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Archival Report

Investigating the Relationship Between Smoking Behavior and Global Brain Volume

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ABSTRACT

BACKGROUND: Previous studies have shown that brain volume is negatively associated with cigarette smoking, but there is an ongoing debate about whether smoking causes lowered brain volume or a lower brain volume is a risk factor for smoking. We address this debate through multiple methods that evaluate directionality: Bradford Hill's criteria, which are commonly used to understand a causal relationship in epidemiological studies, and mediation analysis.

METHODS: In 32,094 participants of European descent from the UK Biobank dataset, we examined the relationship between a history of daily smoking and brain volumes, as well as an association of genetic risk score to ever smoking with brain volume.

RESULTS: A history of daily smoking was strongly associated with decreased brain volume, and a history of heavier smoking was associated with a greater decrease in brain volume. The strongest association was between total gray matter volume and a history of daily smoking (effect size = -2964 mm^3, $p = 2.04 \times 10^{-16}$), and there was a dose-response relationship with more pack years smoked associated with a greater decrease in brain volume. A polygenic risk score for smoking initiation was strongly associated with a history of daily smoking (effect size = 0.05, $p = 4.20 \times 10^{-84}$), but only modestly associated with total gray matter volume (effect size = -424 mm^3, $p = .01$). Mediation analysis indicated that a history of daily smoking mediated the relationship between the smoking initiation polygenic risk score and total gray matter volume.

CONCLUSIONS: A history of daily smoking is strongly associated with a decreased total brain volume.

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Cigarette smoking is associated with numerous harmful health outcomes, including cardiovascular disease, respiratory disease, cancer, and diminished overall health (1–4). The adverse effect of smoking extends into the brain, and this is shown by the association between smoking and dementia (5–7). People who smoke are more likely to have deterioration in gray and white matter, which provides a possible explanation as to why 14% of global Alzheimer's disease cases could be attributable to cigarette smoking (8,9).

Smoking-related behaviors are in part biologically driven. Twin studies have firmly established the importance of genetic factors contributing to the onset of cigarette smoking, heaviness of smoking, and smoking cessation, and smoking initiation has heritability estimates of 44% (10–12). Recent large genome-wide association studies have identified thousands of genetic loci that are associated with smoking-related behaviors (13–15). Differences in responses to nicotinic receptors, nicotine metabolism, and many other genetic factors contribute to the development of smoking behaviors. Models of addiction posit that predisposing neurodevelopmental risk factors promote the onset of cigarette smoking and other addictive behaviors (16,17).

It is known that there are associations between smoking behavior and lower total brain volume and gray and white matter volumes (18). However, a significant question remains about whether these associations represent predisposing features for the risk of developing cigarette smoking or are consequences of cigarette smoking. The UK Biobank presents a unique opportunity to study the association between smoking behaviors and brain features with a large sample of individuals who have completed comprehensive assessments and to shed light on whether associations with brain volumes and smoking behaviors are predisposing factors or adverse consequences of cigarette smoking. Currently, the UK Biobank provides surveys on health behaviors and imaging-derived measures from magnetic resonance imaging on approximately 40,000 participants. In addition, genetic data are available for UK Biobank participants. Our goal was to examine the associations between smoking behaviors, global brain volumes, and genetic variation to provide evidence for the direction of the association between smoking behaviors and brain imaging measures by using traditional epidemiological methods and mediation analysis.

Bradford Hill, an eminent epidemiologist, developed criteria for establishing evidence of causality (19). Hill's criteria for causation, originally developed to specify a causal relationship between smoking behavior and lung cancer, consists of 9

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points: strength of association, consistency across sites and methods, specificity, temporality, biological gradient, plausibility, coherence, experimental evidence, and analogy (related evidence). We used the different smoking measures (history of daily smoking, number of cigarette pack years smoked, and time since smoking cessation) available in the UK Biobank dataset to examine Hill's criteria and to build evidence about whether observed brain differences represent predisposing factors that influence smoking behaviors or are consequences of smoking exposure. We studied: 1) the association between a history of daily smoking and global brain volumes, 2) whether there is a dose-response relationship with greater cumulative exposure to smoking (measured by pack years) associated with changes in brain volumes, 3) whether smoking cessation is associated with a reversal of changes in brain volumes, and 4) whether there are subregions of the brain that are more or less associated with smoking behaviors after correcting for total brain volume changes.

We also incorporated genetic data to further establish the direction of the effect between smoking behaviors and brain volume. To test the association between genetic predisposition to smoking behavior and brain volume differences, we used summary statistics from GSCAN (GWAS and Sequencing Consortium of Alcohol and Nicotine Use) (15), a large genetic study of smoking behaviors, to create a polygenic risk score (PRS) for ever smoking, a summary score of an individual's genetic predisposition. In UK Biobank participants, we examined 1) the association between the PRS for smoking and history of daily smoking and 2) the association between the PRS for smoking and global brain volumes. The lack of a strong association between genetic predisposition to smoking and brain volume differences would add evidence that smoking is negatively related to brain volume rather than that a decrease in brain volume influences smoking behavior. Finally, we used mediation analysis as a tool to study the direction of causation and the strength of daily smoking as a mediator. Converging results from these different methodologies can provide evidence for the direction of effect of the association between smoking behaviors and imaging measures of brain volume. An overview of the study is presented in Figure 1.

METHODS AND MATERIALS

UK Biobank Participants

Our sample included the 2019 UK Biobank released data of participants with imaging data. The UK Biobank study was approved by the National Health Service National Research Ethics Service (11/NW/0382). All participants provided informed consent to participate in the UK Biobank study (Study ID: 47267, 48123).

From the imaging dataset, we removed related individuals up to third-degree relatives (n = 1123) and individuals who withdrew consent following participation. We also excluded participants with neurological conditions (n = 1122) to eliminate potential confounding effects from these conditions (18). See Figure S1 in Supplement 1 for the flow chart of sample processing and Table S1 in Supplement 1 for additional details of participants with neurological conditions. This study follows the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) reporting guideline for cross-sectional studies. See Table 1 for demographic, smoking, and health-related details of the participants (N = 32,094).

Imaging-Derived Measures

Detailed information regarding the UK Biobank image acquisition parameters, preprocessing pipeline, and estimation of brain imaging–derived measures is available elsewhere (https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf) (20). Briefly, T1-weighted scans were acquired at 1-mm isotropic resolution using a Siemens Magnetom Skyra 3T scanner. Following brain extraction and nonlinear registration to Montreal Neurological Institute space with BET and FNIRT tools, respectively, tissue-type segmentation was performed using the FAST tool (20). T1 images were also processed with FreeSurfer. Cortical surface atlases for FreeSurfer modeling were used to extract area, volume, and mean cortical thickness imaging-derived phenotypes (FreeSurfer DKT [Desikan-Killiany-Tourville]). FreeSurfer ASEG (automatic segmentation) tools are used for the extraction of subcortical regions and total measures of the brain (volume of brain, volume of gray

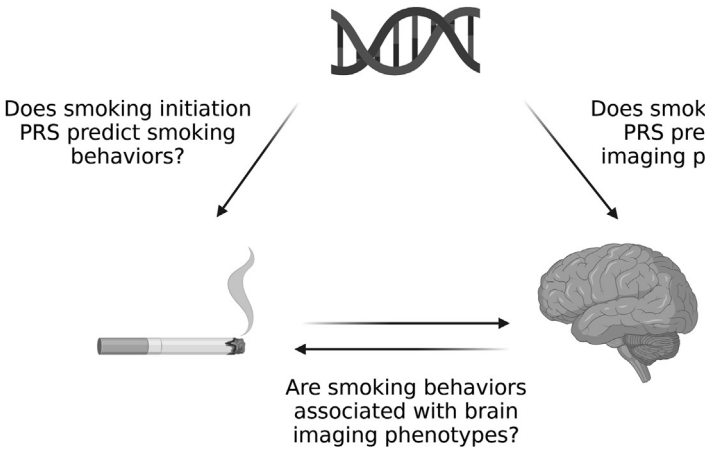

Figure 1. Overview of the study. We examined: 1) the predictive ability of the smoking initiation polygenic risk score (PRS) for smoking for a history of daily smoking; 2) the association between the smoking initiation PRS for smoking initiation and brain measures; and 3) the association between smoking behaviors and brain measures. (Created with BioRender.com.)

matter, volume of white matter, and volume of cerebrospinal fluid [CSF]). Variable IDs (brain measures, covariates) used in these analyses are provided in Table S2 in Supplement 1.

Smoking Behaviors

Smoking phenotypes were defined using data from self-report surveys obtained during in-person assessment center visits at baseline ("instance 0," 2006–2010) and at the neuroimaging visit ("instance 2," 2012–2013). A history of daily smoking (n = 8906) was defined by a consensus of reports of former or current daily smoking on surveys at both time points (visits). Never smoking (n = 23,188) was defined by a lifetime history of never smoking or smoking fewer than 100 cigarettes reported on both surveys. Those with a history of occasional smoking but not daily smoking and those with conflicting smoking status reports on the 2 surveys were excluded from the analysis (n = 7494) so that the distinction between a history of daily smoking and never smoking would be clearer, and the data more reliable. See Figure S2 in Supplement 1 for the sample size and questionnaire details for the imaging subset. See Table S3 in Supplement 1 for the baseline and imaging visit comparison of reported smoking behaviors.

Smoking pack years (number of cigarette packs [1 pack = 20 cigarettes] smoked per day times the number of years smoked) was derived for participants who had a history of daily smoking at the imaging survey. If this value was missing, smoking pack years was taken from the baseline survey. See Figure S3 in Supplement 1 for pack-year distribution in categories.

The age when each participant last smoked was obtained from the imaging survey; if this value was missing, it was taken from the baseline survey. Duration of smoking cessation was derived by subtracting the age last smoked from the participants' age at the imaging assessment.

Standardized imaging confound values (age, age^2, gender, age × gender, head size, head motion resting-state functional magnetic resonance imaging, head motion task-based functional magnetic resonance imaging, date, date^2, site) were curated (21). Additional covariates that might have confounded the association between brain measures and smoking behaviors were included in analyses: average household income, age when completed full-time education, systolic blood pressure, diastolic blood pressure, body mass index, waist-to-hip ratio, weekly dose of alcohol (calculated by converting drink by type into an overall sum of drinks), stress, physical activity, diabetes, cancer, vascular/heart problems, and other health conditions. Additional covariates included 10 ancestral principal components. See Table S4 and Supplemental Text in Supplement 1 for additional information on the selected covariates.

Imputation of missing values for all covariates was first done using participants' reports from the baseline survey. The additional missing values were imputed using R package MICE (Multivariate Imputation by Chained Equations). Additional details on missing data and data wrangling are given in the Supplemental Text in Supplement 1 and Table S5 in Supplement 2.

Genetic Dataset

We used the UK Biobank genetic dataset to retrieve genome-wide data for all participants of European ancestry (dataset version/number = ukb48123). We used GSCAN summary statistics with the UK Biobank sample excluded to create a PRS for ever smoking with variants using PRSice-2 (22,23). The PRS results have been pruned for sites with minor allele frequency > 0.001, imputation quality (effective_N/N) > 0.3, and an effective sample size of at least 10% of the maximum sample size. Insertions and deletions were not included in GSCAN summary statistics and were also not included in the calculation of the PRS. PRSice-2 utilizes a p value selection threshold approach so that according to the different thresholds, only those single nucleotide polymorphisms with a genome-wide association study association p value below a certain threshold are included in the calculation of the PRS. We tested the PRS for ever smoking to determine whether it predicted history of daily smoking in the UK Biobank dataset as well as total brain measures in 1) the total sample, 2) the subset of participants who never smoked, and 3) the subset of participants who reported a lifetime history of daily smoking. See Figure S4 in Supplement 1 for an overview of the study including the genetic dataset.

Statistical Analysis

We performed linear regression analysis using the lm package from R for each question listed below.

Question 1: Is a history of daily smoking associated with global brain measures?

Equation 1: brain volume = history of daily smoking (dichotomous variable) + covariates

The following 2 analyses were undertaken only in those with a history of daily smoking.

Question 2: Is there a dose-response relationship between the heaviness of smoking (defined by pack years smoked) and global brain measures?

Equation 2: brain volume = pack years (continuous variable) + covariates

Question 3: Is there evidence of a positive association between brain volume and time since smoking cessation among those with history of daily smoking?

Equation 3: brain volume = time since smoking cessation (continuous variable for those who smoked daily in the past) + pack years + covariates

Question 4: Is the ever smoking PRS associated with history of daily smoking?

Equation 4: history of daily smoking = ever smoking PRS + covariates

Question 5: Is the ever smoking PRS associated with the global brain measures?

Equation 5: brain volume = ever smoking PRS + covariates

Question 6: Are there regions of the brain more or less associated with daily smoking after correcting for the total brain volume in addition to head size?

Equation 6: brain subregion volume = history of daily smoking + total brain volume + covariates

For questions 1 through 5, a threshold of .05 was set as the level for statistical significance. For question 6, 235 subregions were examined; thus, the threshold for significance was set at a Bonferroni correction of .05/235 = 2.13×10^{-4}.

Mediation Analysis for History of Daily Smoking and Total Gray Matter Volume

Mediation analysis was performed using the "mediation" package in R to measure the strength of the causal mediator

(daily smoking) in the relationship between the PRS for smoking initiation and the outcome (total brain volume) while adjusting for various confounding variables (age, age^2, sex, age × sex, head size, head motion, date, date^2, site, average household income, age when completed full-time education, systolic blood pressure, diastolic blood pressure, body mass index, waist-to-hip ratio, weekly dose of alcohol). The average causal mediated effect, or the statistical significance of the mediator, was calculated using this package. See Figure S5 in Supplement 1 for the model for the mediation analysis.

RESULTS

History of Daily Smoking Was Associated With Global Brain Measures

A history of daily smoking was associated with a decrease in total brain volume, gray matter volume, and white matter volume (Table 2). Decreased volume of gray matter had a strong association with a history of daily smoking (effect size = −2964 mm^3, $p = 2.04 \times 10^{-16}$), along with decreased volume of total brain (effect size = −3360 mm^3, $p = 2.85 \times 10^{-8}$). Volume of white matter was modestly associated with a history of daily smoking (effect size = −802 mm^3, $p = 4.68 \times 10^{-2}$).

Evidence of a Dose-Response Relationship With Pack Years

Among participants with a history of daily smoking, there was evidence of a dose-response relationship between increasing number of pack years smoked and a decrease in brain volume, gray matter volume, and white matter volume (Table 2). Volume of gray matter had a strong association with pack years of smoking (effect size = −84 mm^3, $p = 3.25 \times 10^{-5}$), as well as volume of the total brain (effect size = −129 mm^3, $p = 1.23 \times 10^{-4}$). A modest association was seen with volume of white matter (effect size = −64 mm^3, $p = 0.04$). There was no significant association of pack years smoked with volume of CSF.

Time Since Smoking Cessation Was Not Associated With Total Brain Volume Measures

There was no significant association between the number of years since smoking cessation and total brain volume, total gray matter volume, white matter volume, and CSF volume.

Effect of Genetic Predisposition to Smoking on Total Gray Matter Volume Among the Smoking Population

The ever smoking PRS was strongly associated with history of daily smoking in the UK Biobank dataset (effect size = 0.05, $p = 4.20 \times 10^{-84}$), thus corroborating that these genetic variants collectively predicted this smoking behavior. There was a modest association of the ever smoking PRS with reduced total gray matter volume (effect size = −424 mm^3, $p = .01$) and increased white matter volume (effect size = 367 mm^3, $p = .04$) in the total sample (n = 30,973) (Table 3). There was no evidence of a PRS-brain volume association in the subsets including only participants who had a history of daily smoking.

Table 1. Demographic, Smoking, and Health-Related Variables (Total *N* = 32,094)

	Daily Smoked, n = 8906	Never Smoked, n = 23,188
Age, Years	65.14 ± 7.53	63.21 ± 7.65
Gender[a]		
Woman	3967 (44.5%)	13,049 (56.3%)
Man	4939 (55.5%)	10,139 (43.7%)
Income, £		
<18,000	1283 (14.4%)	2692 (11.6%)
18,000–30,999	2699 (30.3%)	6057 (26.1%)
31,000–51,999	2646 (29.7%)	7039 (30.4%)
52,000–100,000	1790 (20.1%)	5579 (24.1%)
>100,000	488 (5.5%)	1821 (7.9%)
Age Completed Full-Time Education	19.20 ± 3.53	20.10 ± 3.33
Health Conditions		
Diabetes	353 (4.0%)	468 (2.0%)
Cancer	552 (6.2%)	1182 (5.1%)
Vascular/heart problems (heart attack, angina)	2292 (25.7%)	4353 (18.8%)
Other[b]	1595 (17.9%)	3266 (14.1%)
Stress, Illness, Bereavement		
Illness, injury, bereavement, stress	3819 (42.9%)	9451 (40.8%)
None of the above	5087 (57.1%)	13,737 (59.2%)
Body Mass Index	27.29 ± 4.28	26.25 ± 4.20
Waist/Hip Ratio	0.89 ± 0.09	0.86 ± 0.09
Systolic Blood Pressure, mm Hg	141.38 ± 20.06	139.65 ± 19.64
Diastolic Blood Pressure, mm Hg	79.38 ± 10.59	79.31 ± 10.68
Weekly Drinks of Alcohol	12.66 ± 10.86	8.21 ± 7.73
Nonvigorous Physical Activity[c]	3.44 ± 2.30	3.43 ± 2.26
Vigorous Physical Activity[c]	1.81 ± 1.86	1.86 ± 1.81

Values are presented as mean ± SD or n (%).

[a]Gender information extracted from UK Biobank data-field 31, and differed from genetically assigned sex (data-field 22001) in 2 participants.

[b]Participants who answered "yes" to the question: Has a doctor ever told you that you have had any other serious medical conditions or disabilities?

[c]Number of days/week of nonvigorous or vigorous physical activity for 10+ minutes.

Additionally, there was modest evidence of a PRS–white matter volume association in the subset of participants that included only those who had never smoked (effect size = 438 mm^3, $p = .04$).

Mediation Analysis Between Total Gray Matter Volume, Smoking Initiation PRS, and History of Daily Smoking

Because total gray matter volume was modestly associated with the PRS for smoking initiation, we performed a mediation analysis between the ever smoking PRS, total gray matter volume (outcome), and history of daily smoking (mediator). The association between the PRS for ever smoking and total gray matter volume became nonsignificant (effect size: 0.04, $p = .21$) when the mediator, a history of daily smoking, was added (total/indirect causal mediation effect size [average causal

Table 2. Effect Size and *p* Value for Total Brain Measures With the Smoking Phenotypes

Brain Measures	Effect Size	SE	t_{53}	*p* Value
History of Daily Smoking, *N* = 32,094				
Volume of brain	−3,360.95	605.39	−5.55	2.85×10^{-8}
Volume of gray matter	−2,964.18	360.42	−8.22	2.04×10^{-16}
Volume of white matter	−801.74	403.24	−1.99	4.68×10^{-2}
Volume of cerebrospinal fluid	4.93	3.03	1.63	.10
Number of Pack Years of Smoking, *n* = 8622				
Volume of brain	−128.75	33.52	−3.84	1.23×10^{-4}
Volume of gray matter	−83.87	20.17	−4.16	3.25×10^{-5}
Volume of white matter	−63.61	22.28	−2.85	4.32×10^{-3}
Volume of cerebrospinal fluid	0.29	0.17	1.68	.09
Time Since Smoking Cessation, *n* = 8111				
Volume of brain	−5.01	57.08	−0.09	.93
Volume of gray matter	9.86	34.39	0.29	.77
Volume of white matter	15.64	38.04	0.41	.68
Volume of cerebrospinal fluid	−0.55	0.29	−1.89	.06

Covariates: Weekly alcohol use, diastolic and systolic blood pressure, body mass index, waist-to-hip ratio, income, age completed full-time education, diabetes, vascular/heart problems, other health conditions/disabilities, physical activity, stress, and imaging confounds (age, age^2, sex, age × sex, head size, head motion resting-state functional magnetic resonance imaging, head motion task-based functional magnetic resonance imaging, date, date^2, site). Effect sizes are in mm^3. Results with normalized imaging-derived phenotype values are in Table S6 in Supplement 3.

mediated effect] = 0.005, $p < 2 \times 10^{-16}$, total direct causal mediation effect size [average direct effect] = 0.00, $p > .99$).

History of Daily Smoking Was Associated With Cortical Volume and Thickness Measures

The purpose of the subregion analyses was to determine whether certain regions of the brain are more or less associated with a history of daily smoking after adjusting for head size and total brain volume. The correlation between head size and total brain volume was 0.7. In these subregion analyses, a significance level of 2.13×10^{-4} was selected based on a Bonferroni correction for 235 tests.

Of the 186 FreeSurfer DKT measures based on white matter parcellation, 41 subregions were significantly associated with a history of daily smoking, and only 7 (4%) remained significantly associated after correcting for total brain volume. Mean thickness and volume of the superior frontal cortex (both hemispheres), volume of the rostral middle frontal cortex (left hemisphere), volume of the precentral cortex (right hemisphere), and volume of the medial orbital frontal cortex (left hemisphere) were all negatively associated with a history of daily smoking after correcting for total brain volume (Table 4). None of the other cortical regions passed the threshold of significance based on multiple testing and demonstrated a significant association with a history of daily smoking.

Table 3. Effect Size and *p* Value for Total Brain Measures Associated With the Smoking Initiation PRS

Brain Measures	Effect Size	SE	t_{53}	*p* Value
Smoking Initiation PRS (Total Population, *N* = 30,973[a])				
Volume of brain	−36.61	277.32	−0.13	.89
Volume of gray matter	−424.48	165.33	−2.57	.01
Volume of white matter	366.99	184.92	1.98	.04
Volume of cerebrospinal fluid	−1.23	1.39	−0.89	.38
Smoking Initiation PRS, Never Smoked Population, *n* = 22,298				
Volume of brain	157.46	323.90	0.49	.63
Volume of gray matter	−315.65	192.13	−1.64	.10
Volume of white matter	437.84	216.52	2.02	.04
Volume of cerebrospinal fluid	−0.34	1.61	−0.21	.83
Smoking Initiation PRS, Daily Smoked Population, *n* = 8675				
Volume of brain	−124.13	531.72	−0.23	.82
Volume of gray matter	−327.46	320.51	−1.02	.31
Volume of white matter	262.91	353.07	0.74	.46
Volume of cerebrospinal fluid	−4.16	2.72	−1.53	.13

Other PRS thresholds (0.4, 0.3, 0.2, 0.1, 0.05, 0.01, 1×10^{-3}, 1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7}, 5×10^{-8}) are included in Table S6 in Supplement 3. Effect sizes are in mm^3. Results with normalized imaging-derived phenotype values are in Table S6 in Supplement 3.

PRS, polygenic risk score.

[a]Sample size is after filtering for robust genetic information.

Table 4. Effect Size and *p* Value for Total Brain Measures Associated With the FreeSurfer DKT Measures

Brain Measures	Hemisphere	Effect Size	SE	t_{53}	*p* Value
Mean Thickness of Superior Frontal	Left	-5.26×10^{-3}	1.01×10^{-3}	−5.23	1.67×10^{-7}
	Right	-4.07×10^{-3}	9.33×10^{-4}	−4.36	1.29×10^{-5}
Volume of Superior Frontal	Left	−122.14	25.17	−4.85	1.22×10^{-6}
	Right	−61.84	16.11	−3.84	1.24×10^{-4}
Volume of Rostral Middle Frontal	Left	−116.07	28.53	−4.07	4.75×10^{-5}
Volume of Precentral	Right	−61.61	16.55	−3.72	1.98×10^{-4}
Volume of Medial Orbitofrontal	Left	−27.66	6.27	−4.41	1.02×10^{-5}

Covariates: Weekly alcohol use, diastolic and systolic blood pressure, body mass index, waist-to-hip ratio, income, age completed full-time education, diabetes, vascular/heart problems, other health conditions/disabilities, physical activity, stress, and imaging confounds (age, age^2, sex, age × sex, head size, head motion resting-state functional magnetic resonance imaging, head motion task-based functional magnetic resonance imaging, date, date^2, site). Effect sizes are in mm^3. Results with normalized imaging-derived phenotype values are in Table S6 in Supplement 3.

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History of Daily Smoking Was Associated With Increased Ventricle Sizes and Decreased Cerebellum and Subcortical Volume Measures

Of the 49 FreeSurfer ASEG measures, 15 (30%) were significantly associated with a history of daily smoking after correcting for total brain volume (before correction, 24 measures were significantly associated). The volume of white matter hypointensities, choroid plexus in both hemispheres, ventricle choroid, and third ventricle in the whole brain; volume of interior lateral ventricle (left hemisphere); and volume of lateral ventricle (right hemisphere) were positively associated with a history of daily smoking after correcting for total brain volume. An increase in volume of all these regions is an adverse effect. The volume of cerebellum white matter; volume of ventral diencephalon (all both hemispheres); volume of cortex, amygdala, and thalamus (all left hemisphere); and volume of corpus callosum central in the whole brain were negatively associated with a history of daily smoking after correcting for total brain volume (Table 5).

DISCUSSION

We systematically examined the relationship between a history of daily smoking and global brain volume, and the preponderance of evidence supports an adverse association of smoking with brain volume. Daily smoking is associated with a decrease in total brain volume. Using the Hill criteria as a guide to study causation, we found a strong association between a history of daily smoking and brain imaging phenotypes as reported in previous studies. Several studies using different datasets and various analytical methods have identified a strong association between a history of daily smoking and global brain volume, gray matter volume, and white matter volume (18,24–26). We also found a significant biological gradient, with a dose-response effect of a history of more pack years of smoking associated with greater differences in brain volume. In addition, there is evidence of biological plausibility. Daily smoking is associated with many adverse health effects across multiple organ systems, and adding the brain to the list of organs that are adversely affected by smoking is biologically

Table 5. Effect Size and *p* Value for Total Brain Measures Associated With the FreeSurfer ASEG Measures

Brain Measures	Hemisphere	Effect Size	SE	t_{53}	*p* Value
Volume of Choroid Plexus	Left	26.45	2.66	9.95	2.68×10^{-23}
	Right	31.02	2.54	12.20	3.71×10^{-34}
Volume of Inferior Lateral Ventricle	Left	13.86	3.40	4.07	4.63×10^{-5}
Volume of Third Ventricle	Whole	26.32	5.76	4.57	4.86×10^{-6}
Volume of Lateral Ventricle	Right	304.82	75.44	4.04	5.34×10^{-5}
Volume of Ventricle Choroid	Whole	703.66	164.11	4.29	1.81×10^{-5}
Volume of White Matter Hypointensities	Whole	152.49	33.18	4.60	4.32×10^{-6}
Volume of Cerebellum White Matter	Left	−128.20	22.38	−5.73	1.03×10^{-8}
	Right	−120.73	24.60	−4.91	9.23×10^{-7}
Volume of Ventral Diencephalon	Left	−15.02	3.49	−4.31	1.65×10^{-5}
	Right	−16.76	3.37	−4.98	6.41×10^{-7}
Volume of Thalamus-Proper	Left	−23.06	6.15	−3.75	1.78×10^{-4}
Volume of Amygdala	Left	−11.30	2.29	−4.93	8.43×10^{-7}
Volume of Corpus Callosum Central	Whole	−6.56	1.45	−4.54	5.74×10^{-6}

Covariates: Weekly alcohol use, diastolic and systolic blood pressure, body mass index, waist-to-hip ratio, income, age completed full-time education, diabetes, vascular/heart problems, other health conditions/disabilities, physical activity, stress, and imaging confounds (age, age^2, sex, age × sex, head size, head motion resting-state functional magnetic resonance imaging, head motion task-based functional magnetic resonance imaging, date, date^2, site). Effect sizes are in mm^3. Results with normalized imaging-derived phenotype values are in Table S6 in Supplement 3.

ASEG, automatic segmentation.

plausible. There is similar evidence of alcohol causing adverse consequences on the brain, which provides analogical evidence of the harms of smoking (27,28). A recent study investigated the causal relationship between smoking and alcohol and subcortical brain volume variations and concluded that smoking and heavy alcohol consumption can causally reduce subcortical brain volume (29). Another recent study performed Mendelian randomization and found a significant association between genetic liability to ever smoking and decreased gray matter volume (30).

We used genetics as a tool to provide additional evidence that a history of daily smoking may be negatively related to brain volume. Mediation analysis provides convergent evidence highlighting the plausibility of smoking being associated with decreases in brain volume. We found that a PRS for ever smoking was strongly associated with history of daily smoking in the UK Biobank, but minimally associated with total gray and white matter volume. With the additional mediation analysis on the PRS for ever smoking and the total gray matter volume using history of daily smoking as a mediator, we found that the mediation effect was strong, and the association between the PRS and brain volume disappeared. These findings provide additional evidence that smoking is negatively associated with the differences in brain volume.

The complexity of the relationship between smoking history and brain imaging phenotypes underscores the debate regarding causation: are brain differences predisposing to smoking behavior or are the brain differences a consequence of smoking behaviors? There are studies that have suggested that brain differences are a predisposing factor for alcohol consumption rather than reflecting alcohol-induced atrophy (17,31). There is evidence that greater volume or thickness in brain regions (pars opercularis, cuneus) and lower volume in brain regions (basal forebrain, insular gray matter volume, right dorsolateral prefrontal cortex) may contribute to the development of problematic alcohol use (17,31). It is likely that there are also differences in brain measures that are predisposing factors for the initiation of smoking behaviors (32). While we acknowledge that there are studies that support the notion that regional brain differences may be a predisposing factor for alcohol consumption, we focused our investigation on the relationship between smoking behavior and global brain volume. The evidence presented in this study suggests that the changes in total brain volume, total gray matter volume, total white matter volume, and subcortical/cortical regional volumes more likely reflect adverse consequences of a history of daily smoking behavior. In addition, hippocampal volume, an important brain region affected by Alzheimer's disease, is negatively associated with a history of daily smoking. This finding is consistent with smoking, which has been identified as a modifiable risk factor for Alzheimer's disease, accelerating the development of this illness (7). These brain changes seem to be long-lasting, and we found no evidence of an increase in brain volume after smoking cessation.

Finally, in addition to studying the total brain measures, we examined whether subregions of the brain were more or less associated with daily smoking after correcting for total brain volume. For cortical regions, we found that the thickness of the superior frontal cortex was negatively associated with daily smoking, which is consistent with the evidence found in recent studies that smoking is associated with cortical thinning (33,34). Additionally, we identified that the volume of the superior frontal cortex, rostral middle frontal cortex, and precentral gyrus were more negatively associated with daily smoking beyond the overall decrease in total brain volume associated with a history of daily smoking. For the cerebellum, the volume of cerebellum white matter in the left hemisphere was negatively associated with daily smoking, and the volume of the corpus callosum also showed a negative association, as shown in the previous studies (35,36). The volume of the thalamus and amygdala were more negatively associated with daily smoking, as shown in the previous studies (26,33,34,37). We found that the volume of the choroid plexus, lateral ventricle, and third ventricle were more positively associated with daily smoking than the other regions. These areas are the essential parts or paths of the CSF system (38,39), and these findings are consistent with a compensatory increase in CSF volume as total brain volume decreases.

Limitations and Future Directions

The best way to address causation is through triangulation of data and convergent evidence including cross-sectional association, longitudinal data, and experimental paradigms. The UK Biobank dataset is large and provides ample statistical power, and we examined cross-sectional brain imaging data. Longitudinal neuroimaging data from UK Biobank is growing, but it remains limited at this time. Importantly, almost all participants in the UK Biobank study who smoked had quit smoking by the time of the first assessment, which limits longitudinal analyses of the effect of current smoking on subsequent brain imaging measures. There is also the need for prospective developmental data to better understand the complex interplay between behavior and brain structure. The Adolescent Brain Cognitive Development (ABCD) Study, the largest neuroimaging study of brain development conducted to date in the United States, will be best able to disentangle what brain measures represent predisposing factors to substance use and adverse consequences from substance use.

Conclusions

We examined the nature of the relationship between daily smoking and brain imaging phenotypes using traditional epidemiological criteria (Hill's criteria) and genetics tools (PRS and mediation analysis) in a large dataset of participants. There was a dose effect, with a history of heavier smoking being associated with more severe adverse effects. We found minimal evidence that a genetic predisposition to smoking was associated with total brain volume, and this association became nonsignificant when a history of daily smoking was set as a mediator variable. Thus, mediation analysis further supported the effect of smoking leading to decreases in brain volume. We found that a history of smoking was strongly associated with adverse changes in total brain volumes and certain cortical, cerebellar, and subcortical regional volumes. Finally, there was no evidence of an increase in brain volume following smoking cessation. Taken together, these findings provide additional evidence that a history of daily smoking is strongly associated with long-term global adverse consequences in the brain.

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