Original Contribution

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Causal Impact of Type 2 Diabetes Mellitus on Cerebral Small Vessel Disease

A Mendelian Randomization Analysis

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Background and Purpose—The relationship between type 2 diabetes mellitus (T2D) and cerebral small vessel disease (CSVD) is unclear. We aimed to examine the causal effect of T2D, fasting glucose levels, and higher insulin resistance on CSVD using Mendelian randomization.

Methods—Five CSVD phenotypes were studied; 2 were clinical outcomes associated with CSVD (lacunar stroke: n=2191/27 297 and intracerebral hemorrhage [ICH]: n=2254/8195 [deep and lobar ICH]), whereas 3 were radiological markers of CSVD (white matter hyperintensities: n=8429; fractional anisotropy [FA]: n=8357; and mean diffusivity: n=8357). We applied 2 complementary analyses to evaluate the association of T2D with CSVD. First, we used summarized data from genome-wide association study to calculate the effects of T2D-related variants on CSVD with inverse-variance weighted and weighted median approaches. Second, we performed a genetic risk score approach to test the effects of T2D-associated variants on white matter hyperintensities, FA, and mean diffusivity using individual-level data in UK Biobank.

Results—T2D was associated with higher risk of lacunar stroke (odds ratio [OR], 1.15; 95% confidence interval [CI], 1.04–1.28; P=0.007) and lower mean FA (OR, 0.78; 95% CI, 0.66–0.92; P=0.004) but not white matter hyperintensities volume (OR, 1.01; 95% CI, 0.97–1.04; P=0.626), higher mean diffusivity (OR, 1.04; 95% CI, 0.89–1.23; P=0.612), ICH (OR, 1.07; 95% CI, 0.95–1.20; P=0.269), lobar ICH (OR, 1.07; 95% CI, 0.89–1.28; P=0.466), or deep ICH (OR, 1.16; 95% CI, 0.99–1.36; P=0.074). Weighted median and penalized median weighted analysis showed similar effect estimates of T2D on lacunar stroke and FA, but with wider CIs, meaning they were not significant. The genetic score on individual-level data was significantly associated with FA (OR, 0.63; 95% CI, 0.45–0.89; P=0.008) after adjusting for potential confounders.

Conclusions—Our Mendelian randomization study provides evidence to suggest that T2D may be causally associated with CSVD, in particular with lacunar stroke and FA. **(*Stroke*. 2018;49:00-00. DOI: 10.1161/STROKEAHA.117.020536.)**

Key Words: cerebral small vessel disease ■ diabetes mellitus, type 2 ■ insulin resistance ■ Mendelian randomization analysis ■ stroke, lacunar

Cerebral small vessel disease (CSVD) is an age-related disease affecting the small blood vessels of the brain.[1,2] It accounts for at least 25% of all strokes and is the most common cause of vascular dementia.[1,2] Several neuroimaging features are associated with CSVD, including lacunar infarcts, white matter hyperintensities (WMH), enlarged perivascular spaces, microbleeds, and brain atrophy. Intracerebral hemorrhages (ICH), particularly those arising from deep perforating small vessels, are also believed to be caused by CSVD.[2] In addition, diffusion tensor imaging measures, such as fractional anisotropy (FA) and mean diffusivity (MD), are thought to capture microstructural changes of the white matter related to CSVD.[3]

However, the pathogenesis of CSVD is still uncertain,[2] and consequently few effective and mechanism-based treatments for CSVD are available, aside from management of vascular risk factors of CSVD.[2] Understanding which vascular risk factors are truly causal and improved understanding of how

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these pathological processes relate to different neuroimaging features of CSVD has the potential to improve treatment and prevention of CSVD.

Type 2 diabetes mellitus (T2D) is an established risk factor for ischemic stroke and cognitive decline.[4,5] Epidemiological studies have suggested that T2D is associated with lacunar stroke,[6,7] but the relationships of T2D with WMH, ICH, and other radiological markers of CSVD have been inconsistent.[4,6–9] Such studies are also limited by the study design, which can be confounded. Therefore, a definitive causal association between T2D and CSVD is yet to be established. In addition, few studies have specifically studied the relationship between higher insulin resistance and fasting glucose levels and risk of CSVD,[10–12] leaving a gap in knowledge regarding whether either is the driving force behind increased risk of CSVD.

Mendelian randomization (MR), using genetic variants as instrumental variables, is a method that enables stronger claims to be made about the causality of risk factors in disease pathogenesis.[13] It is based on the theory that genetic variants are randomly allocated at meiosis, similar to a randomized controlled trial.[14] Therefore, genetic variants are independent of many other factors that bias observational studies, such as confounding and reverse causation. In the absence of pleiotropy, a significant association in an MR study between an exposure and outcome implies causality. In the present study, we aimed first to use MR to determine whether T2D is causally associated with clinical outcomes associated with CSVD; lacunar stroke and ICH; as well as intermediate radiological markers of CSVD; WMH, FA and MD. Second, we performed exploratory analyses investigating the relationship between higher insulin resistance and fasting glucose levels and risk of CSVD. All analyses are based on the aggregate effects of genetic variants rather than clinically diagnosed T2D.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design, Data Sources, and Ethical Approval

We performed a MR analysis, testing the causal relationship of T2D, fasting glucose, and fasting insulin with 5 manifestations of CSVD (magnetic resonance imaging–confirmed lacunar stroke, ICH [alone and stratified by the location of hemorrhage: deep ICH and lobar ICH], WMH, FA, and MD). Analyses of all CSVD phenotypes were based on subjects of European ancestry only.

For ICH, we used a data set composed of 2254 cases and 8195 controls from 3 studies. One thousand five hundred forty-five cases and 1481 controls were from the Intracerebral Hemorrhage Genetics Collaboration and downloaded from the Cerebrovascular Disease Knowledge Portal (http://cerebrovascularportal.org).[15] Five hundred seventy-five cases and 5750 propensity score–matched controls (matched on age, sex, and ancestry-informative principal components) were from UK Biobank, based on algorithmically defined ICH in White British subjects.[16] One hundred thirty four cases and 964 controls were from the Cambridge ICH Genetics Study. For full details, Methods section in the online-only Data Supplement.

The magnetic resonance imaging–confirmed lacunar stroke data were derived from 2191 lacunar stroke cases with magnetic resonance imaging confirmation from the SIGN-NINDS,[17] WTCCC2,[18] and DNA lacunar genome-wide association studies and 27 297 controls.[19] All cases were obtained based on hospital admissions.

Radiological markers of CSVD were derived from UK Biobank. Procedures for brain imaging acquisition and initial quality check have been described previously and are available on the UK Biobank website (Brain Imaging Documentation V1.3, http://www.ukbiobank.ac.uk).[20] For FA and MD, we analyzed the first principal component of mean FA or MD values across 48 standard space tracts in 8357 individuals. WMH data were based on an analysis of 8429 subjects from UK Biobank. Additional details are provided in the Methods section in the online-only Data Supplement.

Study characteristics for each of the data sets are provided in Table 1.

An IRB or regional review board has approved the use of human subjects in each of the study populations. All patients gave informed consent. UK Biobank received ethical approval from the research ethics committee (REC reference 11/NW/0382). The present analyses were conducted under UK Biobank application number 19463.

Single-Nucleotide Polymorphism Selection

We selected single-nucleotide polymorphisms (SNPs) associated with T2D, fasting insulin, and fasting glucose, which reached genome-wide significance in the largest genome-wide association meta-analyses to date.[21–23] Eighty-four SNPs were included for T2D, 36 for fasting glucose, and 18 for fasting insulin (Table I in the online-only Data Supplement). In instances where SNPs were not available in a data set because of poor imputation quality, we replaced them with proxy SNPs if available (r^2>0.7). We included only 1 SNP from any associated locus. We verified that SNPs were uncorrelated by performing LD clumping (r^2>0.1, 100 kb) using PLINK.[24]

Statistical Analyses

We performed 2 complementary analyses to evaluate the impact of T2D-associated variants on CSVD phenotypes. For our primary analysis of the association of T2D with all CSVD phenotypes using 2-sample MR on summary statistics, we used a significance threshold

Table 1. Cohort Characteristics

CSVD Phenotypes	N	Age, Mean (SD)	% Male	% Diabetes Mellitus	% Hypertension
MRI-confirmed lacunar stroke	2191 cases, 27 297 controls	65 (14)	63	19	71
Intracerebral hemorrhage	2254 cases, 8195 controls	ICH GWAS: 67 (10) Cambridge ICH: 71 (13) UK Biobank: 61 (7)	ICH GWAS: 55 Cambridge ICH: 55 UK Biobank: 57	N/A	N/A
Deep intracerebral hemorrhage	957 cases, 2445 controls				
Lobar intracerebral hemorrhage	722 cases, 2445 controls				
White matter hyperintensity	8429	62 (7)	48	3	46
Fractional anisotropy	8357	62 (7)	48		
Mean diffusivity	8357	62 (7)	48		

CSVD indicates cerebral small vessel disease; and N/A, not available.

of $P<0.0071$, equivalent to Bonferroni correction for 7 independent tests. For radiological markers of CSVD, we performed a confirmatory analysis using individual-level data in UK Biobank:

Two-Sample MR Using Summary Statistics

We assessed the impact of risk factor–associated SNPs on each CSVD phenotype using MR approaches. Our primary analysis used an inverse-variance weighted meta-analysis approach (conventional MR). We then performed secondary analyses using weighted median and penalized weighted median approaches. We assessed the potential role of directional pleiotropy by testing if the intercept from MR-Egger regression was significantly different from zero.

As sensitivity analysis, we performed a look-up of all the SNPs used in our study in Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk/phenoscanner) to evaluate whether these SNPs were associated with other traits at genome-wide significance level which may affect our results. We found 8 SNPs related to T2D (rs2925979, rs2943640, rs3794991, rs3923113, rs429358, rs459193, rs635634, and rs780094), which were also associated with lipids and kidney function, as well as 4 SNPs for fasting glucose (rs174550, rs17762454, rs780094, and rs983309) and 9 SNPs for fasting insulin (rs10195252, rs1530559, rs2126259, rs2745353, rs2943645, rs3822072, rs459193, rs731839, and rs780094). We then reassessed the results after excluding these SNPs. Additionally, for significant findings, we assessed the potential that the association was mediated by body mass index (BMI) by performing a sensitivity analysis, removing 8 SNPs, which are also associated with BMI at genome-wide significance (rs2943640, rs11671664, rs12970134, rs8050136, rs10146997, rs5215, and rs7903146). All analyses were performed using the Mendelian Randomization and gtx libraries in R version 3.3.2 (https://www.R-project.org/).

Two-Sample MR Using Genetic Risk Scores on Individual-Level Data

For each of the 84 SNPs associated with T2D, we constructed a genetic risk score for each individual in UK Biobank by multiplying the log of the odds ratio (OR) for association with T2D by the number of risk alleles and summing this value for each individual. We then used a linear regression model to evaluate the effect of the genetic score on WMH, FA, and MD, with adjustment for genotyping batch, age, sex, BMI, blood pressure, and ancestry-informative principal components to control for these potential confounding factors.

We also assessed their associations by constructing quartiles of the genetic score and calculated the ORs and 95% confidence interval (CI) for the score quartiles using quantile 1 as a reference, thus quantile 2 versus quantile 1, quantile 3 versus quantile 1, and quantile 4 versus quantile 1.

Results

MR Results: Associations of T2D With CSVD Phenotypes

Conventional MR estimates for the effect of T2D on clinical outcomes associated with CSVD (lacunar stroke, ICH, deep ICH, and lobar ICH) and radiological markers of CSVD (WMH, FA, and MD) are displayed in the Figure and Table 2.

T2D was associated with higher risk of lacunar stroke (OR, 1.15; 95% CI, 1.04–1.28; P=0.007) and lower mean FA (OR, 0.78; 95% CI, 0.66–0.92; P=0.004). Conversely, we did not find any significant associations of T2D with WMH volume (OR, 1.01; 95% CI, 0.97–1.04; P=0.626) and higher mean MD (OR, 1.04; 95% CI, 0.89–1.23; P=0.612). Regarding the effect of T2D on ICH, lobar ICH, and deep ICH, none were found to be significant (ICH risk: OR, 1.07; 95% CI, 0.95–1.20; P=0.269; lobar ICH risk: OR, 1.07; 95% CI, 0.89–1.28; P=0.466; deep ICH risk: OR, 1.16; 95% CI, 0.99–1.36; P=0.074). Although nonsignificant, the odds ratio for association with deep ICH was near significant with an OR and direction of effect similar to that seen for lacunar stroke.

Weighted median and penalized median weighted analysis yielded similar effect estimates of T2D on lacunar stroke and FA, but the CIs were wide, so were not significant (Table 2). To assess the robustness and consistency of the results, we also conducted a sensitivity analysis by excluding the SNPs associated with lipids and kidney function at genome-wide significance level. In this sensitivity analysis, the associations of T2D with lacunar stroke and FA remained significant (Table III in the online-only Data Supplement). To assess whether the results were influence by BMI, we repeated the analyses for T2D and lacunar stroke as well as T2D and FA, both of which showed only minor differences after removing the SNPs (lacunar stroke: OR, 1.14; 95% CI, 1.01–1.29; P=0.028 and FA: OR, 0.78; 95% CI, 0.64–0.94; P=0.0095).

MR-Egger regression showed no evidence of directional pleiotropy for the effects of T2D on lacunar stroke (intercept=0.008; P=0.515), ICH (intercept=0.009; P=0.528), deep ICH (intercept=0.022; P=0.218), lobar ICH (intercept=0.014; P=0.539), WMH (intercept=0.003; P=0.518), FA (intercept=−0.003; P=0.901), and MD (intercept=0.004; P=0.869).

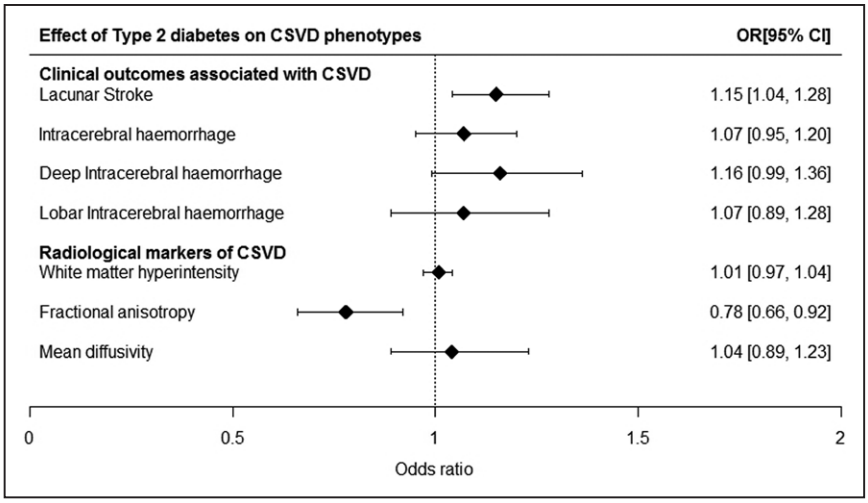

Figure. Mendelian randomization estimates for the effect of type 2 diabetes mellitus (T2D) on cerebral small vessel disease (CSVD) phenotypes. Analyses were performed with conventional Mendelian randomization analysis (inverse-variance weighted method). CI indicates confidence interval; and OR, odds ratio.

Table 2. Mendelian Randomization Estimates for the Effect of T2D on CSVD Phenotypes Using Inverse-Variance Weighted, Weighted Median, and Penalized Weighted Median Methods

	Inverse-Variance Weighted		Weighted Median		Penalized Weighted Median	
CSVD Phenotypes	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
MRI-confirmed lacunar stroke	1.15 (1.04–1.28)	0.007	1.19 (0.98–1.43)	0.075	1.19 (0.98–1.44)	0.076
Intracerebral hemorrhage	1.07 (0.95–1.20)	0.269	1.10 (0.89–1.35)	0.370	1.10 (0.89–1.35)	0.367
Deep intracerebral hemorrhage	1.16 (0.99–1.36)	0.074	1.06 (0.81–1.38)	0.697	1.05 (0.80–1.38)	0.743
Lobar intracerebral hemorrhage	1.07 (0.89–1.28)	0.466	1.15 (0.83–1.61)	0.399	1.16 (0.82–1.62)	0.401
White matter hyperintensity	1.01 (0.97–1.04)	0.626	0.98 (0.92–1.04)	0.528	0.98 (0.92–1.04)	0.534
Fractional anisotropy	0.78 (0.66–0.92)	0.004	0.74 (0.54–1.02)	0.068	0.74 (0.54–1.02)	0.064
Mean diffusivity	1.04 (0.89–1.23)	0.612	1.08 (0.81–1.45)	0.598	1.08 (0.81–1.45)	0.582

Significance threshold was set at $P<0.0071$. CI indicates confidence interval; CSVD, cerebral small vessel disease; OR, odds ratio; and T2D, type 2 diabetes mellitus.

Genetic Risk Score Analyses: Effects of T2D on WMH, FA, and MD

Results of the T2D genetic risk score on WMH, FA, and MD are presented in Table 3. The genetic score including 84 T2D-associated SNPs was significantly associated with FA (OR, 0.63; 95% CI, 0.45–0.89; $P=0.008$) after adjustment for genotyping batch, age, sex, BMI, blood pressure, and ancestry-informative principal components. We note that this result is significant when correcting for the 3 phenotypes studied in this secondary analysis but does not reach $P<0.0071$, the threshold used in our primary analysis. Conversely, the risk score was not significantly associated with WMH (OR, 1.01; 95% CI, 0.94–1.09; $P=0.727$) or MD (OR, 1.09; 95% CI, 0.78–1.52; $P=0.613$).

Stratifying by quartiles of the genetic score, we only found a nominally significant effect of quantile 4 compared with quantile 1 of the score on FA (OR, 0.73; 95% CI, 0.57–0.94; $P=0.015$).Odds ratios±SE for WMH, FA, and MD based on the quartiles of the genetic score were plotted (Figures I through III in the online-only Data Supplement).

Associations of Fasting Glucose and Insulin With CSVD Phenotypes

Fasting glucose and insulin were not associated with any CSVD phenotypes (lacunar stroke, ICH, deep ICH, lobar ICH, WMH, FA, and MD) in the present study using inverse-variance weighted, weighted median, or MR-Egger regression methods (Table II in the online-only Data Supplement).

Discussion

Prevention and management of CSVD is limited by our understanding of causal factors underlying the disease process. T2D is an established risk factor for CSVD, but a causal relationship is yet to be determined, and its impact on different CSVD phenotypes is not well understood. Using genetic data via an MR approach, we assessed the causal relationship between T2D and different CSVD phenotypes. Our primary analysis showed that a genetic predisposition to T2D was related to lacunar stroke and FA. We performed secondary analyses using an alternative weighted median method, which showed similar effects but wider CIs. Evidence indicating that the associations are robust is provided by the fact that (1) the results remained in sensitivity analyses removing pleiotropic SNPs and (2) associations with FA were significant when performing an alternative analysis based on individual-level data in UK Biobank, in which we were able to adjust for potential confounding factors.

The results were consistent with previous observational studies showing a positive association of T2D with risk of lacunar stroke[6,7] and an MR analysis using small artery occlusion strokes based on the TOAST classification (Trial of ORG 10172 in Acute Stroke Treatment).[25] However, the OR values

Table 3. Association of the T2D-Related SNPs With WMH, FA, and MD in UK Biobank Using Linear Regression*

	White Matter Hyperintensity		Fractional Anisotropy		Mean Diffusivity	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Multi-SNP score	1.01 (0.94–1.09)	0.727	0.63 (0.45–0.89)	0.008	1.09 (0.78–1.52)	0.613
Quantile 1	Ref	Ref	Ref	Ref	Ref	Ref
Quantile 2†	0.98 (0.93–1.04)	0.521	0.82 (0.64–1.06)	0.126	0.96 (0.75–1.23)	0.745
Quantile 3†	0.99 (0.94–1.04)	0.604	0.84 (0.66–1.08)	0.173	0.89 (0.69–1.13)	0.337
Quantile 4†	1.01 (0.96–1.06)	0.661	0.73 (0.57–0.94)	0.015	1.03 (0.81–1.32)	0.788

CI indicates confidence interval; FA, fractional anisotropy; MD, mean diffusivity; OR, odds ratio; SNP, single-nucleotide polymorphism; T2D, type 2 diabetes mellitus; and WMH, white matter hyperintensity.

*Adjustment for genotyping batch, age, sex, body mass index, blood pressure, and ancestry-informative principal components.

†We constructed quartiles of the genetic score, and OR and 95% CI for the multi-SNP score quartiles were generated for quantile 2, quantile 3, and quantile 4 relative to quantile 1 (quantile 2 vs quantile 1, quantile 3 vs quantile 1, and quantile 4 vs quantile 1).

of the relationship between T2D and lacunar stroke from a meta-analysis published in 2006[6] ranged from 1.3 to 2.2 in different cohorts, which were larger than our MR analyses (OR, 1.15; 95% CI, 1.04–1.28). This may be explained by the fact that previous observational epidemiological associations may have been influenced by potentially important confounders such as sex, blood pressure, and dietary factors. It should also be noted that the OR in our study refers to the increased risk associated with genetic markers related to T2D. Therefore, as these do not capture the total variance associated with T2D, the estimate is likely to be smaller than those from epidemiological studies.

Studies of the association between T2D and pathogenic subtypes of ICH are limited, but a recent meta-analysis[9] included 19 case–control studies has reported that hemorrhagic stroke was 1.23-fold more prevalent in patients with diabetes mellitus, whereas the association was not observed in 3 population-based cohort studies.[26–28] Our study using an MR approach showed no significant associations of T2D on total ICH risk, and on different location of ICH risk (lobar and deep ICH[29,30]). Although not significant, the effect of T2D on deep ICH was similar to that seen in lacunar stroke, which is consistent with the idea that deep ICH is associated with CSVD. However, we had limited power because of the small sample size for ICH, and this needs to be studied in larger data sets.

To better assess the causality of the associations between T2D and cerebral white matter injury, we used macrostructural (WMH volume) and microstructural (FA and MD) brain magnetic resonance imaging measures for the total brain. First, both MR approach and genetic score analyses did not show any significant associations between T2D and WMH volume, which was similar to the results from 2 recent reviews[6,7] showing uncertainty for the effect of T2D on WMH. In addition, data from a randomized controlled trial study[31] found that intensive control of diabetes mellitus (hemoglobin A1c <6.0%) did not impede WMH progression and conversely caused more serious WMH for unclear reasons.

Second, FA and MD are 2 commonly used diffusion tensor imaging indices, which are highly sensitive to subtle white matter changes.[32] A few observational studies[33–36] have shown a decreased FA and increased MD in T2D patients compared with controls, and the results were largely independent of WMH volume, which suggested that microstructural integrity damage likely precedes WMH, and diffusion tensor imaging indices maybe early markers for brain damage in patients with T2D. The results from the current study partly confirmed the findings from these aforementioned observational studies. We found the significant association between T2D and FA using MR methods, which also remained consistent in genetic score analyses. Surprisingly, T2D was not significantly associated with increased MD, and the reason for this discrepancy is unclear. It has been suggested that FA decrease maybe modulated more directly by myelin alterations, whereas MD is more sensitive to cellularity, edema, and necrosis.[37] Our results might, therefore, point to demyelination being an important factor in T2D patients.

Strengths of our MR analysis include the use of multiple T2D SNPs, which enable us to construct a polygenic score to increase the precision of the estimates. In combination, the SNPs explained between 5% and 10% of the variance of T2D. Ours is the first MR study to investigate the relationship between T2D and CSVD phenotypes, and the design of MR study can prevent reverse causation and potential confounding factors,[13,14] such as lifestyle and dietary preference. However, there are some limitations in our MR study. First, in some of our MR analyses, the sample size was relatively small, which resulted in limited statistical power in the respective analyses, especially for the ICH analysis stratified by hemorrhage location. Second, effects of the genetic variants on T2D were obtained largely from European populations, and all the subjects included in our study were Europeans. Therefore, the results may not be generalizable to other populations. Additionally, we note that differences in baseline characteristics between the T2D and CSVD populations might have subtle influences on the effect estimates, meaning that OR values given here should be interpreted with this caveat. Limitations of MR include the potential for residual pleiotropy that could have influenced the results when T2D-associated SNPs also influence other traits. We note that this is often challenging to rule out with absolute certainty.[38] One possible pleiotropic pathway in this analysis is through elevated BMI. From the results presented here, we cannot rule out partial mediation of the genetic effects through this pathway. Finally, our significant association of T2D with lacunar stroke and FA was not confirmed after conducting weighted median and penalized weighted median methods. However, the weighted median approaches showed a similar effect size. MR studies with larger samples are, therefore, needed to confirm this result in the future.

Conclusions

Our MR study is consistent with a causal association between T2D and CSVD. In particular, we found evidence of associations with lacunar stroke and FA. Further MR studies with larger sample sizes are required to determine this with more certainty and to rule out associations with other CSVD phenotypes.

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Disclosures

None.

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Causal Impact of Type 2 Diabetes Mellitus on Cerebral Small Vessel Disease: A Mendelian Randomization Analysis

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SUPPLEMENTAL MATERIAL

Table I. Single nucleotide polymorphism used as instrumental variables in the Mendelian randomization analyses of type 2 diabetes, fasting glucose, and fasting insulin

SNP	Closest gene	Risk factor	Effect allele	Risk factor Beta
rs10146997	NRXN3/DIO2	T2D	G	0.068
rs10193447	*BCL11A*	T2D	T	0.068
rs10238625	*DGKB*	T2D	A	0.068
rs10507349	RNF6	T2D	A	-0.056
rs1061810	*HSD17B12*	T2D	A	0.077
rs10811661	CDKN2A/2B	T2D	C	-0.156
rs10830963	*MTNR1B*	T2D	G	0.094
rs10842994	KLHDC5	T2D	T	-0.081
rs1111875	HHEX	T2D	T	-0.107
rs11123406	BCL2L11	T2D	C	-0.049
rs111669836	*MAP3K11*	T2D	A	0.068
rs11257655	CDC123	T2D	T	0.077
rs11671664	GIPR	T2D	A	0.074
rs11708067	*ADCY5*	T2D	G	-0.104
rs11759026	*CENPW*	T2D	G	0.095
rs11787792	*GPSM1*	T2D	A	0.130
rs1182436	*MNX1*	T2D	C	0.077
rs12970134	MC4R	T2D	A	0.071
rs13266634	SLC30A8	T2D	T	-0.111
rs13292136	TLE4	T2D	T	-0.129
rs1359790	SPRY2	T2D	A	-0.077
rs1552224	ARAP1	T2D	C	-0.101
rs163184	KCNQ1 [1]	T2D	G	0.079
rs17106184	FAF1	T2D	A	-0.090
rs17168486	DGKB	T2D	T	0.093
rs1801282	PPARG	T2D	G	-0.113
rs2023681	*MTMR3/HORMAD2*	T2D	G	0.122
rs2050188	C6orf10	T2D	T	0.051
rs2237897	*KCNQ1*	T2D	C	0.223
rs2292626	*PLEKHA1*	T2D	C	0.086
rs231360	*KCNQ1*	T2D	T	0.077
rs2706785	TMEM155	T2D	G	0.128
rs2796441	TLE1	T2D	A	-0.064
rs2925979	*CMIP*	T2D	T	0.077
rs2943640	IRS1	T2D	C	0.082
rs329122	JADE2	T2D	A	0.045
rs340874	*PROX1*	T2D	C	0.060
rs35352848	*UBE2E2*	T2D	T	0.086

rs3794991	GATAD2A|CILP2	T2D	T	0.130
rs3821943	*WFS1*	T2D	T	0.095
rs3923113	GRB14	T2D	C	-0.069
rs4238013	*CCND2*	T2D	C	0.095
rs429358	*APOE*	T2D	T	0.122
rs4402960	*IGF2BP2*	T2D	T	0.140
rs4457053	ZBED3	T2D	A	-0.080
rs459193	ANKRD55	T2D	G	0.078
rs4774420	*C2CD4A*	T2D	C	0.077
rs4812829	HNF4A	T2D	A	0.053
rs516946	*ANK1*	T2D	C	0.078
rs5215	KCNJ11	T2D	T	-0.075
rs576674	KL	T2D	A	-0.073
rs60780116	*ACSL1*	T2D	T	0.086
rs622217	SLC22A3	T2D	C	-0.053
rs635634	*ABO*	T2D	T	0.077
rs6813195	TMEM154	T2D	T	-0.064
rs6918311	*SLC35D3*	T2D	A	0.068
rs702634	ARL15	T2D	A	0.050
rs7041847	GLIS3	T2D	G	-0.042
rs7111341	MIR4686	T2D	T	0.051
rs7224685	*ZZEF1*	T2D	T	0.068
rs7428936	*ADAMTS9*	T2D	T	0.068
rs7451008	*CDKAL1*	T2D	C	0.174
rs756852	*KCNQ1*	T2D	G	0.086
rs757209	*HNF1B (TCF2)*	T2D	G	0.086
rs7578597	THADA	T2D	C	-0.138
rs7674212	SLC9B2	T2D	T	-0.063
rs780094	GCKR	T2D	C	0.055
rs78761021	*GLP2R*	T2D	G	0.068
rs7903146	*TCF7L2*	T2D	T	0.329
rs79349575	*GIP*	T2D	A	0.068
rs7957197	HNF1A	T2D	A	-0.077
rs7985179	MIR17HG	T2D	A	-0.089
rs8042680	PRC1	T2D	A	0.063
rs8050136	FTO	T2D	A	0.107
rs8056814	*BCAR1*	T2D	G	0.148
rs810517	*ZMIZ1*	T2D	C	0.086
rs864745	JAZF1	T2D	C	-0.085
rs9271774	*HLA-DQA1*	T2D	C	0.095
rs944801	CDKN2B-AS1	T2D	C	0.071
rs9505118	SSR1	T2D	G	-0.063
rs952471	*HMG20A*	T2D	G	0.077
rs9648716	BRAF	T2D	T	0.076
rs9687833	*ANKRD55*	T2D	A	0.095
rs9940149	ITFG3|HCCA2	T2D	A	-0.062

rs10747083	P2RX2	Fasting glucose	A	0.016
rs10811661	CDKN2B	Fasting glucose	T	0.027
rs10830963	MTNR1B	Fasting glucose	G	0.077
rs10885122	ADRA2A	Fasting glucose	G	0.022
rs11071657	VPS13C/C2CD4A/B	Fasting glucose	A	0.006
rs11558471	SLC30A8	Fasting glucose	G	0.029
rs11603334	ARAP1	Fasting glucose	G	0.019
rs11605924	CRY2	Fasting glucose	A	0.019
rs11619319	PDX1	Fasting glucose	G	0.021
rs11708067	ADCY5	Fasting glucose	A	0.021
rs11715915	AMT	Fasting glucose	C	0.013
rs11920090	SLC2A2	Fasting glucose	T	0.028
rs16913693	IKBKAP	Fasting glucose	T	0.047
rs174550	FADS1	Fasting glucose	T	0.018
rs17762454	RREB1	Fasting glucose	T	0.012
rs2191349	DGKB/TMEM195	Fasting glucose	T	0.029
rs2302593	GIPR	Fasting glucose	C	0.014
rs2657879	GLS2	Fasting glucose	G	0.011
rs340874	PROX1	Fasting glucose	C	0.010
rs3783347	WARS	Fasting glucose	G	0.015
rs3829109	LOC728489	Fasting glucose	G	0.018
rs4506565	TCF7L2	Fasting glucose	T	0.019
rs4607517	GCK	Fasting glucose	A	0.055
rs4869272	PCSK1	Fasting glucose	T	0.018
rs560887	G6PC2	Fasting glucose	C	0.070
rs576674	KL	Fasting glucose	G	0.019
rs6072275	TOP1	Fasting glucose	A	0.016
rs6113722	FOXA2	Fasting glucose	G	0.038
rs6943153	GRB10	Fasting glucose	T	0.016
rs7651090	IGF2BP2	Fasting glucose	G	0.014
rs7708285	ZBED3	Fasting glucose	G	0.009
rs780094	GCKR	Fasting glucose	C	0.028
rs7867224	GLIS3	Fasting glucose	A	0.014
rs7944584	MADD	Fasting glucose	A	0.023
rs9368222	CDKAL1	Fasting glucose	A	0.016
rs983309	PPP1R3B	Fasting glucose	T	0.025
rs10195252	GRB14	Fasting insulin	T	0.018
rs1167800	HIP1	Fasting insulin	A	0.015

rs1530559	YSK4	Fasting insulin	A	0.013
rs17036328	PPARG	Fasting insulin	T	0.014
rs2126259	PPP1R3B	Fasting insulin	T	0.031
rs2745353	RSPO3	Fasting insulin	T	0.015
rs2943645	IRS1	Fasting insulin	T	0.016
rs3822072	FAM13A1	Fasting insulin	A	0.010
rs459193	ANKRD55	Fasting insulin	G	0.019
rs4846565	LYPLAL1	Fasting insulin	G	0.015
rs4865796	ARL15	Fasting insulin	A	0.016
rs6822892	PDGFC	Fasting insulin	A	0.010
rs6912327	C6orf107	Fasting insulin	T	0.015
rs731839	PEPD	Fasting insulin	G	0.017
rs780094	GCKR	Fasting insulin	C	0.022
rs7903146	TCF7L2	Fasting insulin	C	0.022
rs860598	IGF1	Fasting insulin	A	0.012
rs974801	TET2	Fasting insulin	G	0.016

Table II. Mendelian randomization estimates for the effect of Fasting glucose, and insulin on CSVD phenotypes using inverse-variance weighted, weighted median and penalized weighted median methods

	Lacunar stroke OR(95%CI)	P	ICH OR(95%CI)	P	Deep ICH OR(95%CI)	P	Lobar ICH OR(95%CI)	P	WMH OR(95%CI)	P	FA OR(95%CI)	P	MD OR(95%CI)	P
Inverse-variance weighted														
Fasting Glucose	1.10(0.63-1.90)	0.744	0.65(0.36-1.20)	0.167	0.75(0.32-1.74)	0.504	0.71(0.28-1.78)	0.464	1.16(0.97-1.40)	0.099	0.44(0.18-1.08)	0.073	1.85(0.76-4.52)	0.177
Fasting Insulin	1.52(0.45-5.08)	0.500	0.48(0.12-1.86)	0.288	0.18(0.03-1.05)	0.056	0.41(0.06-2.98)	0.378	1.04(0.69-1.54)	0.843	1.61(0.23-11.32)	0.635	0.67(0.09-4.74)	0.685
Weighted median														
Fasting Glucose	1.14(0.53-2.44)	0.733	0.60(0.25-1.44)	0.249	0.47(0.14-1.54)	0.213	2.10(0.53-8.40)	0.294	1.01(0.78-1.32)	0.934	0.62(0.16-2.37)	0.484	1.99(0.49-8.18)	0.338
Fasting Insulin	2.11(0.39-11.39)	0.383	0.18(0.03-1.24)	0.081	0.19(0.02-2.28)	0.192	0.13(0.01-1.86)	0.133	1.10(0.64-1.91)	0.728	0.79(0.05-13.39)	0.868	0.48(0.03-8.17)	0.608
Penalized weighted median														
Fasting Glucose	1.16(0.54-2.48)	0.696	0.60(0.25-1.44)	0.249	0.47(0.14-1.54)	0.211	2.16(0.54-8.55)	0.275	1.01(0.78-1.31)	0.946	0.62(0.16-2.37)	0.484	2.01(0.46-8.77)	0.355
Fasting Insulin	2.38(0.44-12.89)	0.316	0.17(0.02-1.21)	0.077	0.17(0.01-2.04)	0.163	0.12(0.01-1.72)	0.119	1.10(0.64-1.91)	0.728	0.69(0.04-12.01)	0.802	0.38(0.02-6.46)	0.499

Abbreviations:CSVD=cerebral small vessel disease; CI = confidence interval; OR = odds ratio,ICH=Intracerebral haemorrhage, WMH=White matter hyperintensity, FA=Fractional anisotropy, MD=Mean diffusivity.

*MR-Egger regression estimates could not be identified because the SNPs had similar magnitudes of association with fasting insulin.

Table III. Mendelian randomization estimates for the effect of each risk factor on CSVD phenotypes using inverse-variance weighted for sensitivity analyses*

	Lacunar stroke		ICH		Deep ICH		Lobar ICH		WMH		FA		MD	
	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P
Type 2 diabetes	1.17(1.05-1.31)	0.004	1.08(0.96-1.22)	0.193	1.16(0.98-1.38)	0.081	1.10(0.92-1.33)	0.300	1.02(0.98-1.06)	0.264	0.77(0.64-0.91)	0.002	1.08(0.91-1.27)	0.397
Fasting Glucose	1.03(0.58-1.84)	0.908	0.64(0.34-1.20)	0.166	0.87(0.36-2.08)	0.747	0.80(0.04-17.86)	0.889	1.17(0.97-1.41)	0.106	0.40(0.16-1.00)	0.051	1.75(0.69-4.42)	0.238
Fasting Insulin	5.68(0.86-37.46)	0.071	0.86(0.11-6.82)	0.889	0.92(0.05-15.41)	0.953	0.81(0.04-17.87)	0.889	1.23(0.66-2.28)	0.509	2.62(0.13-54.20)	0.534	1.54(0.07-32.28)	0.780

Abbreviations:CSVD=cerebral small vessel disease; CI = confidence interval; OR = odds ratio, ICH=Intracerebral haemorrhage, WMH=White matter hyperintensity, FA=Fractional anisotropy, MD=Mean diffusivity.

*Excluded the SNPs associated with lipids and kidney function.

Table IV Studies included in the MRI-confirmed lacunar stroke collaboration

Cohorts	Cases	Controls
DNA LACUNAR	917	
GENESIS (SR/ADDS)	299	
PRESERVE	46	
UK-WTCCC2	250	
GERMANY-WTCCC2	37	
MILANO	9	
ASGC	23	
SIGN:BRAINS	5	
SIGN:GEOS	4	
SIGN:GCNKSS	27	
SIGN:MIAMISR	13	
SIGN:GASROS	27	
SIGN:ISGS	28	
SIGN:KRAKOW	7	
SIGN:LEUVEN	45	
SIGN:BASICMAR	36	
SIGN:SAHLSIS	31	
SIGN:SPS3_EUR	345	
SIGN:GRAZ	42	
1958 BIRTH COHORT, NATIONAL BLOOD SERVICE		5175
DNA LACUNAR		968
KORA		797
SIGN:HRS		9286
SIGN:GRAZ		816
SIGN:LEUVEN		453
SIGN:KRAKOW		716
SIGN:ADHD		411
SIGN:MALMO		1362
SIGN:OAI		3201
SIGN:GEOS		519
SIGN:ASGS		1200
SIGN:HABC		1586
SIGN:INMA		807
TOTAL EUROPEAN	**2191**	**27297**

Standard thorough quality control was performed on each dataset separately. The data were then aligned to the forward strand and imputed to the HRC reference panel. SNPs with MAF<0.01 or INFO<0.5 were then removed and association analysis was carried out using RVTESTS including age, sex, study group, and the first 10 principal components. The inflation of test statistics (λ) was equal to the inflation expected for the sample size.

Figure I. Odds ratios ± standard error for WMH (logWMH volume) based on multi-SNP score (Quantile2 vs Quantile 1, Quantile 3 vs Quantile 1 and Quantile 4 vs Quantile 1)

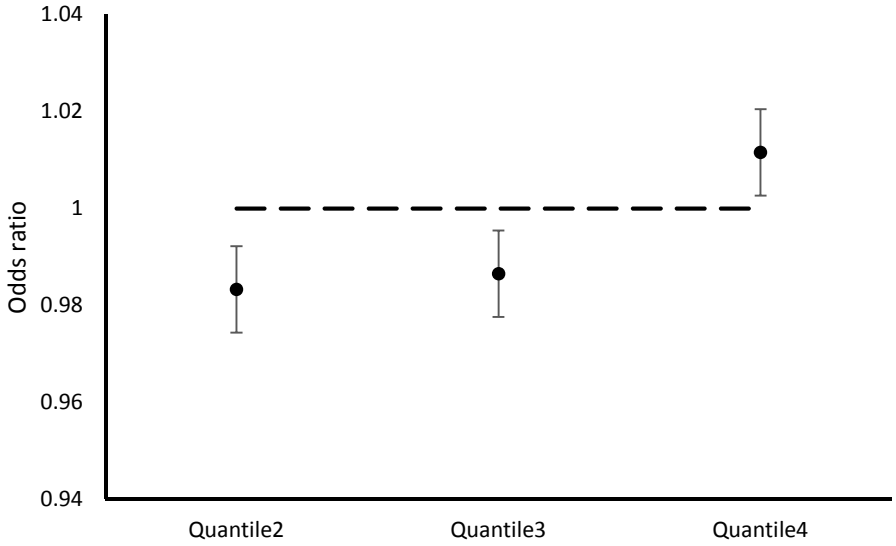


Figure II.Odds ratios ± standard error for FA based on multi-SNP score(Quantile2 vs Quantile 1, Quantile 3 vs Quantile 1 and Quantile 4 vs Quantile 1)

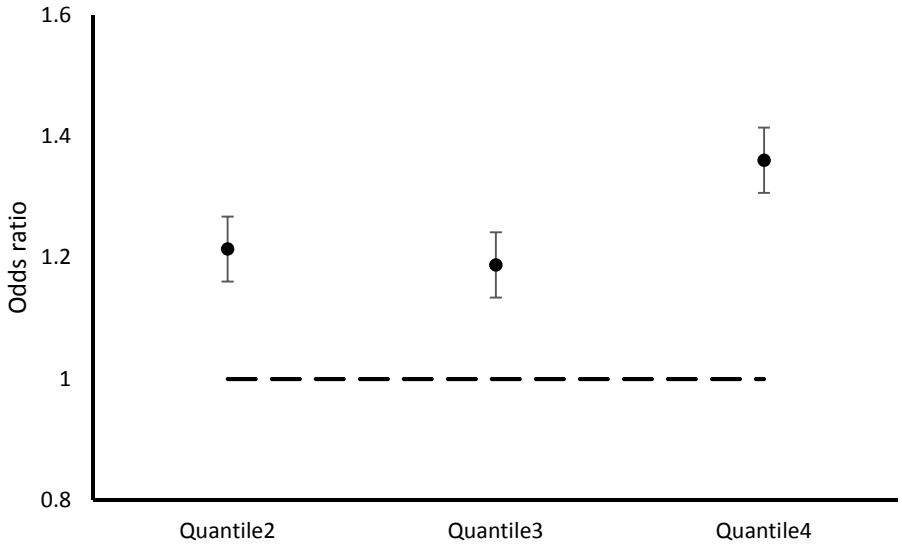
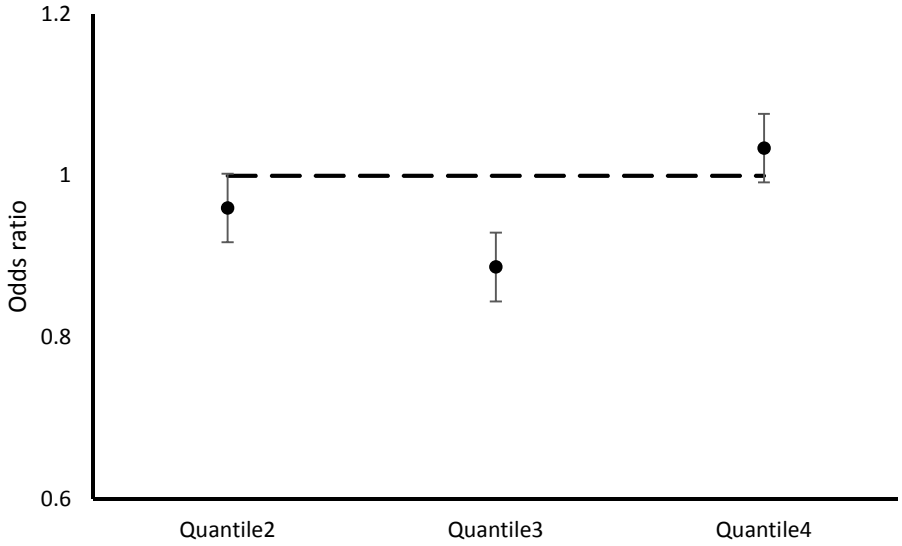


Figure III. Odds ratios ± standard error for MD based on multi-SNP score (Quantile2 vs Quantile 1, Quantile 3 vs Quantile 1 and Quantile 4 vs Quantile 1)



Supplementary methods

UK Biobank genome-wide association study of FA, MD, and WMH

Study population and ethical approval

UK Biobank is a prospective study that recruited 500,000 community-dwelling participants from across the UK between 2006 and 2010, aged 40–69 years (http://www.ukbiobank.ac.uk). The study collects extensive data from questionnaires, interviews, health records, physical measures, biological samples and imaging.

A subset of the participants also underwent brain MRI. In the present study we used the second release of MRI data, which included 9,045 subjects who underwent brain MRI, on average 6.6 years (SD 1.0 years) after initial recruitment at mean age 55.5 years (SD 7.4 years) and had usable T2 FLAIR or DTI images. Patients with a baseline diagnosis of stroke (ICD-9/-10 or self-report or health-record linkage), multiple sclerosis, Parkinson's disease, dementia, any other neurodegenerative problem or no genetic data were excluded. A further three participants with consistently extreme outlying tract-averaged water diffusion biomarker values were removed following visual inspection of the data by the authors, leaving 8,568 individuals for the current analysis.

UK Biobank received ethical approval from the research ethics committee (REC reference 11/NW/0382). All participants provided informed consent to participate. The present analyses were conducted under UK Biobank application number 19463.

MRI

Procedures for brain imaging acquisition and initial quality check have been described previously and are available on the UK Biobank website (Brain Imaging Documentation V1.3, http://www.ukbiobank.ac.uk).[1]

In brief, all brain MRI data were acquired on a single standard Siemens Skyra 3T scanner (Siemens Medical Solutions, Germany) using the standard Siemens 32-channel RF receiver head coil. Sagittal T1-weighted scans were acquired using a 3D magnetization-prepared rapid acquisition gradient-echo (MPRAGE) sequence (resolution 1 x 1 x 1 mm, field of view 208 x 256 x 256, TI/TR=880/2000 ms). Sagittal T2-weighted fluid attenuated inversion recovery (FLAIR) scans were obtained using a 3D SPACE sequence (resolution 1.05 x 1.0 x 1.0 mm, field of view 192 x 256 x 256, TI/TR=1800/5000 ms). DTI scans were acquired with a spin-echo echo-planar imaging sequence and multi-shell acquisition (b0 = 0 s/mm^{-2}, b = 1,000 s/mm^{-2}, b = 2,000 s/mm^{-2}, 100 distinct diffusion-encoding directions, 2 mm isotropic voxels, field of view 104 x 104 x 72).

White matter hyperintensities

White matter hyperintensities (WMH) were automatically segmented using the combined T1 and T2-FLAIR data as input in the BIANCA tool (Brain Intensity AbNormality Classification Algorithm).[2]BIANCA is a fully-automated supervised method for WMH detection, based on the k-nearest neighbour algorithm, which gives the probability per voxel of being WMH. The total WMH volume was calculated from the voxels exceeding a probability of 0.9 of being WMH and located within a white matter mask. Obtained values were adjusted for the total intracranial volume and log transformed because of their skewed distribution.

Fractional anisotropy and mean diffusivity

Following gradient distortion correction and further correction for head movement and eddy currents, diffusion tensors and scalar diffusion parameters (ie, FA and MD) were calculated by feeding the b=1000 shell (50 directions) into DTIFIT within the FSL software.[3] The FA maps were fed into tract-based spatial statistics (TBSS) which aligns the FA map onto a standard-space white matter skeleton. The resulting standard-space warp is applied to all other DTI outputs. MD images were projected onto the skeleton, using the FA derived projection parameters. Subsequently the skeletonised images were averaged across a set of 48 standard-space tract masks, similar to the processing applied in the ENIGMA project (http://enigma.ini.usc.edu/protocols/dti-protocols).[4,5] Principal component analysis (PCA) was applied on the 48 tracts to extract a latent measure. The first principal component of FA (FA.PC1) and MD (MD.PC1) was used in subsequent analyses as dependent variable.

Genetic data

We used the June 2017 release of the imputed genetic data from UK Biobank (downloaded on June 3, 2017). Details of the design of the arrays, sample processing and stringent quality control have been described elsewhere.[6]In brief, two closely related arrays from Affymetrix, the UK BiLEVE Axiom array (9.9% of individuals) and the UK Biobank Axiom array, were used to genotype approximately 805,426 markers with good genome-wide coverage. Phasing was performed using SHAPEIT3 and imputation to a merged HRC reference panel (39,131,578 autosomal SNPs) and UK10K & 1000 Genomes Phase 3 panel was carried out using the IMPUTE4 package.[6–8] Imputed genotypes were available for 487,442 individuals in this study. From the resulting dataset, we excluded (1) individuals that did not segregate with European samples based on PCA analysis, (2) individuals with high levels heterozygosity and missingness (>5%), (3) individuals whose reported sex was inconsistent with sex inferred from the genetic data. In addition, only SNPs imputed from the HRC panel were included in this analysis.

Statistical analysis

In this analysis, we first subset the genetic data on the individuals that also had MRI imaging data. We performed a genome-wide association study of FA, MD and log(WMH), using SNPTEST v2.5.4-beta3 including age at MRI, sex, body mass index (BMI), genotyping batch, and the first 10 ancestry informative principal components as covariates.

Intracerebral Haemorrhage Meta-analysis

Contributing Studies

1. Cambridge ICH Genetics Study.

Intracerebral Haemorrhage cases were recruited based on hospital admissions at St. George's Hospital, London and Addenbrooke's Hospital, Cambridge as part of the St. George's Stroke Register and GENESIS studiesbetween 2002-2012. All cases were confirmed radiologically.

Unrelated Caucasian controls, free of clinical cerebrovascular disease, were obtained by random sampling, stratified for age and sex, from general practice lists from the same geographical location as the patients. All patients and controls underwent a standardized clinical assessment and completed a standardized study questionnaire.

The genetic dataset, which included other individuals not eligible for this analysis, was genotyped on the Illumina HumanCoreExome array. SNPs were excluded with MAF<0.01, genotype missingness>3%, HWE p<1e-6 in controls, strand ambiguity (A/T or C/G) or evidence of differential missingness by case-control status (p<0.05). Individuals were excluded if they had missingness>3%, excess or reduced heterozygosity, showed evidence of relatedness with another individual (pi-hat>0.1875), or failed a "sex-check" in PLINK. EIGENSTRAT was used to remove non-caucasian individuals, and was then repeated to calculate ancestry-informative principal components. The remaining 269,691 autosomal SNPs and 2,603 individuals were then imputed to the haplotype reference consortium build 2016.1 using the Michigan Imputation Server.[7]Post-imputation, SNPs were removed with poor imputation quality (INFO<0.5) or low minor allele frequency (MAF<.005).

Analysis of the relationship between genomewide SNP dosages and ICH was performed using rvtests[9],adjusting for age, sex and ancestry informative-principal components.

2. Intracerebral Haemorrhage Genetics Collaboration

The intracerebral Haemorrhage Genetics Collaboration is composed of 1,545 cases and 1481 controls from studies from the United States and Europe. Full details of the population, genotyping and imputation are available elsewhere.[10]

3. UK Biobank ICH GWAS

We used the June 2017 release of the imputed genetic data from UK Biobank (downloaded on June 3, 2017). Details of the design of the arrays, sample processing and stringent quality control have been described elsewhere.[6]In brief, two closely related arrays from Affymetrix, the UK BiLEVE Axiom array (9.9% of individuals) and the UK Biobank Axiom array, were used to genotype approximately 805,426 markers with good genome-wide coverage. Phasing was performed using SHAPEIT3 and imputation to a merged HRC reference panel (39,131,578 autosomal SNPs) and UK10K & 1000 Genomes Phase 3 panel was carried out using the IMPUTE4 package.[6–8] Imputed genotypes were available for 487,442 individuals in this study.

From the resulting dataset, we excluded (1) individuals not designated as 'White British' based on central PCA analysis, (2) individuals with high levels heterozygosity and missingness (>5%), (3) individuals whose reported sex was inconsistent with sex inferred from the genetic data. In addition, only SNPs imputed from the HRC panel were included in this analysis.Post-imputation, SNPs were removed with poor imputation quality (INFO<0.5) or low minor allele frequency (MAF<.005).

We used algorithmically defined intracerebral haemorrhage as our outcome. We derived a set of controls at a 10:1 ratio based on propensity score matching on age, sex and ancestry-informative principal components.

Analysis of the association between genome-wide genotype dosages and the resulting 575 cases and 5750 controls was performing using rvtests[9]adjusting for age, sex, and ancestry-informative principal components.

We note that haemorrhage location information was not available in UK Biobank, meaning subgroup (deep or lobar) analyses could therefore not be performed.

Meta-analysis Methods

The contributing datasets were analysed using a fixed-effects inverse variance weighted method using METAL.[11] Genomic Control was used to control for any residual inflation. Only SNPs present in all 3 contributing datasets were considered in this analysis.

References

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