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Association of sickle cell trait with measures of cognitive function and dementia in African Americans

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ABSTRACT

Objective: The incidence and prevalence of cognitive decline and dementia are significantly higher among African Americans compared with non-Hispanic Whites. The aim of this study was to determine whether inheritance of the sickle cell trait (SCT) i.e. heterozygosity for the sickle cell mutation increases the risk of cognitive decline or dementia Among African Americans.

Methods: We studied African American participants enrolled in the Atherosclerosis Risk in Communities study. SCT genotype at baseline and outcome data from cognitive assessments at visits 2, 4 and 5, and an MRI performed at visit 5 were analyzed for the association between SCT and risk of cognitive impairment and/or dementia.

Results: There was no significant difference in risk factors profile between participants with SCT ($N = 176$) and those without SCT ($N = 2532$). SCT was not independently associated with a higher prevalence of global or domain-specific cognitive impairment at baseline or with more rapid cognitive decline. Participants with SCT had slightly lower incidence of dementia (HR = 0.63 [0.38, 1.05]). On the other hand, SCT seems to interact with the apolipoprotein E ε4 risk allele resulting in poor performance on digit symbol substitution test at baseline (z-score = −0.08, $P_{interaction} = 0.05$) and over time (z-score = −0.12, $P_{interaction} = 0.04$); and with diabetes mellitus leading to a moderately increased risk of dementia (HR = 2.06 [0.89, 4.78], $P_{interaction} = 0.01$).

Conclusions: SCT was not an independent risk factor for prevalence or incidence of cognitive decline or dementia, although it may interact with and modify other putative risk factors for cognitive decline and dementia.

1. Introduction

Cerebral small vessel disease (CSVD) is a vascular risk factor associated with the development of cognitive abnormalities, overall cognitive decline and dementia [1]. The prevalence of CSVD and risk factors for CSVD are significantly higher among African Americans compared to non-Hispanic Whites [2]. This disparity exists even after adjusting for traditional dementia risk factors and relevant covariates [3,4]. Several studies have reported on the contribution of genetic variation, such as the *APOE* ε4 genotype, to the incidence and

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prevalence of dementia and cognitive decline in both African Americans and non-Hispanic Whites [5–7]. But reports on the impact of the *APOE* ε4 genotype on the incidence and prevalence of dementia and/or cognitive decline in African Americans or people of African ancestry has been mixed and does not explain the observed disparity in vascular dementia and cognitive dysfunction between African Americans and non-Hispanic Whites [5–8].

A genetic variant and potential risk factor for cognitive decline and dementia in African Americans, which might partly contribute to the observed disparity, is heterozygosity for the sickle cell mutation or sickle cell trait (SCT). Until recently, this variant has been thought to be clinically benign. In a recent study, we showed that SCT was an independent risk factor for incident and prevalent chronic kidney disease (CKD) and albuminuria [9,10]. Furthermore, studies have shown that African Americans with SCT have a significantly higher risk for atrial fibrillation and other cardiovascular phenotypes [11,12]. Both CKD [3,13,14] and atrial fibrillation [15,16] have been reported to be significantly associated with an increased risk of cognitive decline and dementia. A recent study also showed that young African Americans were significantly more likely to have evidence of silent cerebral infarcts on MRI compared to healthy age and sex matched controls.

Sickle cell trait, or heterozygosity for the sickle cell mutation, refers to the inheritance of one sickle β-globin gene and one normal β-globin gene. It is present in 8% or 3.5 million African Americans. The homozygous or double heterozygote state is known as sickle cell disease (SCD). The sickle β-globin mutation results from a point mutation in the β-globin gene, leading to the substitution of valine for glutamic acid at the 6th position of the β-globin chain [17]. Sickle cell disease is associated with CSVD such as silent cerebral infarction [18], which correlates with poor cognitive outcomes [19]. Furthermore, a recent study reported significantly lower cortical and subcortical brain volumes in patients with SCD (i.e. individuals who are homozygous or double heterozygotes for the sickle cell mutation) after adjusting for relevant risk factors [20]. These findings led us to hypothesize that African Americans with SCT would have a significantly higher risk for prevalent and incident cognitive decline as well as a higher incidence of dementia than African Americans without SCT, adjusted for relevant covariates. We tested our hypothesis in the African American participants of the Atherosclerosis Risk in Communities (ARIC) study.

2. Method

2.1. Study population

The ARIC study is a community-based cohort including 15,792 participants recruited from 4 sites across the United States between 1987 and1989. For this analysis, we included African American participants with information on sickle cell trait (SCT) from two ARIC sites (Total $N = 3010$): Forsyth County, North Carolina and Jackson, Mississippi. Participants in the ARIC study has had 5 exams (visit 1: 1987–1989, visit 2: 1990–1992, visit 3: 1993–1995, visit 4: 1996–1998, visit 5: 2011–2013) and were contacted by telephone annually (semi-annually since 2012) [21]. All participants were invited for a cognitive assessment at visit 2, visit 4, and visit 5. Brain MRI was performed for a subset of participants at visit 5 as part of an ancillary study, the ARIC-Neurocognitive Study (ARIC-NCS) [22,23]. Participants who were missing relevant covariates data or who did not provide consent for genetic study were excluded from this analysis (see Fig. 1). The ARIC study was approved by the Institutional Review Boards (IRBs) of the participating institution, while the present analysis was approved by ARIC's Publication and Presentation Committee.

2.2. Assessment of sickle cell trait status

Sickle cell trait was defined based on the presence of the minor allele of the rs334 single nucleotide polymorphism encoding the *HBB* p.Glu7Val mutation. Genotyping for SCT was performed via direct genotyping using Taqman®, as reported elsewhere [9,24,25]. Participants who were homozygous for hemoglobin S (HbSS) or who were compound heterozygotes were excluded from the study. Those with SCT were the exposure group while participants who did not have SCT or who had wild-type hemoglobin were the control and thus the comparison group.

2.3. Outcome

2.3.1. Cognitive assessment

Cognitive performance was assessed by three tests which were: (1) The Delayed Word Recall Test (DWRT) - designed to measure short term memory, (2) Digit Symbol Substitution Test (DSST) - designed to measure processing speed, and (3) Word Fluency Test (WFT) - designed to measure executive function [26–28]. Z scores for all cognitive test scores from each visit were generated by standardizing with visit 2 mean and standard deviation. The mean of the three z scores was calculated and standardized in the same way to create a composite cognition z score [21].

2.3.2. Assessment of grey matter volume in different brain regions (MRI brain ROIs)

One thousand nine hundred and six (1906) non-demented participants with mild cognitive impairment (59% women; 25% African-American; mean age 76.6 years) in the ARIC study underwent quantitative MR imaging for white matter hyperintensities (WMH) and infarcts. The Freesurfer imaging analysis pipeline was used to determine regional cerebral volumes [22,23]. Participants without contraindications were selected for a brain MRI according to the following criteria; (1) all people who had previous scans in 2004 to 2006, (2) those with low cognitive test scores\declines on longitudinally administered tests, and (3) an age-stratified random sample of the remaining individuals. Sampling fractions for the random sample were set for participants < 80 and ≥ 80 years of age to approximate the age distribution of those selected from the cognitively suspect group and were modified slightly over the course of the study to achieve a goal of $\approx$2000 total MRI scans. The final sample was 1980 and 74 out of 1980 (4%) were excluded because of cognitive impairment sufficient to suspect dementia (Mini-Mental Status Examination scores, < 21 if white and < 19 if African–American). Brain MRI was conducted on 3 Tesla scanners using a common set of sequences. White matter hyperintensities (WMH) burden was measured quantitatively in cubic centimeters using an algorithm at Mayo Clinic, Rochester MN [29]. We selected 6 regions of interest (ROIs) based on relevance to cognition: frontal ROI; temporal ROI; parietal ROI; occipital ROI; hippocampal ROI; and Alzheimer disease (AD)-relevant brain area [22,23]. Mean cortical volumes were evaluated for ROIs in this study. All ROI volumes were recorded in cubic centimeters. The final sample size for the analysis in this section was 421 because we only included African Americans which were 25% (495) of the MRI sample, but 74 (14.9%) were excluded for various reasons including missing relevant covariate data as described above.

2.3.3. Diagnosis of dementia

Dementia diagnosis was based on a combination of (1) the in-person cognitive assessment at ARIC-NCS/visit 5, including a longer more detailed neurocognitive battery, not limited to the three tests described above, and also accompanied by an informant interview; (2) the telephonic instrument of cognitive status-modified (TICS-m) and (3) an informant interview for those not undergoing the in-person assessment, in addition to longitudinal cognitive scores. Hospital or death certificate codes only were used in situations where informant interviews could not be obtained and the participant was not examined at visit 5; for this category, we used the International Classification of Diseases, Ninth Revision (ICD-9) hospital discharge code. Diagnosis of dementia for

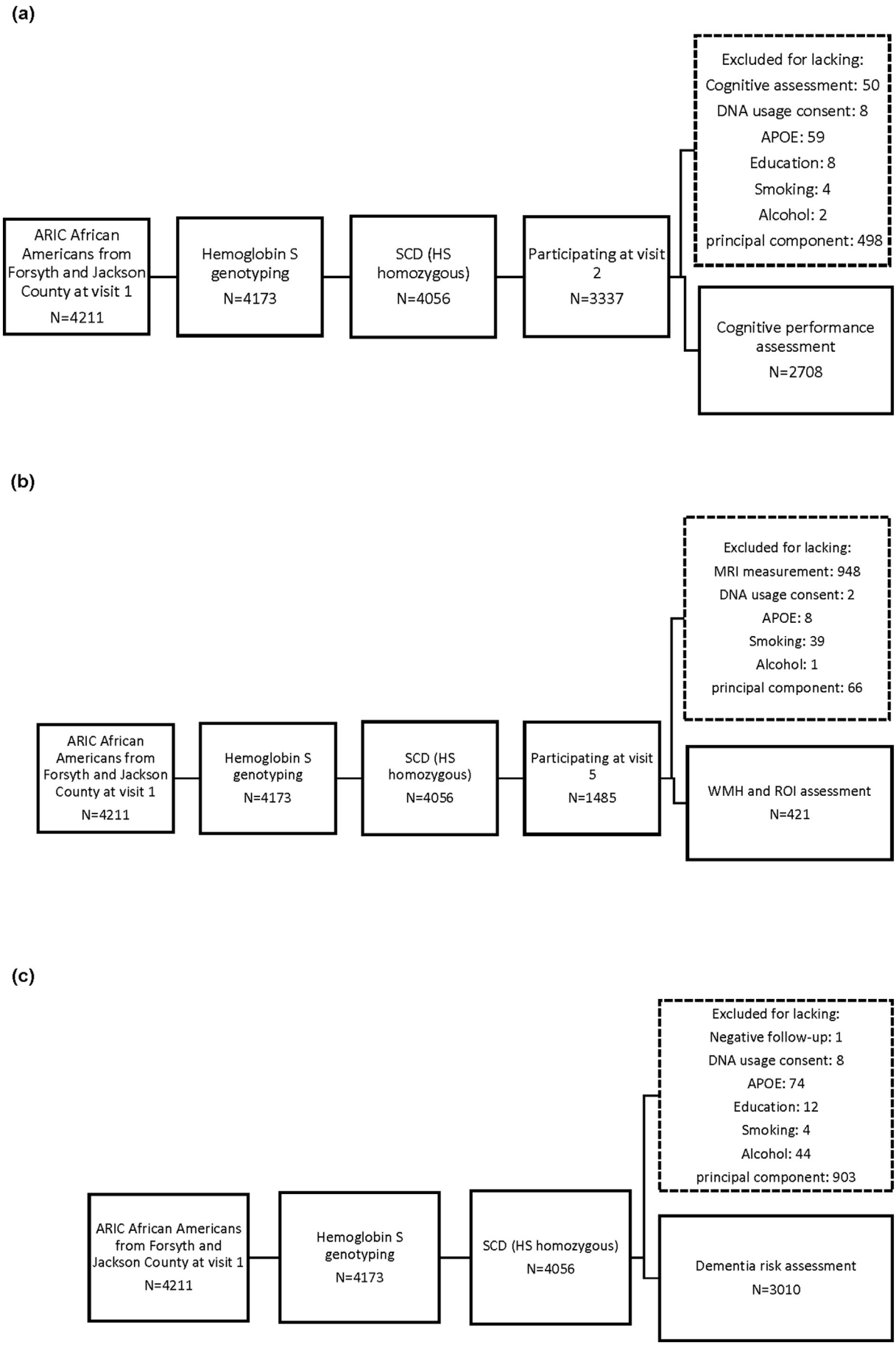

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Fig. 1. Charts indicate participants who were included in each major analysis. In each box, we have indicated the reasons why a specific number of participants were excluded. Fig. 1a shows those included in the cognitive function analysis, Fig. 1b show the number of participants included in the WMH and ROIs analysis, while Fig. 1c shows the number of participants included in the Dementia risk assessment/incident of dementia analysis. WMH = White Matter Hyperintensity, ROI = Region Of Interest.

those participants with the complete in-person visit 5 assessments were adjudicated by a panel of 3 independent reviewers, the details of the adjudication process have been previously described and published [28].

2.4. Covariates

Race and education were self-reported at ARIC visit 1. Other demographic and lifestyle information including age, sex, smoking status (defined as current, former or never), and alcohol drinking status (defined as current, former or never) were assessed at each visit. Weight and height were measured and BMI was calculated as weight (kg)/height [2] (m^2). The *APOE* ε4 risk allele genotyping and classification has been described elsewhere [30]. Global genetic ancestry was estimated for each participant using principal components of genetic ancestry [31,32], and the first 10 components (eigenvectors) included in each model to adjust for population sub-structure. Participants were determined as having diabetes if they had fasting blood glucose ≥126 mg/dL or non-fasting blood glucose ≥200 mg/dL, self-reported physician diagnosis of diabetes, or were taking medication for diabetes. Hypertension status was defined based on systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or self-reported physician diagnosis of hypertension or history of use of antihypertensive medications. Stroke was defined as self-reported history of stroke at visit 1 or adjudicated hospitalized events during follow-up.

2.5. Statistical analysis

Statistical analysis was performed using SAS software (version 9.4 [SAS Institute Inc., Cary, NC]) and two-sided tests. Distribution of demographic characteristics were compared between the SCT group and the control group using *t*-test and χ^2 statistics. Concurrent measures (such as cognitive assessment at visit 2 or brain volumes measures) were used in cross-sectional analyses while baseline measures (such as age, sex and educational status) were used in longitudinal models. We used visit 2 data as baseline covariates for the longitudinal models of cognitive tests, visit 5 data as baseline for MRI measures, and visit 1 data as baseline for analysis of incidence of dementia.

We estimated the association of SCT with cognitive test z scores at visit 2 and MRI derived cerebral measures at visit 5 using multiple linear regression. For MRI derived measures (only available in a subset of participants), WMH burden and hippocampal ROI were right skewed and therefore were log-transformed. Generalized estimating equations with unstructured covariance structure between visits were used to evaluate 20-year cognitive performance trajectories in and between each group. Linear spline terms were incorporated with knot at 6 years (corresponding to time of visit 4). We used inverse probability of attrition weighting (IPAW) to adjust for selective attrition due to death and non-participation at visit 4 and visit 5 using the method described previously [27]. The logistic regression predicting attrition included all the covariates listed above as well as interaction terms between covariates. All models involving WMH and ROI volumes were adjusted for total intracranial volume.

Cox proportional hazards models were used to compare rates of incident dementia between the two groups (SCT, no SCT). Time to dementia was defined as the time elapsed between visit 1 and the date of dementia diagnosis. Individuals were censored if they developed dementia, were lost to follow-up, or were administratively censored (September 1, 2013), whichever occurred earlier. Hazard ratios (HRs) and 95% confidence intervals of dementia outcome were calculated for the SCT group compared to the control group.

Nested models were applied in the analyses. Model 1 included age at baseline, sex, principal components of global genetic ancestry (to adjust for population substructure), and *APOE* genotype as covariates. Model 2 additionally adjusted for baseline characteristics of BMI, educational level, smoking status, alcohol drinking status, diabetes and stroke prevalence. In longitudinal analysis and survival analysis, we further adjusted for incidence of stroke as a time varying covariate in model 2.

Based on an a priori hypothesis of a possible interaction between SCT and the *APOE* ε4 risk allele, we assessed whether the effect of SCT on cognitive outcomes would differ according to the presence or absence of the *APOE* ε4 risk allele. For this analysis, the presence or absence of *APOE* ε4 risk allele was expressed as a dichotomous variable and we introduced an interaction term between SCT and *APOE* ε4 risk allele. Similarly, we examined potential modifying effects of diabetes mellitus on the relationship of SCT with incidence of dementia. This test is based on the putative link between diabetes mellitus and cognitive impairment even in the absence of overt cerebral injury. Results for interactions were reported as *p* values and, when significant at the conventional 0.05 level, we conducted stratified analyses.

2.6. Data availability

The de-identified data used for this study is immediately available from the ARIC data coordinating center, after appropriate federal and ARIC imposed data use agreement are satisfied.

3. Result

A total of 4211 African Americans participants were recruited in ARIC visit 1. Of this, 4173 were genotyped for the sickle β-globin gene mutation or hemoglobin S (HbS). After excluding those that were homozygous for sickle β-globin gene mutation (i.e. HbSS) or are double heterozygotes such as HbSC, we were left with 4056 participants. Of this number, 3337 participated in ARIC visit 2. A total of 2708 out of 3337 were included in the 20-year cognitive function decline analysis, after excluding 629 participants who were either missing relevant covariates, did not provide consent for genetic study or data usage for subsequent ancillary studies or did not have baseline cognitive assessment scores. In addition, 421 (i.e. 25% of those who got MRI) were included in the WMH and ROIs analysis (after excluding 116 participants missing covariates or not consenting to genetic data usage and 948 without MRI data at the ARIC-NCS visit 5). Finally, 3010 people recruited at visit 1 were eligible for the evaluation of dementia risk (after excluding 1045 participants with missing covariates, principal components or no consent and 1 person because of prevalent dementia at baseline) (Fig. 1).

Baseline characteristics at visit 2 among SCT participants compared to those with a normal genotype (no SCT) are shown in Table 1. Participants with and without SCT had a mean age of 56 years and female proportion of around 60%. There was also a similar distribution of smoking status, alcohol use, *APOE* genotype distribution, prevalent diabetes, and prevalent and incident stroke between participants with SCT ($N = 176$) and those without SCT ($N = 2532$). Baseline characteristics at visit 1 were similar to visit 2 and are shown in Supplemental Table 2. SCT participants at visit 5 had a similar average age of 76 years, a lower female proportion, a lower prevalence of stroke, and were less likely to smoke compared to the group without SCT (Supplemental Table 1).

Table 1
Selected characteristics of participants stratified by sickle cell genotype at visit 2: The Atherosclerosis Risk in Communities Study, 1990–1992. These are, the participants in the baseline cognitive analysis group in Table 2.

SCT status[a]	No SCT	SCT	p value
N	2532	176	
Follow up after visit 2, in years, mean (SD)	10.1 (8.9)	10.8 (9.0)	0.38
Age, years, mean (SD)	56.1 (5.7)	56.4 (5.7)	0.47
Female, %	63.9	62.5	0.71
Education level, %			0.26
Less than high school	37.8	40.9	
High school or vocational school	28.2	30.1	
College, graduate school, or professional school	34.0	29.0	
BMI, kg/m^2, mean (SD)	30.1 (6.2)	29.6 (5.7)	0.33
Smoking status, %			0.23
Current	26.1	20.5	
Former	29.5	30.1	
Never	44.4	49.4	
Alcohol use, %			0.21
Current	33.4	39.2	
Former	31.5	26.1	
Never	35.1	34.7	
Prevalent diabetes, %	25.2	21.6	0.29
Prevalent stroke, %	2.7	3.4	0.63
Incident stroke, %	5.0	4.2	0.66
APOE genotype, %			0.43
44	4.1	4.6	
24 or 34	36.1	31.3	
Other	59.8	64.2	

[a] No SCT indicates homozygous hemoglobin A; SCT indicates sickle cell trait.

3.1. Cognitive performance

The fully adjusted means of baseline z score for global cognition at visit 2 were similar in African Americans with SCT and those without SCT (difference − 0.03, 95%CI: −0.12, 0.06), with comparable results for the individual cognitive tests (DWRT, DSST, and WFT) (Table 2). The 20-year decline in global cognition z score was nearly identical among African Americans with SCT compared to the control group before (Supplemental Table 3) and after IPAW (Table 3). Similar results were observed for the DWRT, DSST, and WFT.

3.2. WMH and ROIs

Overall, volumes of WMH and ROIs were similar between participants with and without SCT. The average parietal ROI volume among participants with SCT was 2.67 cm^3 larger than the average volume in the control group (95%CI: 0.24, 5.11, p = .03). Additionally, participants with SCT also had a slightly larger temporal ROI (1.49, 95%CI: −1.19, 4.17, p = .27) volume and AD-related brain area (0.76, 95%CI: −0.76, 2.28, p = .33), but these differences were not statistically significant. The volumes of WMH, frontal and occipital ROI, and hippocampal volumes were very similar in participants with SCT and without SCT (Table 4).

3.3. Dementia incidence

Over a median follow up of 22.9 years, 16 and 388 cases of dementia were identified among participants with and without SCT, respectively. As shown in Table 5, participants with SCT had a lower incidence of dementia compared to participants without SCT (HR: 0.63, 95%CI: 0.38, 1.05), but this was not statistically significant (p = .08).

3.4. Assessment of interaction between SCT and APOE ε4 risk allele

We observed a significant interaction between SCT and *APOE* genotype for the association with DSST, with worse scores at baseline (z-score = −0.08, $p_{interaction}$ = 0.05) and faster 20-year score decline (z-score = −0.12, $p_{interaction}$ = 0.04) associated with SCT in those with the *APOE* ε4 risk allele compared to those without the allele. DSST score at visit 2 and the decline between visit 2 and visit 5 for each *APOE* category are shown in Supplemental Table 4. No interaction of SCT and *APOE* genotype for other cognitive outcomes or incidence of dementia was observed.

3.5. Interaction between SCT and Diabetes Mellitus

In multivariable analysis among participants with diabetes, the incidence of dementia was higher (HR = 2.06 [95%CI = 0.89–4.78]) among participants with SCT compared to those without SCT. On the other hand, among participants without diabetes mellitus there was a significantly lower (HR = 0.45 [95%CI = 0.23–0.87]) incidence of dementia among those with SCT compared to those without SCT ($P_{interaction}$ = 0.01) (Supplemental Table 5).

4. Discussion

In this study, we evaluated whether SCT was an independent risk factor for cognitive function at middle age, cognitive decline in later life, and/or the incidence of dementia in African Americans. Overall,

Table 2
Adjusted Z score means of cognitive function tests (global score, DWRT, DSST, WFRT) and their differences by sickle cell genotype groups among participants at visit 2: The Atherosclerosis Risk in Communities Study, 1990–1992.

SCT status[a]	Model	No SCT		SCT		Difference[b]		p value
N		2532		176				
Global z-score	Model 1	−0.55	(−0.60, −0.50)	−0.57	(−0.68, −0.45)	−0.02	(−0.13, 0.09)	0.71
	Model 2	−0.51	(−0.56, −0.47)	−0.54	(−0.64, −0.45)	−0.03	(−0.12, 0.06)	0.54
DWRT[c] z-score	Model 1	−0.31	(−0.38, −0.24)	−0.40	(−0.56, −0.23)	−0.09	(−0.25, 0.07)	0.25
	Model 2	−0.30	(−0.37, −0.23)	−0.40	(−0.56, −0.24)	−0.10	(−0.26, 0.05)	0.19
DSST[d] z-score	Model 1	−0.94	(−1.00, −0.89)	−0.87	(−1.01, −0.74)	0.07	(−0.06, 0.20)	0.28
	Model 2	−0.91	(−0.96, −0.86)	−0.84	(−0.96, −0.73)	0.06	(−0.04, 0.17)	0.25
WFT[e] z-score	Model 1	−0.38	(−0.45, −0.32)	−0.43	(−0.59, −0.27)	−0.04	(−0.20, 0.11)	0.58
	Model 2	−0.34	(−0.40, −0.28)	−0.38	(−0.52, −0.25)	−0.05	(−0.18, 0.08)	0.49

Model 1: Adjusted for age, sex, principal components and *APOE* genotyping status.
Model 2: Additional adjustment for BMI, educational level, alcohol, smoking, diabetes, hypertension and prevalent stroke.
[a] No SCT indicates homozygous hemoglobin A; SCT indicates sickle cell trait.
[b] Difference indicates the difference of scores in SCT group compared with HbAA group.
[c] Delayed Word Recall Test.
[d] Digit Symbol Substitution Test.
[e] Word Fluency Test.

Table 3
Adjusted cognitive function Z score declines and differences in decline between sickle cell genotype groups among participants over 20 years after *inverse probability of attrition weighting*[*]: The Atherosclerosis Risk in Communities Study, 1990–2013.

SCT status[a]	Model	No SCT		SCT		Δ decline[b]		p value
N		2532		176				
Global z-score	Model 1	−0.64	(−0.73, −0.55)	−0.67	(−0.85, −0.49)	−0.03	(−0.21, 0.15)	0.71
	Model 2	−0.46	(−0.72, −0.20)	−0.53	(−0.83, −0.24)	−0.07	(−0.24, 0.10)	0.40
DWRT[c] z-score	Model 1	−0.96	(−1.15, −0.78)	−1.20	(−1.54, −0.85)	−0.24	(−0.58, 0.11)	0.18
	Model 2	−1.09	(−1.64, −0.55)	−1.36	(−1.95, −0.77)	−0.27	(−0.59, 0.06)	0.11
DSST[d] z-score	Model 1	−0.52	(−0.59, −0.45)	−0.50	(−0.66, −0.35)	0.02	(−0.15, 0.18)	0.85
	Model 2	−0.37	(−0.56, −0.17)	−0.39	(−0.63, −0.15)	−0.02	(−0.17, 0.13)	0.79
WFT[e] z-score	Model 1	−0.37	(−0.47, −0.27)	−0.32	(−0.52, −0.12)	0.06	(−0.14, 0.25)	0.58
	Model 2	0.06	(−0.22, 0.34)	0.07	(−0.26, 0.39)	0.01	(−0.17, 0.20)	0.90

Model 1: Adjusted for age, sex, principal components and *APOE* genotyping status.
Model 2: Additional adjustment for BMI, educational level, alcohol, smoking, diabetes, prevalent stroke and incident stroke during the follow-up.
[*] Excluding visit 5 data for participants who did not attend visit 4.
[a] No SCT indicates homozygous hemoglobin A; SCT indicates sickle cell trait.
[b] Δ decline is the difference in SCT group compared with HbAA group in the change of scores over 20 years.
[c] Delayed Word Recall Test.
[d] Digit Symbol Substitution Test.
[e] Word Fluency Test.

Table 4
Adjusted average white matter hyperintensity (WMH) lesions and region of interest (ROI) volumes associated with SCT among participants at visit 5: The Atherosclerosis Risk in Communities Study, 2011–2013. Large WMH are detrimental to cognitive function or performance. Note that larger cortical volumes are good for cognitive functioning and performance.

SCT status[a]	Model	No SCT		SCT		Difference[b]		p value
N		389		32				
WMH[*]	Model 1	9.56	(9.43, 9.69)	9.51	(9.22, 9.80)	−0.05	(−0.33, 0.23)	0.73
	Model 2	9.59	(9.42, 9.75)	9.56	(9.25, 9.87)	−0.03	(−0.03, 0.25)	0.85
Volumetric ROI (cm^3)								
Frontal cortical	Model 1	143.28	(141.72, 144.84)	143.51	(140.09, 146.93)	0.23	(−3.10, 3.56)	0.89
	Model 2	143.50	(141.52, 145.48)	143.87	(140.22, 147.52)	0.37	(−2.96, 3.70)	0.83
Temporal cortical	Model 1	97.13	(95.88, 98.38)	98.40	(95.66, 101.14)	1.27	(−1.40, 3.94)	0.35
	Model 2	97.09	(95.49, 98.68)	98.58	(95.64, 101.51)	1.49	(−1.19, 4.17)	0.27
Parietal cortical	Model 1	98.19	(97.04, 99.34)	100.67	(98.15, 103.18)	2.47	(0.02, 4.92)	0.05
	Model 2	97.87	(96.42, 99.31)	100.54	(97.88, 103.21)	2.67	(0.24, 5.11)	0.03
Occipital cortical	Model 1	36.91	(36.29, 37.54)	36.84	(35.47, 38.20)	−0.08	(−1.41, 1.25)	0.91
	Model 2	36.70	(35.91, 37.50)	36.81	(35.35, 38.26)	0.10	(−1.23, 1.43)	0.88
Hippocampal[*]	Model 1	8.79	(8.77, 8.81)	8.81	(8.76, 8.85)	0.01	(−0.03, 0.06)	0.51
	Model 2	8.79	(8.77, 8.82)	8.81	(8.76, 8.86)	0.02	(−0.03, 0.06)	0.51
AD-relevant brain area	Model 1	55.31	(54.60, 56.02)	55.99	(54.44, 57.55)	0.68	(−0.83, 2.19)	0.38
	Model 2	55.26	(54.36, 56.17)	56.02	(54.35, 57.68)	0.76	(−0.76, 2.28)	0.33

Model 1: Adjusted for age, sex, principal components and *APOE* genotyping status.
Model 2: Additional adjustment for BMI, educational level, alcohol, smoking, diabetes, prevalent stroke, intracranial volume and incidence of stroke during the follow-up.
[*] WMH and hippocampal volumes were log-transformed.
[a] No SCT indicates homozygous hemoglobin A; SCT indicates sickle cell trait.
[b] Difference indicates the difference of volumes in SCT group compared with HbAA group.

our results show that there was no evidence of worsened or worsening cognitive performance or increased risk of dementia in individuals with SCT compared to those without SCT. On the contrary, SCT was associated with slightly better scores on neurocognitive tests, and a nominally lower risk of dementia in African Americans. Specifically, we noted that participants with SCT did not perform any worse on baseline cognitive assessment tests compared to those without SCT. In addition, SCT was not associated with worsened performance in domain specific cognitive assessment tests. Furthermore, SCT was not a significant predictor of decline in global and domain specific cognitive function over 20 years (Tables 2 and 3). Our study findings corroborate those reported from the REason for Geographic And Racial Differences in Stroke (REGARDS) study, where they also observed that SCT was not a predictor of incident, prevalent or decline in cognitive function [33,34].

Furthermore, when we examined the role of SCT in structural/anatomical changes in the brain, we noted a similar trend as for cognitive assessment. Sickle cell trait was not associated with differences in WMH volume, or frontal cortical, temporal cortical, occipital cortical or hippocampal volumes among participants with SCT compared to those without SCT. Similarly, there was no significant difference in Alzheimer's disease (AD) relevant brain areas among participants with SCT compared to those without SCT. This observation is in concert with our baseline findings of similar prevalence of stroke among participants with SCT compared to those without SCT and supported by a recent finding that SCT was not an independent genetic risk factor for stroke among African Americans [35]. Surprisingly, we noted that participants with SCT had larger average parietal cortical volume compared to those without SCT (Table 4). The exact biological mechanism for this and the prior observations is not immediately apparent, and could just be a spurious finding. However, recent studies have shown that ischemic preconditioning from either a remote source such as limb ischemia using cuffs [36–39] or direct subclinical cerebral

Table 5
Hazard ratios (HRs) and 95% confidence intervals (CIs) for incident dementia according to sickle cell genotype among participants: The Atherosclerosis Risk in Communities Study, 1987–2013.

SCT status[a]	No SCT	SCT	*P*-values
N	2813	197	
dementia cases, n	388	16	
person-years	56,746	3912	
Incidence rate (per 1000 person-years)	6.8	4.1	
	Hazard ratio (95% CI)		
Cox regression			
Model 1	1	0.62 (0.37, 1.02)	0.06
Model 2	1	0.63 (0.38, 1.05)	0.08

Model 1: Adjusted for age, sex, principal components and *APOE* genotyping status.
Model 2: Additional adjustment for BMI, educational level, alcohol, smoking, diabetes, prevalent stroke and incident stroke during follow-up.
[a] No SCT indicates homozygous hemoglobin A; SCT indicates sickle cell trait.

ischemia as seen after a transient ischemic attack (TIA) [40–43], is associated with improvement in cognitive function, decrease in infarct volume and overall clinical recovery after a stroke. Thus, we speculate here that the brain of participants with SCT might have undergone ischemic preconditioning, remote and direct from many years of the subclinical repeated cerebral ischemia as a result of an slight abnormality in the oxygen dissociation curve [44], relative anemia and/or transient repeated cerebral microvascular obstruction by sickle erythrocytes respectively. Other pathologies, as was recently suggested [45,46], but which were not part of the focus of this study and manuscript might be responsible for the higher parietal cortical thickness among participants with SCT. Further evidence supporting this hypothesis is the finding that participants with SCT were 37% less likely to develop dementia compared to those without SCT (Table 5), although it should be noted that the number of dementia cases with SCT is small ($N = 16$). These overall findings contradict previous reports in individuals with sickle cell disease (SCD). Among individuals with SCD, studies have repeatedly shown that adults [47] and children [48] with SCD were more likely to have smaller global and region-specific brain volumes and were also more likely to develop silent cerebral infarcts and cognitive deficits compared to normal healthy controls without SCD or SCT. Our study findings are in contrast to this, but it is also possible that patients with SCD had more severe ischemia that results in overt injury instead of preconditioning. Thus, this study raises many questions, especially on the mechanism of the observed association of SCT with lower risk of dementia and cognitive deficits. The primary results of our study showed no association with cognitive function or decline, only in the interaction of SCT with *APOE* ε4 for DSST scores over time or SCT with diabetes mellitus for incidence of dementia. The marginal interactions with larger confidence intervals in the SCT groups suggest that these estimates and the direction of the observed associations are less robust and should be interpreted with caution.

When we assessed the impact of an interaction between SCT and the *APOE* ε4 risk allele on cognitive function, we noted that the presence of the *APOE* ε4 risk allele significantly modified the effect of SCT on the DSST component of the cognitive assessment. We observed that participants with both the *APOE* ε4 risk allele and SCT had lower cognitive assessment scores on the DSST compared to those with only the *APOE* ε4 risk allele but without SCT. Similarly, individuals with SCT and the *APOE* ε4 risk allele experienced a faster decline in cognitive function over 20 years based on the DSST score domain compared to those with only the *APOE* ε4 risk allele but without SCT. In both cases, participants with SCT and no *APOE* ε4 risk allele had slightly slower cognitive decline based on the DSST compared to those without SCT and the *APOE* ε4 risk allele, suggesting an association of SCT with better cognitive function (Supplemental table 4). The biological basis for this observed association is not known at this time and more/larger studies are needed to replicate this finding. However, our hypothesis is that in the presence of the *APOE* ε4 risk allele the insult is deleterious rather than resulting in ischemic preconditioning as would be expected when SCT alone is present. It is not clear why only the DSST component was associated with the interaction. But in a recent study, it was shown that only the DSST component of the global cognitive assessment was abnormal among adults with SCD [49], suggesting that the impact on cognition, of the co-inheritance of SCT and no *APOE* ε4 risk allele might be similar to that of SCD.

We also observed that non-diabetic participants with SCT had a 55% lower incidence of dementia compared to those without SCT. On the other hand, among participants with diabetes mellitus, those with SCT had approximately a two-fold higher risk of developing dementia compared to those without SCT, thus suggesting a possible interaction between SCT and diabetes mellitus. A similar reasoning as for the interaction between SCT and *APOE* ε4 risk allele can be applied here. Furthermore, in a recent study [50], it was shown that among African Americans with SCT, the HbA1c estimate was significantly lower for a given serum glucose concentration compared to those without SCT. This underestimation likely impacts glucose control, especially when it is based on HbA1c levels. This might be one of the reasons for the higher incidence of dementia among African Americans with SCT and diabetes in this study. We maintain that our possible biological explanations in both cases are purely speculative and further investigations are needed to replicate these findings and to determine the potential biological mechanisms behind the observed interactions.

This study has utilized a large prospective cohort to determine whether SCT was a genetic risk factor for cognitive decline among African Americans. While most of our findings were robust, our study had some limitations which deserve mentioning. The final sample available for analysis was about 64% of the total sample of African Americans enrolled into ARIC. It is not clear how data from the 36% of participants not available for analysis would have impacted the results of our study. However, overall our results were in agreement with those from REGARDS (a larger study) cited above. Similar concern arises for MRI data, which due to budgetary constraints, was only available from 421 participants. This limits the conclusions that can be drawn from the analysis of the anatomical impact of SCT on global and regional brain volumes and larger studies are needed to ensure that future results and conclusions are more robust. Further, the small sample of dementia cases with SCT ($N = 16$), individuals with co-inheritance of the *APOE* ε4 risk allele and SCT, and those with both SCT and diabetes mellitus, limits the conclusions that can be drawn from the interaction tests. These interaction findings need to be replicated in a much larger sample. Unfortunately, it was not possible for the authors to do this, as it will involve starting a new cohort and collecting similar cognitive and MRI outcomes. Finally, the SCD and SCT phenotypes can be modified by other hemoglobinopathies such as alpha-thalassemia trait. Genotyping for and testing such interaction was beyond the scope of this manuscript. Also, the low prevalence (2–3%) of alpha thalassemia trait among African Americans, meant that we will not be adequately powered to detect any effect if they exist.

In conclusion, we observed that SCT was not an independent risk factor for prevalent or incident cognitive decline, but it could potentially interact with and modify other genetic risk factors for dementia and cognitive dysfunction. Finally, by an as of now unknown mechanism, participants with only SCT seems to have a lower risk for cognitive decline and dementia, but additional studies are needed to support a more robust conclusion on this point and to tease out the potential mechanisms underlying the observed reduction in risk associated with SCT.

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Statement of authorship contribution

AA and HIH conceived and designed the study. NC and AA performed data analysis guided by HIH. NC and CC wrote the initial manuscript draft. VKD, AK, RS, NK, RFG, MLG, JB, EB, BGW, THM and HIH provided critical reviews of subsequent iterations of the manuscript. All authors listed contributed enough to merit authorship.

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Appendix A. Supplementary data

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