemia and cardiovascular disease) [1, 39–42]. To address this challenge, we estimated the causal effect of smoking by Mendelian randomization (MR) analysis. Several addiction and neuropsychiatric research studies have employed MR analysis to investigate the causal effects of smoking behaviors on stroke, amyotrophic lateral sclerosis and psychiatric and behavioral disorders [14, 43–46].

The current study aimed to investigate the causal effects of smoking behaviors [i.e. smoking status (SS) and cigarette per day (CPD)] on BAG using the UK Biobank (UKB) cohort. We hypothesized that smoking behaviors, such as being a smoker or smoking a larger number of cigarettes per day, would significantly cause impairment in brain microstructures reflected by an increase in BAG among the participants in UKB. The estimated causal effect can reveal the overall effect of smoking and its biological consequences (e.g. cardiovascular condition worsening) on brain aging and thus unfolds the potential health benefits of the intervention and prevention for tobacco smoking.

METHODS

UKB cohort and variables

Our data were extracted from the UKB, a large-scale biomedical database consisting of phenotype and genotype, and imaging details of approximately 500 000 participants aged 40–69 years [47]. The initial measurement was conducted in 2006–10, and the repeat of baseline assessment started in 2013 [48]. We used the initial baseline measurement of smoking behavioral data in 2006–10 when conducting our current research to avoid poor data quality due to common loss to follow-up problem [48]. The neuroimaging data we used were collected since 2014 [48]. We performed quality control and restricted our analyses to participants with European ethnic backgrounds who had data available. To generate an

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accurate BAG estimation for normal brain aging, we excluded participants with health conditions (e.g. stroke, brain injury, brain cancer and psychiatric illnesses) to reduce the distortion of the fractional anisotropy (FA) measurements due to these diseases [49]. The study cohort included 23 624 subjects with neuroimaging data [10 665 males and 12 959 females with a mean age of 54.18 years, 95% confidence interval (CI) = 54.08, 54.28]. Supporting information, Fig. S1 shows a flow diagram of the number of participants for different analytical step. In each step we included participants with complete data, and thus no missing data presented in the analysis. As the primary research question and analysis plan were not pre-registered on a publicly available platform, the results of this study are considered exploratory.

Smoking behavior

This study focused up on two smoking behaviors (phenotypes): SS and CPD, following the commonly used definitions in the literature [50, 51]. SS was a binary variable classifying participants as current smokers and non-smokers who had never smoked according to the UKB data field 2016 (SS). The CPD was a quantitative variable representing the average number of cigarettes smoked per day for tobacco users, characterizing the level of nicotine craving in current smokers. We defined the CPD (ranges from 0 to 60) of current smokers based on three UKB data fields (IDs: 2887, 3456 and 6183) following the commonly used procedure in smoking genome-wide association studies (GWAS) [50, 51]. For those subjects who smoked fewer than one CPD, the CPD values were rounded down and recoded to 0; for those who smoked more than 60 CPD, the CPD values were truncated recoded to 60.

Neuroimaging data

We used diffusion magnetic resonance imaging (dMRI) data for the 23 624 participants for whom genotype and SS/CPD data were also available (see Supporting information, Fig. S1). The UKB acquired and processed these imaging data following its imaging protocol and pipeline [52]. White matter microstructure integrity was measured by FA derived based on the pre-processed dMRI, and then the per-tract mean values of 40 FA tracts were calculated [52]. The locations and names of the 40 FA tracts are provided in Supporting information, Data S1.

Genotype data

We used genotype data from the UKB cohort sequenced through two genotyping chip types, Affymetrix UK BiLEVE Axiom and UKBB Axiom® arrays. The data involved more than 90 million single-nucleotide variants (SNVs) among all participants [53]. More details regarding the quality control of genotype data are available in Supporting information, Appendix S1.

Analysis overview

Our analysis consisted of two steps (see Figure 1). The first step was to establish the function to compute the outcome variable, adjusted BAG, based on FA data and chronological age using machine learning techniques in a training set with only non-smokers (Figure 1a). In this step, we ensured the accuracy of the estimation and locked the optimal

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predictive model through an internal fivefold cross-validation (CV) within the training set. This predictive model was eventually applied to calculate the adjusted BAG of participants in another non-overlapping set consisting of both non-smokers and smokers (i.e. testing set) (see Figure 1b). In the second step, we conducted a two-sample MR analysis to investigate the causal effects of smoking behaviors on adjusted BAG [54]. The first sample consisted of UKB participants (n = 185 972) with only smoking behavioral data (exposure) but no brain imaging data (outcome: adjusted BAG). The second sample included a non-overlapping set of UKB participants (n = 12 907) who had neuroimaging data and the outcome data for adjusted BAG.

Adjusted BAG

The goal of the ML model was to compute a scalar brain age metric based on 40 FA measures. The UKB non-smokers with neuroimaging data were randomly split into training and testing sets by a 1:1 ratio, as shown in Supporting information, Fig. S1. The training set included 10 717 non-smokers for building an unbiased brain age estimating function for the general population [28]. The testing set consisted of 10 746 non-smokers and 2161 smokers serving as one of the samples used in the two-sample MR analysis. Among the smokers in the testing set, 1597 of them had CPD data available. Descriptive statistics were provided for training and testing sets separately, including mean and standard error for continuous variables and frequency and percentage for categorical variables.

Within the training set, we implemented ML analysis through an internal fivefold CV to achieve the optimal predictive performance using random forest (RF) regression [55, 56]. Based on the performance of the fivefold CV, we tuned the parameters of RF and then determined the set of FA measures via recursive feature selection according to their variable importance (i.e. Gini importance) based on accuracy criteria, including the coefficients of correlation (R) and mean absolute error [MAE (year)] [57, 58].

Given the optimal predictive model, we estimated the predicted brain age ($\widehat{B}$) and obtained BAG by subtracting chronological age (Y) by the predicted brain age ($\Delta G = Y - \widehat{B}$). This estimated BAG can be systematically biased because the prediction tends to overestimate brain age at low chronological age and underestimate brain age at high chronological age (see Supporting information, Fig. S6) [33, 34, 37]. Therefore, we regressed the BAG on chronological age to adjust the bias following a common bias adjustment procedure [33, 59–61]. We then obtained the adjusted predictive brain age ($\widehat{B}_{adj}$) and the adjusted BAG ($\Delta G_{adj} = Y - \widehat{B}_{adj}$). A positive adjusted BAG implies accelerated brain aging, while a negative adjusted BAG implies decelerated brain aging [19]. We further inspected the ΔG_{adj} data between current smokers (SMK) and non-smokers (non-SMK), summarizing results of distribution comparison and effect size of group differences (i.e. Cohen's d) in box-plot and forest plot. Hereafter, we conducted the rest of the analyses to evaluate the potential causal effects of smoking phenotypes (i.e. SS and CPD) on ΔG_{adj} in this study. In addition, we explored the association between the adjusted BAG and cognitive function measured from our previous study [62] at the 0.05 significance level. The cognitive function was represented by the intelligence g-factor estimated via factor analysis based on cognitive

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traits related to four domains in the UKB cohort: processing speed, perceptual reasoning, executive function and fluid intelligence [62].

Estimating causal effect by MR analysis

We evaluated the causal effects of smoking phenotypes (i.e. SS and CPD) on ΔG_{adj} by performing a two-sample MR analysis [63], and a *P*-value of 0.05 indicates a significant causal relationship. To implement the analysis (see details in Supporting information, Fig. S1 and Appendix S1), we selected IVs for MR analysis based on smoking behavior GWAS using the first sample ($n = 185\,972$) in the UKB after linkage disequilibrium pruning and clumping (removing genetic variants with $r^2 > 0.50$ within a 50-kb window). We performed IV–outcome association analysis using the second sample ($n = 12\,907$) in the UKB, which is not non-overlapped with the first sample. The UKB participants in our analysis were family unrelated with European ethnic background. We selected genetic variants with genetic-exposure *P*-value $<5 \times 10^{-8}$, genetic-outcome *P*-value adjusted by the Benjamini–Hochberg false discovery rate method (BH*p*) > 0.05 and genetic-confounder BH*P* > 0.05 as the IVs for MR analysis. We provide the details of the IVs in Supporting information, Data S5.

Given the IVs selected, we implemented the two-sample MR analysis using the R package *MendelianRandomization* (version 0.5.1) [64]. We used the MR method with generalized weighted model (gen-IVW) by Burgess *et al.* [65] (see details in Supporting information, Data S5). Cochran's *Q*-test, which provides evidence for heterogeneity in the causal effects between SNPs, was conducted alongside the MR analysis. We further carried out sensitivity analyses to examine the robustness of causal effects of SS and CPD on ΔG_{adj}. The MR methods used in sensitivity analyses are MR-weighted-median [66], MR-PRESSO [67], MR-MIX [68] and contamination mixture method (MRconmix) [69]. MR-weighted-median provides consistent estimates when there are some invalid IVs using a weighted median estimator; however, it requires at least 50% of the weight derived from valid IVs [66]. MR-PRESSO removes IVs that tested to be outliers in the MR analysis, therefore generating MR estimates with less variability (tighter confidence intervals) [67]. Its standard deviation is restricted to be smaller than or equal to the standard deviation from MR-IVW [67]. This method also provides a global test to assess whether the IVs used in the MR model have significant horizontal pleiotropy [67]. MR-Mix is another method that uses a mixture model to incorporate those IVs having horizontal pleiotropy [68]. MRconmix provides robust analysis when invalid IVs present by constructing a likelihood function according to variant-specific causal estimates, assuming different normal distributions for valid and invalid IVs [69]. Additionally, we performed the leave-one-out analysis besides the sensitivity analysis to assess whether an IV was driving the causal relationship between the exposure and the outcome [70]. We also performed MR-Egger and evaluated the reliability of MR-Egger results via its I^2 statistics [measurement of the 'NO Measurement Error' (NOME) assumption]. Moreover, we performed MR analysis to examine the other possible causal direction using BAG as the exposure and smoking behaviors (SS and CPD) as the outcome (see Appendix S2 for BAG GWAS and IV selection). In addition to UKB cohort, we performed the IV selection using summary statistics of CPD from the GSCAN (no

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GSCAN summary statistics available for SS) [39] and also performed the MR analysis using these IVs for CPD.

RESULTS

We summarized the participants' demographic characteristics and health conditions in training and testing sets separately for non-smokers and smokers (Table 1; see Supporting information, Fig. S2 for their distributions). The training and the testing sets had balanced demographic factors such as age, body mass index (BMI) and sex. As shown in Table 1, the mean BMI, mean age and proportions of categorical characteristics were similar between smokers and non-smokers, showing that there is no systematic difference in the distribution of sample characteristics between different groups. Also, we explored the demographic factors and found that these factors were distributed similarly between participants included and excluded from our MR study (see Table 1). We provided frequency of additional health conditions between training and testing sets (Supporting information, Fig. S1) and the prevalence of different self-reported health conditions in Supporting information, Data S2. Most conditions had a low prevalence within the testing set.

Model construction for calculating BAG

We constructed the ML model to calculate BAG from the training set using RF, locked the optimal model and applied it to the testing set. The optimal ML model chosen included 16 FA measures (see Supporting information, Data S1 and Fig. S3). The optimal model achieved excellent prediction performance in both training and testing sets: $R = 0.97$ and $MAE\ (\text{year}) = 2.21$ for training set (see Figure 1a); $R = 0.94$ and $MAE\ (\text{year}) = 2.80$ for non-smokers and $R = 0.94$ and $MAE\ (\text{year}) = 2.89$ for smokers in the testing set, respectively (see Figure 2a). ΔG_{adj} had a significant association with SS [$\widehat{\beta} = 0.88;\ 95\%$ confidence interval $(\text{Cl}) = 0.74,\ 1.02;\ P - \text{value} = 2.81 \times 10^{-36}$] and CPD ($\widehat{\beta} = 0.05;\ \text{Cl} = 0.03,\ 0.07;$ $P\text{-value} = 5.0049 \times 10^{-6}$) at the 0.05 significance level, as shown in Table 2. Such differences between smokers and non-smokers were consistent in all age categories (i.e. 40–49, 50–59 and 60–69 years) (see Figure 2b for the *P*-values). On average, mean ΔG_{adj} of nonsmokers was 0.66 $(\text{Cl} = 0.40,\ 0.93)$, 1.03 (0.77, 1.28) and 1.10 (0.81, 1.38) years younger than the ΔG_{adj} of smokers in aged 40–49, 50–59 and 60–69 categories, respectively (see Cohen's *d* in Figure 2c for the effect size). Also, ΔG_{adj} was significantly associated with cognitive function ($\widehat{\beta} = -0.04;\ \text{CI} = -0.06,\ -0.02;\ P - \text{value} = 6.52 \times 10^{-7}$, data not shown), given the cognitive function represented by the intelligence *g* calculated in our previous work [62].

Causal effects of smoking behaviors on brain aging

We selected 248 and 54 genetic variants as IVs in the MR models for SS and CPD, respectively, following the criteria in Equations (1)–(3) in Supporting information, Data S5. These IVs were well aligned with findings in existing studies, such as the gene cluster CHRNA5–CHRNA3–CHRNB4 linked with CPD [71–73]. We further explored the gene information of these IVs via an SNP annotation portal, the functional annotation of variant on-line resource (FAVOR) (http://favor.genohub.org/, accessed 12 July 2022) (see Supporting information, Data S3) [74].

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We performed MR analyses to test the estimated causal effect ($\hat{\theta}$) of the exposures using the selected IVs given ΔG_{adj} as the outcome. As shown in Table 2 and Figure 3b, the primary MR method [i.e. gen-IVW (UKB)] showed a significant causal effect of SS on ΔG_{adj} ($\hat{\theta} = 0.21;\ \mathrm{CI} = 6.5 \times 10^{-3},\ 0.41;\ P - \mathrm{value} = 0.04$). Also, CPD had a significant causal effect on ΔG_{adj} (UKB: $\hat{\theta} = 0.16;\ \mathrm{CI} = 0.06,\ 0.26;\ P - \mathrm{value} = 1.3 \times 10^{-3}$; GSCAN: $\hat{\theta} = 0.16; \mathrm{CI} = 0.18,\ 0.31;\ P - \mathrm{value} = 0.03$), indicating that the causal effect of CPD were robust across cohorts [see gen-IVW (GSCAN) in Figure 3b]. The results showed that smokers were 0.21 years older in brain age than comparable non-smokers at the same chronological age, and having an extra cigarette per day increased brain age by an additional 0.16 years.

Moreover, sensitivity analyses showed robust causal effects of SS and CPD separately on ΔG_{adj} (see Figure 3b). According to the MR-PRESSO's global test, one of the methods in sensitivity analyses, the selected IVs did not show significant horizontal pleiotropic effects on both SS and CPD ($P - \mathrm{value} = 1.00$). Cochran's Q-test suggested no evidence of weak or pleiotropic effects of IVs in MR analyses for CPD ($P - \mathrm{value} = 0.94$), but there were weak effects of IVs for SS ($P - \mathrm{value} = 0.00$). The leave-one-out analysis indicated that no single IV was driving the causal relationship (see Supporting information, Figs S4 and S5 and a complete list of causal estimates in Supporting information, Data S4). The I^2 statistics suggested that MR-Egger had sufficiently low reliability for CPD $\left(I^2 = 0.0\%\right)$. For SS, $I^2 = 52\%$ also suggested violation of the NOME assumption in MR-Egger. As MR-Egger is highly sensitive to the violation of the assumptions [75], we decided to focus upon results using other MR methods. Overall, most of the MR analyses showed evidence of significant causal effects of SS and CPD on BAG (see Figure 3b). We found no sufficient evidence of causal effect of BAG on smoking behaviors (SS: $P - \mathrm{value} = 0.07$; CPD: $P - \mathrm{value} = 0.09$). We further performed MR analysis using the instruments for CPD in non-smokers and found insignificant effect (P-value of gen-IVW = 0.11).

DISCUSSION

Our study investigated the potential causal effects of smoking behaviors on the brain aging process using the imaging-genetics data from the UKB cohort. Our findings confirmed the causal effect of smoking on accelerated neural degeneration during aging interpreted by age year. Also, the results revealed that the overall brain white matter deterioration was due to aging accelerated by smoking behaviors. The accelerated neural decline has been associated with multiple clinical symptoms of dementia, Alzheimer's disease and vasculature diseases [15, 18]. Findings have important implications for clinical interventions, highlighting how reductions in smoking may have important neurological health benefits.

We identified significant causal effects of SS and CPD on BAG based on MR analysis, consistent with the strong associations reported in previous work [17, 62, 76, 77]. For example, the pack-year was reported as the significant risk factor associated with BAG, given an increase of 0.36 months of adjusted BAG associated with one pack-year [17]. Similarly, we observed that a person who was a current smoker or had a larger number

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of cigarettes per day at the time of data collection would have a higher BAG value than a person who was a non-smoker or had fewer cigarettes in the UKB cohort. In addition, our study revealed that smoking led to a significant increase in brain aging and consequently raised the risk of age-related diseases and symptoms (e.g. memory and cognitive function decline) which may, in part, explain the previous findings of abnormal brain aging and cognitive functions. Smoking can lead to multiple complications (e.g. encouraging acute cardiovascular events and increasing insulin resistance) [78–80]. Subsequently, these smoking-related biological conditions can lead to accelerated brain aging [38, 81–83]. Numerically, the estimated causal effects of smoking behaviors on brain aging are distinct from the association effects. The former provides a more accurate estimate of the potential health benefits by preventing smoking.

Moreover, the IVs used in our analysis coincided with previously reported smoking-behavior-related genes (see Supporting information, Data S1 for the complete list). For example, in chromosome 9 (CHR9), genes *FAM163B* and *GAPVD1* had significant associations with CPD and smoking initiation, respectively [39, 51]. Genes *REV3L* (CHR6), *CHRNB3* (CHR8), *AS3MT* (CHR10), *NCAM1* (CHR11), *CHRNA3–CHRNA5–CHRNB4* (CHR15) and *CYP2T1P* (CHR19) had association with smoking initiation, smoking cessation, nicotine dependence and smoking history [7, 39, 71, 72, 84–94]. These existing studies supported the validity of IVs used in our MR analysis and reliability of the MR results. In addition, the sensitivity analyses reassured that there was no evidence for significant horizontal pleiotropy corresponding to these IVs.

Results must be interpreted in the context of study limitations. First, the UKB sample was biased towards certain sampling characteristics: (1) being older, being female and living in less socioeconomically deprived areas compared to samples in other study cohorts; (2) the participants in UKB tended more likely to have obesity, smoke cigarettes, drink alcohol on a daily basis and have fewer self-reported health conditions compared to the general population [95]. Secondly, our current work was restricted to one ethnic background aged 40–69 years. Some other factors may potentially affect our MR findings, such as population structure, family and assortative mating [96]. To control these potential effects, we adjusted the effect of population structure by adding principal components to GWAS analysis and removed participants that were family related; the effect of assortative mating was not accounted because our data had limited information to assess this effect. Exposure to secondhand smoke can be a potential confounder of the study, the impact of which will be investigated in future studies as the data become available in UKB. We encourage future studies to examine the causal relationship using different cohorts and validate the generalization of the results in a broader range of age and ethnic backgrounds.

In conclusion, our study provided a thorough analysis of causal inference for smoking behaviors on BAG and revealed robust and consistent causal effects in a large study cohort. These results reinforced the evaluation of the impact coming from smoking and assisted in guiding future studies. This study provided a greater understanding of the causal effects of smoking behaviors on brain aging and cognitive disorders, connecting previous findings of neuroimaging and cognitive function [62, 76, 77]. Our results have important implications in how behavior may also improve neurological health status.

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Supplementary Material

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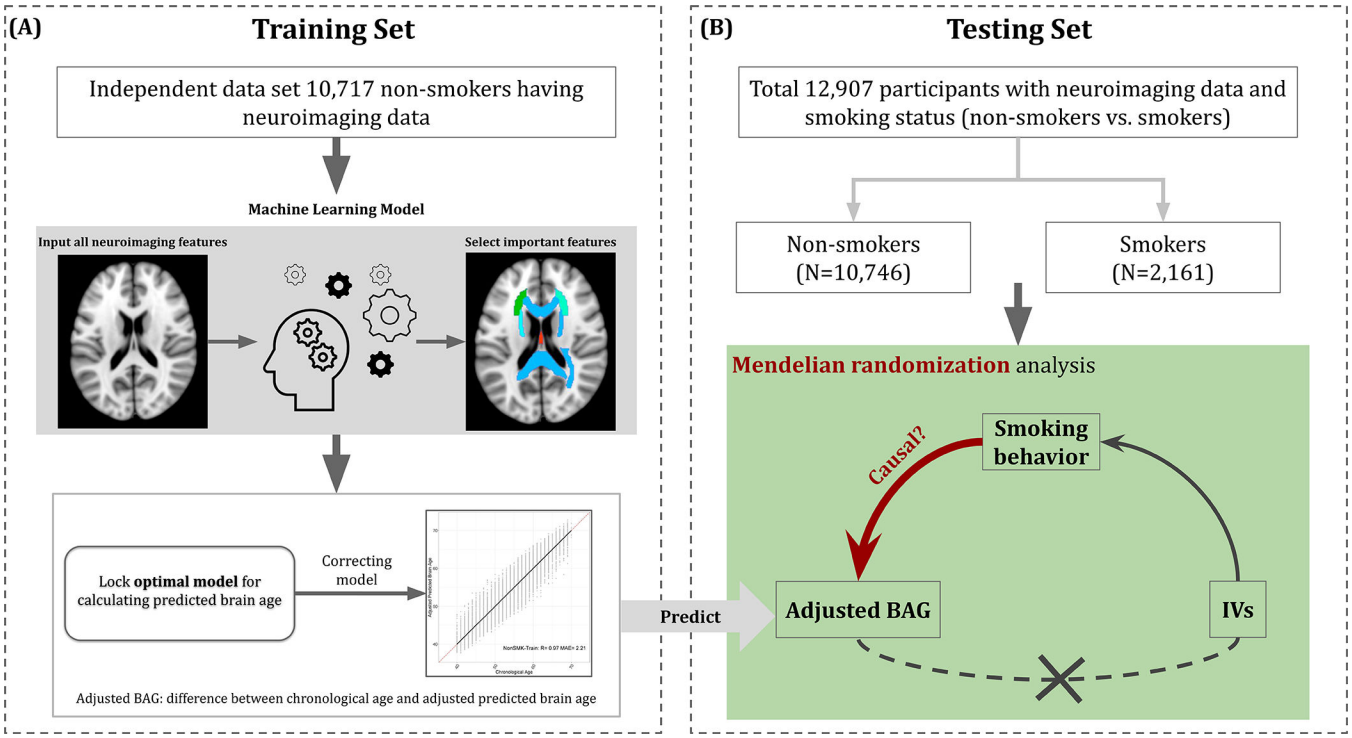

FIGURE 1.

Overview of analysis procedures. (a) Within the non-overlapping training set, (i) the optimal predictive model for estimating predicted brain age was obtained using machine learning model; (ii) the predictive model was corrected to reduce the estimation bias and further used to estimate the adjusted predictive brain age; and (iii) the outcome variable [i.e. adjusted brain age gap (BAG)] was calculated by subtracting the chronological age by the adjusted predictive brain age. (b) In the testing set, the adjusted BAG was estimated based on the corrected predictive model in (a), and the causal effect of smoking behavior (e.g., smoking status) on adjusted BAG was evaluated through Mendelian randomization analysis. Smoking status was a binary trait consisting of nonsmokers and smokers

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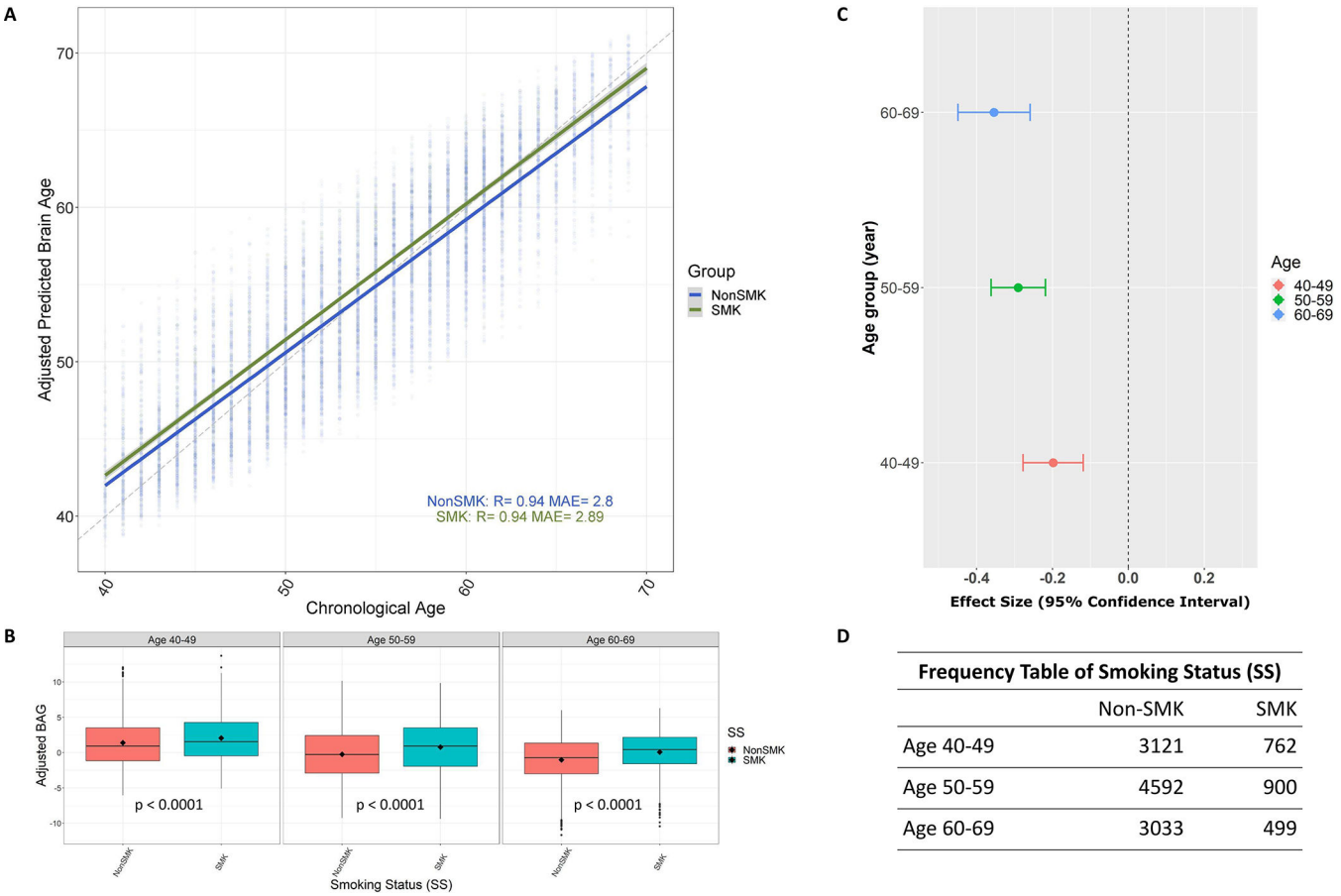

Frequency Table of Smoking Status (SS)		
	Non-SMK	SMK
Age 40-49	3121	762
Age 50-59	4592	900
Age 60-69	3033	499

FIGURE 2.

Adjusted brain age gap (BAG) and its relationships with smoking status in the testing set. (a) The relationship between the adjusted predicted brain age ($\widehat{Y}_{adj}$) and chronological age in different smoking statuses (SS) (R = coefficients of correlation; MAE = mean absolute error). (b) The distribution of adjusted BAG (ΔG_{adj}) between different SS groups in separate chronological age categories, where *P* indicates the *P*-value corresponding to the comparison between nonsmokers (non-SMK) and smokers (SMK) in each category. (c) The effect size with the 95% confidence interval for testing the difference between non-SMK and SMK within each age category based on (b). (d) The number of participants in each age group corresponding to comparisons between non-SMK and SMK in (b)

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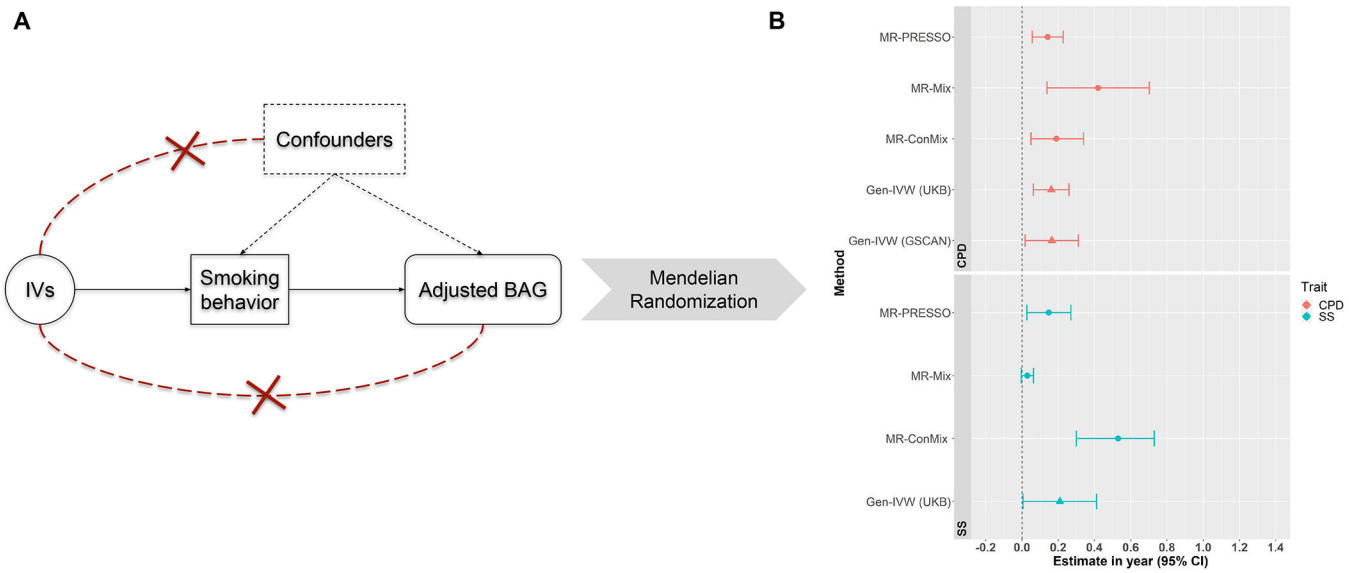

FIGURE 3.

Mendelian randomization and the results. (a) The three fundamental instrumental variable (IV) assumptions in the Mendelian randomization (MR) analysis: (i) IVs are significantly associated with the exposure [i.e. smoking statuses (SS) or cigarettes per day (CPD)]; (ii) the exposure is not significantly associated with confounders of the exposure–outcome association; and (iii) IVs can affect the outcome variable only through the exposure. (b) The causal effect estimate with a 95% confidence interval using different MR methods for smoking traits SS and CPD separately, based on the IVs selected following the three IV assumptions. Gen-IVW (marked with a triangle) is the primary MR method (i.e. weighted generalized linear regression) and the other methods (marked with a dot) are the MR methods used in the sensitivity analysis. BAG = brain age gap

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Table 1.

Demographic characteristics of participants in training and testing data sets

		Training Set	Testing Set	
Variable	**Level**	**NonSMK (N=10,717)**	**NonSMK (N=10,746)**	**SMK (N=2,161)**
BMI (kg/m^2)	Mean (SE)	26.30 (4.08×10^{-2})	26.26 (4.02×10^{-2})	26.48 (8.70×10^{-2})
Age (years)	Mean (SE)	54.30 (7.15×10^{-2})	54.28 (7.12×10^{-2})	53.08 (1.57×10^{-2})
Townsend deprivation index at recruitment	Mean (SE)	−2.13 (2.57)	−2.18 (2.54)	−0.90 (3.19)
Age completed full time education (years)	Mean (SE)	17.28 (2.34)	17.27 (2.35)	16.94 (2.35)
Average total household income before tax (n, %)	Less than 18,000	930 (8.68)	899 (8.37)	145 (6.71)
	18,000 to 30,999	1023 (9.55)	1038 (9.66)	314 (14.53)
	31,000 to 51,999	2012 (18.77)	2017 (18.77)	455 (21.06)
	52,000 to 100,000	2883 (26.9)	3042 (28.31)	635 (29.38)
	Greater than 100,000	3012 (28.1)	2872 (26.73)	495 (22.91)
	Do not know/ Prefer not to answer	838 (7.82)	803 (7.47)	105 (4.86)
Sex (n, %)	Female (%)	6009 (56.07)	5978 (55.63)	972 (44.98)
	Male (%)	4708 (43.93)	4768 (44.37)	1189 (55.02)
Hypertension (n, %)	Yes (%)	1213 (11.32)	1252 (11.65)	259 (11.98)
	No (%)	9504 (88.68)	9494 (88.35)	1902 (88.01)
Diabetes (n, %)	Yes (%)	33 (3.08×10^{-3})	43 (4.00×10^{-3})	12 (5.55×10^{-3})
	No (%)	10684 (0.99)	10703 (0.99)	2149 (0.99)
Stroke (n, %)	Yes (%)	93 (8.68×10^{-3})	86 (8.00×10^{-3})	25 (1.16×10^{-3})
	No (%)	10660 (0.99)	10624 (0.99)	2136 (0.99)

SE = standard error; n = frequency; % = percentage within each column; NonSMK = non-smoker; SMK = smoker.

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Table 2.

Results of Mendelian randomization and association analyses in the study sample.

	Association		**Mendelian randomization**	
	$\hat{\beta} \pm$ SE **(95% CI)**	***p*-value**	$\hat{\theta} \pm$ SE **(95% CI)**	***p*-value**
SS	0.88 ± 0.07 (0.74, 1.02)	2.81×10^{-36}	0.21 ± 0.10 (6.5×10^{-3}, 0.41)	0.04
CPD	0.05 ± 0.01 (0.03, 0.07)	5.49×10^{-6}	0.16 ± 0.05 (0.06, 0.26)	1.30×10^{-3}

SE = standard error; CI = confidence interval; SS = smoking status; CPD = cigarette per day.