SUPPLEMENTAL MATERIAL

Table S1: Minimum detectable difference between the control and SCT cohorts for each structural, hemodynamic, and oxygen metabolic metrics, assuming 80% power for each variable based on current study sample

	Standard deviation	Minimum detectable difference *	Absolute difference of mean between HbAA and HbAS
Normalized brain volume			
Whole brain	0.05	0.04	0.01
Gray matter	0.04	0.03	0.01
White matter	0.02	0.02	0.0002
Microstructural integrity			
NAWM			
FA	0.02	0.02	0.01
MD	0.04	0.03	0.004
"At-risk" NAWM			
FA	0.02	0.02	0.01
MD	0.04	0.03	0.01
Hemodynamic and metabolic metrics			
Whole brain			
CBF	10.08	8.53	0.31
OEF	0.02	0.02	0.01
$CMRO_2$	0.44	0.38	0.003
Gray matter			
CBF	13.23	11.20	0.004
OEF	0.03	0.02	0.01
$CMRO_2$	0.63	0.54	0.02
NAWM			
CBF	7.61	6.44	2.26
OEF	0.02	0.02	0.003
$CMRO_2$	0.32	0.27	0.09
"At-Risk" NAWM			
CBF	6.95	5.88	0.51
OEF	0.02	0.02	0.004

$CMRO_2$	0.29	0.26	0.02
Cerebral vasculopathy, N (%)	N/A	7 (26%)	0 (0%)
Aneurysm, N (%)	N/A	7 (26%)	0 (0%)
Silent infarct, N (%)	N/A	7 (26%)	3 (10.7%)
SCI volume, mL	1.81	1.52	0.67
SCI volume, subgroup, mL †	2.85	4.04	1.50

Abbreviations: SCI, silent cerebral infarct; NAWM, normal appearing white matter; FA, fractional anisotropy; MD, mean diffusivity, expressed in unit of $10^{-3}mm^2s^{-1}$; CBF, cerebral blood flow, expressed in unit of mL/min/100g; OEF, oxygen extraction fraction; $CMRO_2$, cerebral metabolic rate of oxygen, expressed in mL/min/100g.

* Calculated as the product of 1) the standard deviation for individual continuous outcome variable and 2) the minimal effect size we were 80% powered to detect based on our study sample.

† Within participants with SCI on MRI

Table S2. Vascular risk factors at time of enrollment *

	HbAA (N=24)	HbAS (N=25)	*p*
Hypertension, n (%)	2 (8)	0	0.24
Diabetes, n (%)	1 (4)	1 (4)	1
Hyperlipidemia, n (%)	0 (0)	0 (0)	1
Migraine, n (%)	1 (4)	1 (4)	1

*Risk factors were defined as present if requiring medication

Table S3: Brain structure, hemodynamic, and oxygen metabolic metrics in adults with and without sickle cell trait, adjusted for age, sex, medication usage

Outcome variable	β [95% CI] SCT	*p* value*
Normalized brain volume		
Whole brain	-0.003 [-0.027, 0.022]	0.82
Gray matter	-0.002 [-0.02, 0.015]	0.79
White matter	0.00 [-0.013, 0.012]	0.95
Microstructural integrity		
NAWM		
FA	-0.003 [-0.016, 0.011]	0.71
MD	0.002 [-0.020, 0.024]	0.87
"At-risk" NAWM region		
FA	-0.006 [-0.020, 0.008]	0.37
MD	0.004 [-0.018, 0.026]	0.72
Hemodynamic and metabolic metrics		
Whole brain		
CBF	-0.105 [-6.258, 6.048]	0.97
OEF	-0.007 [-0.019, 0.004]	0.20
$CMRO_2$	0.006 [-0.27, 0.29]	0.97
Gray matter		
CBF	-0.725 [-8.714, 7.264]	0.86
OEF	-0.007 [-0.021, 0.006]	0.26
$CMRO_2$	-0.017 [-0.41, 0.38]	0.93
NAWM		
CBF	1.16 [-3.24, 5.55]	0.39
OEF	-0.004 [-0.015, 0.007]	0.81
$CMRO_2$	0.06 [-0.13, 0.25]	0.53
"At-risk" NAWM region		

CBF	-0.058 [-4.27, 4.15]	0.98
OEF	-0.004 [-0.16, 0.007]	0.47
$CMRO_2$	0.01 [-0.18, 0.19]	0.96
SCI volume, mL	0.34 [-0.68, 1.37]	0.5

Abbreviations: SCI, silent cerebral infarct; NAWM, normal appearing white matter; FA, fractional anisotropy; MD, mean diffusivity, expressed in unit of $10^{-3}mm2s^{-1}$; CBF, cerebral blood flow, expressed in unit of mL/min/100g; OEF, oxygen extraction fraction; $CMRO_2$, cerebral metabolic rate of oxygen, expressed in mL/min/100g.

* *p*-value and regression coefficients for sickle cell trait association with outcome variable in linear regression model adjusting for age, sex, and medication use. Raw p-values reported with significance determined using the Benjamini-Hochberg procedure to maintain a false discovery rate of 0.05

Table S4: Participant characteristics of HbSS cohort compared to HbAA and HbAS cohorts

	HbAA (N= 24) Median (IQR)	HbAS (N = 25) Median (IQR)	HbSS (N = 26) Median (IQR)	*p*‡	*p* §		
					HbAA vs HbAS	HbAS vs HbSS	HbAA vs HbSS
Age	31.5 (28.5, 36.5)	35 (31, 39)	23.5 (19, 29)	<0.0001	0.27	<0.0001	0.0024
Female (%)	19 (79)	18 (72)	10 (38.5)	0.0074	0.74	0.02	0.0047
Race, Black (%)	24 (100)	25 (100)	26 (100)	1	-	-	-
Hemoglobin, g/dl	12.35 (11.55, 13)	12.6 (12, 13.6)	8.25 (7.5, 9.5)	<0.0001	0.489	<0.0001	<0.0001
Hemoglobin A, %	97.25 (97.05, 97.4)	59.7 (57.6, 61.7)	0 (0, 0)	<0.0001	<0.0001	<0.0001	<0.0001
COHb, %	1.0 (0.7, 2.7)	1.3 (0.8, 2.3)	3.2 (2.8, 3.8)	<0.0001	1	0.002	<0.001
MetHb, %	1.1 (0.4, 1.4)	1.6 (0.8, 1.9)	2.5 (1.7, 2.8)	<0.0001	0.25	0.002	<0.0001
Hemoglobin S, %	0 (0, 0)	37 (35.1, 39.1)	80.8 (71.2, 89.1)	<0.0001	<0.0001	<0.0001	<0.0001
Hemoglobin F, %	0.4 (0, 0.4)	0.4 (0.4, 0.4)	9.1 (3.2, 17.3)	<0.0001	0.02	<0.0001	<0.0001
SpO2, %	100 (99, 100)	99 (98, 100)	97.5 (95, 99)	0.0007	0.38	0.02	0.002
CaO2, mL/dL	16.18 (15.43, 16.96)	16.14 (15.54, 17.66)	9.91 (9.25, 11.94)	<0.0001	0.93	<0.0001	<0.0001
Silent infarct, N (%)	8 (33.3%)	11 (44.0%)	13 (50%)	-	0.56	0.78	0.27
Aneurysm, N (%)	1 (4.2%)	1 (4%)	7 (26.95%)	-	1	0.0496	0.05
Vascular risk factors							
SBP, mmHg	116.5 (108.5, 129)	120 (113.5, 130.5)	114.5 (106.5, 122)	0.1684	-	-	-
DBP, mmHg	73.5 (67.5, 84)	77.5 (72, 83.5)	66 (61, 69)	<0.0001	0.72	<0.0001	0.005
Cr, mg/dL	0.8 (0.7, 0.9)	0.9 (0.8, 0.9)	0.6 (0.6, 0.7)	<0.0001	0.13	0.0001	0.01
Glucose, mg/dL	91 (78, 96)	94 (78, 99)	87 (78, 97)	0.8532	-	-	-
LDL, mg/dL	92.5 (77.5, 105.5)	88 (70, 111)	51 (43, 57)	<0.0001	0.90	<0.0001	<0.001
HbA1C, % *	5.2 (5, 5.6)	5.3 (5, 5.4)	NA	0.8093	-	-	-
Current smokers (%) †	7 (32)	5 (26)	- (-)	0.6176	-	-	-

* Data not available for HbSS cohort.

† Data available for 22 HbAA, 19 HbAS, and 4 HbSS participants.

‡ Continuous and categorical variables were compared across three cohorts using Kruskal-Wallis or Fisher exact tests, respectively.
§ Multiple pairwise comparisons between two cohorts were calculated using Dwass-Steel-Critchlow-Fligner test to provide family-wise error rate correction.

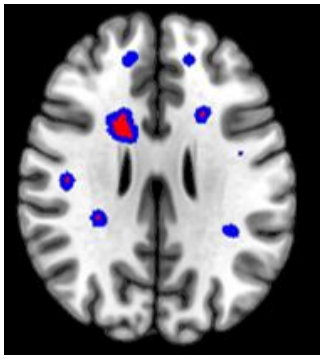

Figure S1. “At-risk” region across HbAA and HbAS cohort

A single at-risk mask was created from on the infarct heatmap of HbAA and HbAS participants. Red areas represent infarct mask of the total cohort, thresholded to 2 or more participants with infarct in a voxel. Blue represents at-risk region, with 3mm contouring surrounding the infarct mask of the total cohort. To prevent the expansion of the at-risk mask into gray matter and ventricles, we restricted the mask to normal appearing white matter when calculating FA, MD, OEF and CBF.

STROBE Statement—checklist of items that should be included in reports of case-control studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 2, line 50	"Young adults with and without SCT (N=49) underwent brain MRI …"
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2, line 50-56	
Introduction				
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 3, line 88-103	"Despite a growing body of research …" to "…patients with SCA to SCT status"
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4-5, line 107-112	"Based on the recent, larger…" to "…volume of silent cerebral infarcts (SCIs) in SCT."
Methods				
Study design	4	Present key elements of study design early in the paper	Page 5, line 119-120	"Adult …were prospectively enrolled"
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5-6, line 119-145	"Adult …were prospectively enrolled…" to "…underwent brain MRI on a 3 Tesla MR system"

Participants	6	(*a*) *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	HbAS: Page 5, line 119-127 HbAA: Page 5, line 119-127 HbSS: page 5, line 127-133	"Adult …were prospectively enrolled…" to "…on clinical examination were also excluded" "Adult …were prospectively enrolled…" to "…on clinical examination were also excluded" "An independent cohort…unrelated to SCA" to "… minimize imbalance in socioeconomic status"
		(*b*) *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 6-9, line 134-208	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*For both control and case cohorts:* Page 6-9, line 134-208	

Bias	9	Describe any efforts to address potential sources of bias	Page 9, line 211-213; page 9-10, line 217-224	"Multiple pairwise comparisons between…" and "An *a priori* power analysis…"
Study size	10	Explain how the study size was arrived at	page 9-10, line 217-224	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 9, line 209-217	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 9, line 209-217	
		(*b*) Describe any methods used to examine subgroups and interactions	Page 9, line 214-216	"To examine any association…"
		(*c*) Explain how missing data were addressed	N/A	
		(*d*) *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A	This is not a matched case-control study.
		(*e*) Describe any sensitivity analyses	page 9-10, line 217-224	"An *a priori* power analysis…"
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 10, line 231-232	"Forty-nine…brain MRI"
		(b) Give reasons for non-participation at each stage	n/a	
		(c) Consider use of a flow diagram	n/a	

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 10, line 231-239, and Table 1, Supplemental Tables II and III	"Forty-nine…" to "… significance (0.9 mg/dL vs 0.8 mg/dL, $p = 0.053$)."
		(b) Indicate number of participants with missing data for each variable of interest	n/a	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	n/a	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	n/a	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Table 2, Figure 1	
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 11-12, line 241-282	
		(*b*) Report category boundaries when continuous variables were categorized	N/A	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a	
Discussion				
Key results	18	Summarise key results with reference to study objectives	Page 13, line 285-293	"In this prospective MRI study…" to "…who are otherwise healthy."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 15, line 337-349	"Our study has several limitations."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 14 line 310-318	"Unexpectedly, within our subset…" to "…traditional vascular risk factors."
			Page 14, line 319-334	"our results suggest no alternations…" to "…participants with SCT"
			Page 15, line 339-342	"Based on our sample size…" to "…our continuous imaging variables"
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 15-16, line 350-3357	
Other information				

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 16, line 360-362

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.