

Supplementary Table S1. Clinical characteristics of the subgroups.

Characteristic	CSVD-S (n=41)	CSVD-M (n=52)	HCs (n=48)	P value (K-W test/ANOVA/ χ^2)	P value (post hoc)		
					CSVD-S vs. CSVD-M	CSVD-S vs. HC	CSVD-M vs. HC
Gender	27 M/14 F	24 M/28 F	19 M/29F	0.039 χ^2	-	0.013	
Age (y)	65 (60.5~69)	66.5 (57~68)	61 (58~65)	0.063 ^a	-	-	-
Education (y)	11.05±3.14	11.46±3.74	11.67±3.24	0.69 ^b	-	-	-
BMI(kg/m²)	25.42±2.90	24.75±2.77	24.86±2.91	0.51 ^b	-	-	-
Alcohol, n (%)	21(51.22%)	15 (28.85%)	11 (22.92%)	0.013 χ^2	-	0.006	-
Smoke, n (%)	16 (39.02%)	10 (19.23%)	11 (22.92%)	0.08 χ^2	-	-	-
Hypertention, n (%)	29 (70.73%)	16 (30.77%)	12 (25%)	< 0.001 χ^2	< 0.001	< 0.001	-
Diabetes, n(%)	20(48.78%)	18 (38.46%)	17 (47.92%)	< 0.210 χ^2	-	-	-
Hyperlipidemia, n(%)	23 (56.10%)	18 (34.61%)	18 (37.5%)	0.126 χ^2	-	-	-
MoCA	22.46±2.96	25.48±3.31	26.00±3.45	< 0.001 ^b	< 0.001	< 0.001	-
SCWT	173.64±66.1 0	139.67±48.3 9	131.55±49.4 1	0.001 ^b	0.01	0.001	-
Lacune	24 (58.53%)	1 (1.92%)	0 (0%)	< 0.001 χ^2	< 0.001	< 0.001	-
WMH	40 (97.56%)	48 (92.31%)	0(0%)	< 0.001 χ^2	-	<0.001	< 0.001
PVS	36 (87.8%)	13 (25%)	0 (0%)	< 0.001 χ^2	< 0.001	< 0.001	< 0.001
CMBs	26 (63.41%)	6 (11.54%)	0 (0%)	< 0.001 χ^2	< 0.001	< 0.001	< 0.001
CSVD-Total score							
0	0	29	48				
1	0	23	0				
2	17	0	0				
3	15	0	0				
4	9	0	0				

Notes: CSVD-S = patients with CSVD with severe CSVD burden scores; CSVD-M = patients with CSVD with mild or moderate CSVD burden scores; HCs = healthy controls.

χ^2 : chi-square test, a: *Kruskal–Wallis* test (K–W test), b: One-way ANOVA.

MoCA: Montreal Cognitive Assessment, SCWT: Stroop Color Word Test.

Clinical Characteristics

Supplementary Table S1 provides a summary of the clinical characteristics of the subgroups. It includes information on the sex, age, education level, medical history, and cognitive status of the participants. There were no significant differences in age among the three groups ($P=0.063$), and significant sex differences were observed only between the CSVD-S and HC groups ($P=0.013$). Additionally, education level, BMI, smoking history,

diabetes history, and hyperlipidemia history did not differ significantly among the groups. However, there were significant differences in hypertension history between the CSVD-S and CSVD-M groups and between the CSVD-S and HC groups ($P < 0.01$), whereas alcohol history was significantly different only between the CSVD-S and HC groups ($P = 0.006$).

Significant differences were also observed in the MoCA and SCWT scores among the three groups. The CSVD-M ($P < 0.001$, $P = 0.01$) and HC ($P < 0.001$, $P = 0.001$) groups presented better MoCA and SCWT scores than did the CSVD-S group, with no significant difference between the CSVD-M and HC groups.

Supplementary Table S2. Susceptibility Value (ppb [$\times 10^{-9}$]) Differences Among the Three Groups

ROI	CSVD-S n=44	CSVD-M n=62	HCs n=61	F	P*	Statistical Value		
						PairWise Test		
						a	b	c
Thalamus	-2.51 $\pm$ 13.20	-1.72 $\pm$ 14.12	-0.91 $\pm$ 13.36	0.154	0.857	-	-	-
Globus pallidus	195.11 $\pm$ 53.39	150.16 $\pm$ 56.71	140.29 $\pm$ 48.93	13.11	< 0.001*	< 0.001	< 0.001	-
Putamen	95.84 $\pm$ 32.24	75.97 $\pm$ 42.58	75.53 $\pm$ 36.00	4.15	0.018*	0.038	0.037	-
Caudate nucleus	84.35 $\pm$ 25.34	66.48 $\pm$ 33.29	68.18 $\pm$ 29.13	4.821	0.009*	0.014	0.035	-
Red nucleus	139.01 $\pm$ 45.73	121.76 $\pm$ 47.17	120.75 $\pm$ 39.49	1.593	0.100	-	-	-
Substantia nigra	160.78 $\pm$ 45.96	131.58 $\pm$ 53.84	123.43 $\pm$ 42.52	7.295	0.001*	0.012	0.001	-

Notes: Data are presented as the mean and standard deviation.

Pairwise test: a = CSVD-S vs. CSVD-M, b = CSVD-S vs. HC, c = CSVD-M vs. HC.

CSVD-S = patients with CSVD with severe CSVD burden scores; CSVD-M = patients with CSVD with mild or moderate CSVD burden scores; HCs = healthy controls.

*FDR correction, $P < 0.05$.

Susceptibility Value Analysis across ROIs.

The results of the one-way ANOVA indicated a significant difference in the mean susceptibility values of the GP ($P < 0.001$), the Put ($P = 0.018$) and the CN ($P = 0.009$) among the three groups. The post hoc tests revealed that the mean susceptibility values in the GP, the Put, and the CN were higher in patients with CSVD-S than in those with CSVD-M and HC group, and these differences were statistically significant (for CSVD-S vs. CSVD-M and for CSVD-M vs. HC, all the P values < 0.05). Notably, no significant differences were detected in the mean susceptibility levels of these brain regions between the CSVD-M and HC group. Additionally, in the subgroup analysis, we observed that the substantia nigra susceptibility values in the CSVD-S subgroup were significantly higher than those in the CSVD-M and HC subgroups, a difference not observed in the original grouping analysis. This newly detected difference may be attributed to the more stringent matching of key factors, such as age, in the

subgroup analysis. Such control likely allowed the sensitivity of the substantia nigra to CSVD pathological burden to become more apparent.

Supplementary Table S3. Determinants of the mean susceptibility values of altered brain regions: Results of multiple linear regression analysis.

	Factors	B	Standardized B	T	P	R of Model	P of Model
Thalamus	Diabetes	6.154	0.219	2.291	0.024	0.074	0.196
Globus pallidus	Age	4.203	0.465	5.610	< 0.001*	0.282	< 0.001
Putamen	Age	2.541	0.429	5.346	< 0.001*	0.329	< 0.001
	Diabetes	20.54	0.267	3.286	0.001*		
	Alcohol	-16.329	-0.204	-2.290	0.024		
Caudate nucleus	Age	1.763	0.376	4.496	< 0.001*	0.271	< 0.001
	Diabetes	15.727	0.258	3.051	0.003*		
Red nucleus	Age	2.584	0.365	4.286	< 0.001*	0.244	< 0.001
	Diabetes	27.067	0.294	3.413	0.001*		
Substantia nigra	Age	3.097	0.395	4.723	< 0.001*	0.270	< 0.001

Notes: *Bonferroni correction, $P < 0.0056$

Regions of interest analysis

After applying the Bonferroni correction, multiple linear regression analysis revealed that several brain regions demonstrated statistically significant associations with specific factors at a threshold of $P < 0.005$. Specifically, age was significantly associated with susceptibility values in the globus pallidus ($P < 0.001$), putamen ($P < 0.001$), caudate nucleus ($P < 0.001$), red nucleus ($P < 0.001$), and substantia nigra ($P < 0.001$), indicating that age is a robust determinant of susceptibility values across multiple brain structures associated with CSVD. In addition, diabetes was significantly associated with susceptibility in the putamen ($P = 0.001$), caudate nucleus ($P = 0.003$), and red nucleus ($P = 0.001$), highlighting diabetes as an important factor influencing susceptibility in these specific brain regions. These results from these subgroup analyses were consistent with the original group comparisons, demonstrating that the relationships between susceptibility values and age and diabetes remained stable even when controlling for age differences. The newly discovered associations between age and the susceptibility values of the Red Nucleus and Substantia Nigra in the subgroup analysis may be due to the age-matching process reducing the influence of other confounding factors, thereby clarifying the relationships between age and the susceptibility values of these specific brain regions. This new finding suggests that the susceptibility values of the Red Nucleus and Substantia nigra may be more susceptible to the effects of age than we previously anticipated. However, the subgroup analysis also revealed a negative correlation between alcohol consumption and susceptibility values in the putamen ($P = 0.024$), although this association did not reach significance after the Bonferroni correction. The previously significant positive correlation between smoking history and the magnetic susceptibility values of Putamen in the original group was not clearly demonstrated in this subgroup analysis, suggesting that the

impact of smoking on the putamen may not be as strong as we previously assumed after controlling for other variables.

Supplementary Table S4. Mediation analysis results

Paths	Estimated effect	95% Confidence interval		P
		lower	upper	
Indirect effect				
Age →CSVD total scores	0.164	-0.001	0.329	0.0518
CSVD total scores →striatum	0.246	0.105	0.387	0.0007
Direct effect				
Age →CSVD total scores →striatum	0.468	0.328	0.609	< 0.001

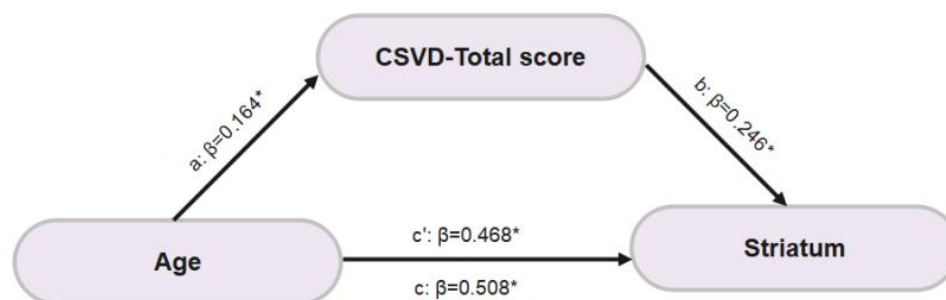


Figure S1. Mediation analysis model. The CSVD total score is a mediating variable between age and the mean susceptibility values of the striatum, suggesting that age is associated with the mean susceptibility values of the striatum and that the effect of age on the mean susceptibility values of the striatum is mediated by the CSVD total score.

Mediation analysis

Given the broad and significant influence of age on susceptibility values in gray matter nuclei, especially the striatum, we conducted a mediation analysis using age as the independent variable, striatum susceptibility as the dependent variable, and the CSVD total score as the mediating variable to explore their interrelationships further. According to the findings, the estimated effect of age on striatum susceptibility mediated by the CSVD total score was 0.164 ($a = 0.164$), with an indirect effect P value of 0.0518, which was slightly above the 0.05 threshold. While this indirect pathway does not reach full significance compared with the original intergroup statistics, it still suggests a meaningful influence of age on striatum susceptibility via the CSVD total score, with practical implications. Given that smaller sample

sizes can reduce statistical power, a slightly relaxed significance level ($P < 0.1$) can sometimes be considered to account for this limitation. Therefore, despite the current P value of 0.0518, the effect may be considered weakly significant under these conditions. Moreover, the estimated effect of the CSVD total score on striatum susceptibility was 0.246, with a P value of 0.0007, underscoring the pathway's significance and indicating that the CSVD total score has a marked effect on striatum susceptibility. Additionally, the direct effect of age on striatum susceptibility via the CSVD total score was 0.468 ($P < 0.001$), which was highly significant. The significance of both the direct effect and the CSVD total score pathway on striatum susceptibility suggests a stable relationship at each node of the “age $\rightarrow$ CSVD total score $\rightarrow$ striatum” pathway, forming a relatively complete causal chain. This structure supports the potential for an indirect effect of age on striatum susceptibility via the CSVD total score.

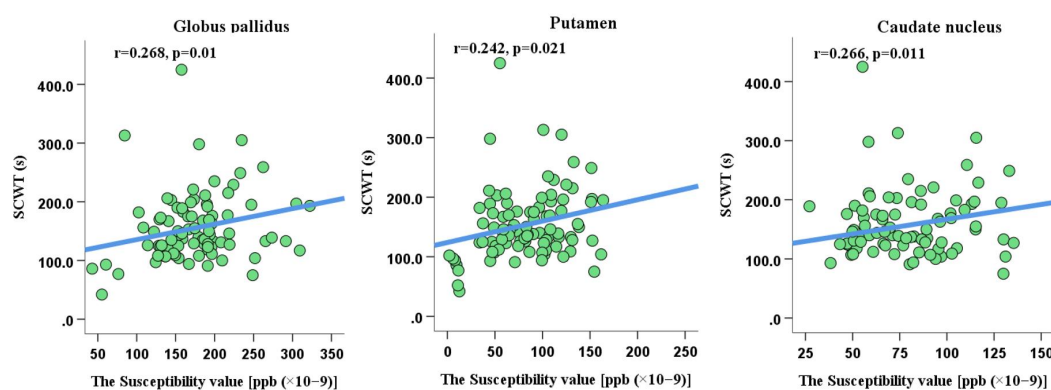


Figure S2. Correlations between the mean susceptibility values of altered brain regions and the SCWT in patients with CSVD.

Comparison of Cognitive Performance

The correlation analysis revealed a significant positive association between the mean susceptibility values of the CSVD subjects and their scores on the SCWT assessment in the following regions: the GP, the Put, and the CN.