Stroke

CLINICAL AND POPULATION SCIENCES

# Genetically Determined Smoking Behavior and Risk of Nontraumatic Subarachnoid Hemorrhage

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**BACKGROUND AND PURPOSE:** Animal and observational studies indicate that smoking is a risk factor for aneurysm formation and rupture, leading to nontraumatic subarachnoid hemorrhage (SAH). However, a definitive causal relationship between smoking and the risk of SAH has not been established. Using Mendelian randomization (MR) analyses, we tested the hypothesis that smoking is causally linked to the risk of SAH.

**METHODS:** We conducted a 1-sample MR study using data from the UK Biobank, a large cohort study that enrolled over 500 000 Britons aged 40 to 69 from 2006 to 2010. Participants of European descent were included. SAH cases were ascertained using a combination of self-reported, electronic medical record, and death registry data. As the instrument, we built a polygenic risk score using independent genetic variants known to associate ($P<5\times10^{-8}$) with smoking behavior. This polygenic risk score represents the genetic susceptibility to smoking initiation. The primary MR analysis utilized the ratio method. Secondary MR analyses included the inverse variance weighted and weighted median methods.

**RESULTS:** A total of 408 609 study participants were evaluated (mean age, 57 [SD 8], female sex, 220 937 [54%]). Among these, 132 566 (32%) ever smoked regularly, and 904 (0.22%) had a SAH. Each additional SD of the smoking polygenic risk score was associated with 21% increased risk of smoking (odds ratio [OR], 1.21 [95% CI, 1.20–1.21]; $P<0.001$) and a 10% increased risk of SAH (OR, 1.10 [95% CI, 1.03–1.17]; $P=0.006$). In the primary MR analysis, genetic susceptibility to smoking was associated with a 63% increase in the risk of SAH (OR, 1.63 [95% CI, 1.15–2.31]; $P=0.006$). Secondary analyses using the inverse variance weighted method (OR, 1.57 [95% CI, 1.13–2.17]; $P=0.007$) and the weighted median method (OR, 1.74 [95% CI, 1.06–2.86]; $P=0.03$) yielded similar results. There was no significant pleiotropy (MR-Egger intercept $P=0.39$; MR Pleiotropy Residual Sum and Outlier global test $P=0.69$).

**CONCLUSIONS:** These findings provide evidence for a causal link between smoking and the risk of SAH.

**GRAPHIC ABSTRACT:** An online graphic abstract is available for this article.

**Key Words:** genetic variation ■ hemorrhagic stroke ■ intracranial aneurysm ■ subarachnoid hemorrhage



Nontraumatic subarachnoid hemorrhage (SAH) is an uncommon subtype of stroke that carries high morbidity and mortality. SAH affects mainly middle-aged individuals, leading to substantial morbidity in persons with long survival.[1] Smoking is a well-known risk factor for multiple cardiovascular conditions. Several observational studies have shown that smoking is associated with an increased risk of SAH,[2–4] with stronger associations observed for women.[5] However, a definitive causal relationship between smoking and SAH risk is difficult to establish as it would be unethical to perform clinical trials utilizing a harmful intervention such as smoking.

Population genetics offers powerful tools to evaluate causality for nongenetic exposures.[6] Genetic variants

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## Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **MR** | Mendelian randomization |
| **OR** | odds ratio |
| **PRS** | polygenic risk score |
| **SAH** | subarachnoid hemorrhage |
| **SNP** | single-nucleotide polymorphism |

known to be strongly associated with smoking behavior can be used as instruments in Mendelian randomization (MR) analyses to evaluate the causal relationship between smoking and risk of SAH. These genetic variants are randomly distributed during meiosis and are therefore relatively immune to confounding by environmental factors. A previous study has shown a causal relationship between smoking and ischemic stroke using this approach[7] but, to date, similar studies have not been pursued for SAH. We therefore conducted a MR study to test the hypothesis that smoking is causally linked to an increased risk of SAH.

# METHODS

## Data Availability

Data from the UK Biobank is publicly available upon request.

## Study Design

The UK Biobank is a large cohort study that enrolled over 500 000 persons aged 40 to 69 years from across the United Kingdom. It ascertained numerous population characteristics and collected biological samples and genetic information.[8] This large prospective study received approval by the appropriate institutional review board. All participants provided informed consent. For this study, we included participants from genetically confirmed European ancestry only.

## Outcome Data

First-ever SAH cases were ascertained using the algorithmically defined outcomes available at the UK Biobank. This ascertainment process combines data from (1) the baseline interview conducted when study participants were enrolled in the study, (2) nation-wide monitoring systems (Hospital Episode Statistics [England], Scottish Morbidity Records [Scotland], and Patient Episode Database [Wales]) that capture data from all admissions, before and after enrollment in the study; and (3) death register data. The last 2 data sources use *International Classification of Diseases (ICD)* codes to capture information on specific conditions. For SAH, the *ICD-9* code 430.X and the *ICD-10* 160.X were used. To avoid misclassification that could lead to bias, we only used SAH events with an associated hospital admission.

## Genetic Data

UK Biobank participants were genotyped using UK Biobank Axiom Array. Standard quality control procedures were performed centrally by the UK Biobank research team, as previously reported. Imputation was also performed centrally, using a reference panel composed by the UK10K haplotype[9] and 1000 Genomes Phase 3[10] reference panels,[11] and the same algorithm implemented by the IMPUTE2 program.[12] We implemented postimputation quality control filters, including minor allele frequency <1% and information score <0.7 and used principal component analysis to account for population structure.

## Instrumental Variable

MR analysis constitutes a special case of instrumental variable analysis. For this study, the instrument was a polygenic risk score (PRS) that represents the genetic propensity to smoke. A recent genome-wide association meta-analysis of 1.2 million persons identified several independent single-nucleotide polymorphisms (SNPs) associated with smoking initiation (defined as an individual ever smoking regularly).[13] We used summary statistics from this study to build a smoking-related PRS using independent ($r^2$ <0.1) SNPs known to be associated with smoking initiation at genome-wide levels ($P<5\times10^{-8}$). All selected SNPs were aligned to the GRCh37 assembly of the human genome. To assure common directionality of effects, for each SNP, the allele associated with an increase in propensity to smoke was identified and utilized as the tested allele in downstream analyses. The PRS for each individual is the sum of the product of the risk allele counts for each locus multiplied by the allele's reported effect on the propensity to smoke.

## Statistical Analysis

We present discrete variables as counts (percentage [%]) and continuous variables as mean (SD) or median (interquartile range), as appropriate.

### *Nongenetic Observational Analysis*

We fitted bivariate and multivariable logistic regression models to assess the relationship between smoking initiation (ie, ever smoking regularly) and risk of SAH. We also conducted bivariate and multivariable regression analysis modeling the exposure as an ordinal variable defined by pack/y of smoking (0, 0.05–20, 20–40, and >40). Multivariable models included age, sex, and hypertension as covariates.

### *Polygenic Risk Score Analysis*

Using individual-level genetic data, we fitted multivariable logistic regression models to assess the relationship between the smoking-related PRS and both smoking initiation and SAH risk, adjusting for age, sex, and the first 4 genetic principal components. These association tests determine the risk of smoking initiation and the risk of SAH, respectively, associated with an increase of 1 SD in the PRS.

### *Primary MR Analysis*

We used the βs of the association tests between the smoking-related PRS and risk of smoking initiation and SAH to perform the MR ratio method.

### *Secondary MR Analyses*

Using summary statistics for each SNP included in the instrument, we implemented the inverse variance weighted and weighted median (WM) MR methods. These summary statistics were obtained by testing for associations between each SNP and both

smoking initiation and SAH risk within the UK Biobank, adjusting for age, sex, and the first 4 genetic principal components.

### *Pleiotropy*

Given the possibility that genetic variants influence the risk of SAH through pathways other than the exposure of interest (horizontal pleiotropy), we conducted pleiotropy analysis to identify and account for this potential bias. Using the same summary statistics, we implemented the MR-Egger[14] and MR Pleiotropy Residual Sum and Outlier[15] approaches to evaluate the presence of horizontal pleiotropy.

### *Stratified Analyses*

Because sex and hypertension[2–5] are well-known risk factors for SAH, we implemented stratified analyses by these variables and tested for interaction between these risk factors and the smoking-related PRS by adding product terms to the regression models.

### *Software*

We used PLINK (v1.9)[16] to conduct quality control procedures, PRS analysis, and single-SNP association testing, and the MR-PRESSO packages in R 3.6 to complete MR analyses. A 2-tailed $P<0.05$ was considered statistically significant for the single test utilized to evaluate the primary hypothesis that genetic propensity to smoke increases the risk of SAH.

## RESULTS

Of the 502 536 study participants enrolled in the UK Biobank, we excluded those who were not from European ancestry (n=92 907), had low quality genetic information based on standard quality control parameters (n=2012), or withdrew consent (n=48). Our final sample included a total of 408 609 persons (mean age at recruitment 57 years [SD 8], female sex 220 937 [54%]). Baseline features are depicted in Table 1.

### Smoking and Nontraumatic Subarachnoid Hemorrhage in the UK Biobank

In line with prior studies, we found a strong association between smoking and risk of SAH. Of the 408 609 study participants included in the analysis, 132 566 (32%) ever smoked regularly and 904 (0.22%) had SAH. In bivariate analysis, smoking initiation was associated with a 75% increased risk of SAH (odds ratio [OR], 1.75 [95% CI, 1.53–1.99]; $P<0.001$). In multivariable analysis adjusting by sex, age, and hypertension, smoking initiation was associated with a 78% increased risk of SAH (OR, 1.78 [95% CI, 1.56–2.03]; $P<0.001$). The relationship between smoking and SAH risk appeared to be linear according to pack/y of smoking, ranging from a 27% increased risk in those 0.05 to 20 pack/y to 2.5-fold increased risk in those smoking >40 pack/y (Table 2).

### Genetic Susceptibility to Smoke

Genetic susceptibility to smoking was strongly associated with both smoking initiation and risk of SAH. The smoking-related PRS included 126 SNPs (Table I in the Data Supplement). The mean minor allele frequency across all SNPs was 31% (SD 13%), including 5 (4%) low-frequency variants (ie, those with a minor allele frequency <5%). Each additional SD of this smoking PRS was associated with a 21% increased risk of smoking initiation in the UK Biobank population (OR, 1.21 [95% CI, 1.20–1.21]; $P<0.001$). Similarly, each additional SD of the smoking PRS was associated with a 10% increased risk of SAH (OR, 1.10 [95% CI, 1.03–1.17]; $P=0.006$).

### MR Analysis of Smoking and the Risk of SAH

In the primary analysis utilizing the ratio method, genetic susceptibility to smoking was associated with a 63% increase in risk of SAH (OR, 1.63 [95% CI, 1.15–2.31]; $P=0.006$). Secondary analyses using the inverse variance weighted method (OR, 1.57 [95% CI, 1.13–2.17]; $P=0.007$) and the WM method (OR, 1.74 [95% CI, 1.06–2.86]; $P=0.03$) yielded comparable results. There was no significant horizontal pleiotropy (Table 3), as evaluated by both the MR-Egger intercept ($P=0.39$) and the MR-PRESSO global test ($P=0.69$; Figure). MR analyses stratified by pack/y confirmed the association between genetic susceptibility to smoking and SAH risk across all evaluated strata but did not yield the linear relationship observed in the epidemiological part of our analysis. Compared with never smokers, the MR results were similar for those who smoked 0.05 to 20 pack/y (OR, 1.63

**Table 1. Baseline Characteristics**

| Characteristic | Overall (n=408 609) | Controls (n=407 705) | SAH (n=904) | *P* value |
|---|---|---|---|---|
| Age, y; mean (SD) | 56.9 (8.0) | 56.9 (8.0) | 57.87 (7.29) | <0.001 |
| Male sex, n (%) | 187 672 (45.9) | 187 336 (45.9) | 336 (37.2) | <0.001 |
| Ever smoked, n (%) | 132 566 (32.4) | 132 154 (32.4) | 412 (45.6) | <0.001 |
| Smoking, pack/y; mean (SD) | 7.2 (15.0) | 7.2 (15.0) | 12.12 (19.24) | <0.001 |
| Hypertension, n (%) | 108 595 (26.6) | 108 265 (26.6) | 330 (36.5) | <0.001 |
| Hyperlipidemia, n (%) | 50 469 (12.4) | 50 340 (12.3) | 129 (14.3) | 0.088 |
| Diabetes, n (%) | 19 245 (4.7) | 19 208 (4.7) | 37 (4.1) | 0.425 |
| Atrial fibrillation, n (%) | 3174 (0.8) | 3165 (0.8) | 9 (1.0) | 0.575 |

SAH indicates subarachnoid hemorrhage.

**Table 2. Observational, Nongenetic Association Between Smoking and Risk of SAH**

| | Risk of subarachnoid hemorrhage | | | |
|---|---|---|---|---|
| | Bivariate analysis | | Multivariable analysis | |
| Exposure | OR (95% CI) | *P* value | OR (95% CI) | *P* value |
| Smoking status | | | | |
| Never smoked | 1 | … | 1 | … |
| Former smoker | 1.49 (1.28–1.72) | <0.001 | 1.47 (1.27–1.71) | <0.001 |
| Current smoker | 2.60 (2.17–3.11) | <0.001 | 2.80 (2.33–3.35) | <0.001 |
| Lifelong exposure | | | | |
| 0 pack/y | 1 | … | 1 | … |
| 0.05–20 pack/y | 1.25 (1.03–1.49) | 0.02 | 1.27 (1.05–1.52) | 0.01 |
| 20–40 pack/y | 2.02 (1.68–2.41) | <0.001 | 2.03 (1.69–2.44) | <0.001 |
| >40 p/y | 2.51 (1.99–3.13) | <0.001 | 2.58 (2.04–3.24) | <0.001 |

OR indicates odds ratio; and SAH, subarachnoid hemorrhage.

[95% CI, 1.01–2.62]; *P*=0.04), 20 to 40 pack/y (OR, 1.65 [95% CI, 1.13–2.41]; *P*=0.009), and >40 pack/y (OR, 1.56 [95% CI, 1.08–2.25]; *P*=0.02).

## Stratification by Sex and Hypertension

In stratified analyses (Table 4), the point estimate for the association between the smoking PRS and SAH risk was higher and significant in women (OR, 1.14 [95% CI, 1.05–1.24]) compared with men (OR, 1.02 [95% CI, 0.92–1.14]). Similarly, the point estimate for the association between smoking PRS and SAH risk was higher and significant for hypertensives (OR, 1.12 [95% CI, 1.01–1.25]) compared with normotensives (OR, 1.07 [95% CI, 0.99–1.17]). We obtained similar results when conducting stratified MR analyses (Table 4). Despite these results, neither the formal test for interaction for sex (interaction *P*=0.22) nor the one for hypertension (interaction *P*=0.49) was statistically significant.

# DISCUSSION

We found that a stronger genetic predisposition to smoking is significantly associated with an increased risk of SAH and that this association might be stronger in women and persons with hypertension. These findings provide important evidence to support a causal relationship between smoking and SAH.

Observational evidence has consistently shown a robust and dose-dependent relationship between smoking and the risk of SAH.[2,3,5,17–19] Smoking cessation, on the other hand, seems to decrease the risk of SAH, but some degree of the attributable risk may be irreversible, especially in former heavy smokers.[20,21] Moreover, laboratory and experimental studies in animal propose oxidative stress and inflammation as the mediating mechanisms behind this link.[22,23] In line with these studies, epidemiological work has found that the incidence in SAH is currently decreasing in parallel with decreases in the smoking rate.[24–26] In our study, we found a robust association between smoking and risk of SAH with a clear dose-response relationship that ranged from 27% increase in SAH risk in those smoking <20 p/y to almost a 3-fold increase in those smoking >40 p/y. This recapitulation of known smoking-SAH associations in our study population constituted a necessary condition to conduct genetic analysis aimed at evaluating the casual association between genetically determined smoking and risk of SAH.

**Table 3. MR Results**

| MR method | Data type | OR (95% CI) | *P* value |
|---|---|---|---|
| Association tests | | | |
| Ratio method | Individual level data | 1.63 (1.15–2.31) | 0.006 |
| Inverse variance weighted | Summary statistics | 1.57 (1.13–2.17) | 0.007 |
| Weighted median | Summary statistics | 1.74 (1.06–2.86) | 0.028 |
| MR-PRESSO | Summary statistics | 1.56 (1.14–2.15) | 0.006 |
| Pleiotropy tests | | | |
| MR-Egger intercept | Summary statistics | 1.01 (0.98–1.05) | 0.39 |
| MR-PRESSO global test | Summary statistics | … | 0.69 |

MR indicates Mendelian randomization; MR-Egger, MR using Egger regression; MR-PRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier; OR, odds ratio; PRS, polygenic risk score; and IVW, Inverse variance weighted.

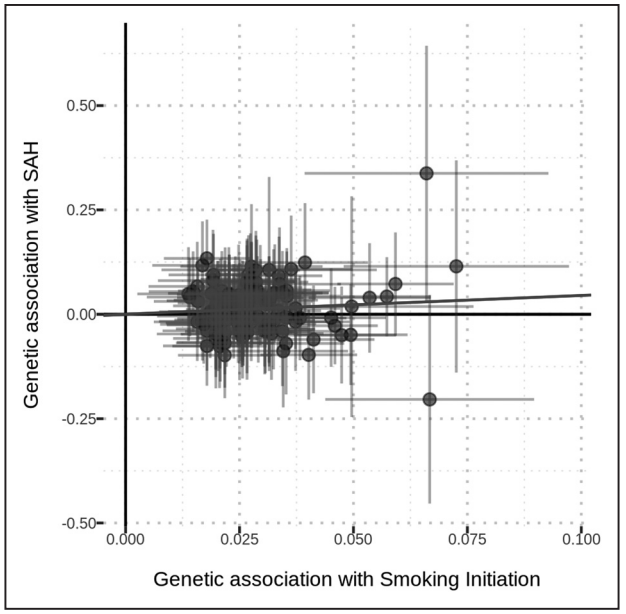


**Figure. Mendelian randomization plot.**
The plot presents the effect estimates of association tests between the single-nucleotide polymorphisms included in the instrument and smoking initiation (*x* axis) and risk of subarachnoid hemorrhage (SAH; *y* axis).

However, deriving causality from the observational evidence outlined above is problematic because of the possibility of bias introduced by confounding factors. Addressing this question through experimental studies in humans (ie, randomized clinical trials) would be unethical given the known harms produced by smoking. Population genetics provides powerful tools to overcome these limitations in causal inference. Genetic variants known to associate with smoking can be used as instruments in MR analyses aimed to evaluate this causal relationship, as these variants are randomly distributed during meiosis and ought to be exempt from confounding by environmental exposures. Using this approach, we found that genetic susceptibility to smoking initiation was associated with a 60% increase in the risk of SAH. Importantly, sensitivity analyses using more conservative methods aimed to decrease the possibility of horizontal pleiotropy yielded similar results. While the interaction analyses between the genetic susceptibility to smoking initiation and sex and hypertension, 2 other important determinants of SAH, were not statistically significant, the higher point estimates for these associations in women and hypertensives point to an important future direction of research.

The most important strengths of our study are its large sample size and the availability of individual-level genetic data from all study participants. In terms of limitations, the utilization of *ICD* codes to ascertain SAH cases in the UK Biobank may have introduced some misclassification of the outcome. However, while certainly limited in its ability to fully capture the diagnostic nuances of SAH cases, the *ICD* system does have appropriate codes to differentiate traumatic and nontraumatic SAH (we used the latter). In addition, even if present, the aforementioned misclassification is likely randomly distributed across levels of the exposure, thus introducing bias toward the null. A second important limitation is the absence of an independent dataset to replicate the presented results. The relatively low incidence of SAH in Western populations limits the amount of cases with available genetic data. Nevertheless, because this study was not intended at risk loci discovery, it could be argued that independent replication for this specific analysis is not strictly needed. Another important limitation is that our study was limited to genetically determined Whites. As a result, our results cannot be immediately extrapolated to other racial/ethnic populations. Because of the disproportion of available genetic data in Whites versus other race/ethnic groups is becoming a serious barrier to conduct genetic studies in non-White persons, this particular limitation should be addressed soon by follow-up studies.

SAH is an uncommon disease with a limited number of studies with available genetic information. As a result, international collaborations will be fundamental to replicate, externally validate, and extend the results of our study. In addition, these large data resources would provide the ideal framework to evaluate whether our results can be used to develop precision medicine approaches aimed at detecting persons at high risk of intracranial aneurysms. This is an appealing research avenue, as genetic information could identify persons that may benefit from early screening with vessel imaging studies, like computed tomography or magnetic resonance angiography.

**Table 4. Stratified Analyses by Sex and Hypertension**

| Stratification variable | Polygenic risk score analysis OR (95% CI) | Mendelian randomization analysis OR (95% CI) | Interaction *P* value |
|---|---|---|---|
| Sex | | | |
| Female | 1.14 (1.04–1.24) | 1.99 (1.29–3.08) | 0.22 |
| Male | 1.02 (0.92–1.14) | 1.14 (0.64–2.02) | |
| Hypertension | | | |
| Yes | 1.12 (1.01–1.25) | 2.04 (1.06–3.95) | 0.49 |
| No | 1.07 (0.99–1.17) | 1.45 (0.95–2.21) | |

OR indicates odds ratio.

## CONCLUSIONS

We used MR analyses to evaluate whether genetic predisposition to smoking initiation is causally related to the risk of SAH in a large population of British persons. We found that a stronger genetic predisposition to smoking is significantly associated with an increased risk of SAH. These findings provide important evidence







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to support a causal relationship between smoking and the risk of SAH.

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### Supplemental Materials

Supplementary Table I
Reference 13

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