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Variation in Neurodegeneration-Linked Brain Regions in Young Adult APOE E4 Carriers With Spina Bifida

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ABSTRACT

Objective: Possible pleiotropic effects of apolipoprotein E4 (APOE E4) in individuals with congenital brain malformations are relatively unknown. Our goal was to determine if neurodegeneration-linked brain region volumes differ significantly between E4 carriers and noncarriers in young adults with spina bifida (SB).

Methods: Eleven individuals (> 18 years), genotyped for APOE, underwent neuroimaging and neurocognitive evaluation. Primary analysis: Magnetic resonance imaging (MRI) data from 10 a priori neurodegeneration-risk regions of interest were compared between E4 carriers and noncarriers, adjusting for age, sex, and total intracranial volume (FDR-adjusted $p < 0.05$). Secondary analyses: Age-adjusted neurocognitive standard scores were compared between groups ($p < 0.05$). Post hoc analyses of NeuroQuant-derived regional brain volumes were examined for combined group differences in young adults with SB.

Results: Comparison of a priori risk region volumes revealed significantly lower left amygdala volumes (FDR-adjusted $p = 0.04$) in young adult E4 carriers ($n = 4$) relative to noncarriers ($n = 7$). Neurocognitive data were not significantly different between the groups. A possible trend was detected for enlarged parietal volumes in E4 carriers ($p = 0.07$), while volumetric extremes (> 95% or < 5%) were detected for the anterior cingulate (100% of cases; $p = 0.001$), frontal cortices (90% of cases), hippocampus (80% of cases), and entorhinal cortices (70% of cases).

Interpretation: Early left amygdala volumetric reduction was found in E4 carriers; combined group volume comparisons revealed frontal and temporal lobe differences in young adults with SB relative to age- and sex-matched volumetric estimates. This pilot investigation does not appear to support E4 conferring a pleiotropic benefit in young adults with SB but rather supports further investigation of MRI volumetrics as a possible biomarker for this population.

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1 | Introduction

Spina bifida aperta or myelomeningocele (henceforth, SB) is the most common type of open neural defect and is associated with brain malformations and neurocognitive impairments [1–9]. In persons with SB, the prevalence of apolipoprotein E (APOE) allelic expression for heightened late-onset Alzheimer disease (LOAD) genetic risk (e.g., APOE E4⁺ carriers) does not differ significantly from the general population [10]. However, it remains unclear if APOE allelic expression in individuals with SB may differentially impact regional brain structure or function during early, middle, and late life [10–16].

Using a prospective clinical sample of young adults with SB (age range of 19–46 years with a mean age of 28 ± 7.8), we sought to determine if APOE E4 carrier status had a similar impact on brain volumes in LOAD risk regions and if neurocognitive functioning is differentially impacted between E4 allele carriers and noncarriers.

Patients with SB have many magnetic resonance imaging (MRI) abnormalities, such as structural changes in the posterior fossa and smaller cerebellar hippocampal and putamina but not amygdala volumes [5, 17–26]. In addition, brain malformations of the cerebellum, hindbrain, midbrain, corpus callosum, and cortex and degree of ventriculomegaly and abnormal connections detected on MRI correlate with impaired neuropsychological abilities [3, 27–38].

APOE is an intracellular cholesterol and fatty acid transport protein that has multiple isoforms, of which the E4 isoform is associated with Alzheimer disease (AD) as well as with other neurodegenerative diseases. E4 is also associated even in the premorbid stages with abnormalities in the structure and function of the medial temporal lobe and related areas [12, 39–48]; healthy young APOE E4 allele carriers exhibit greater bilateral mesial temporal lobe activity relative to noncarriers to accomplish the same episodic memory encoding task [11]. Young adult APOE E4 carriers also demonstrated greater functional connectivity between the mesial temporal lobe, posterior cingulate, and other peri-limbic regions relative to non-E4 controls [11]. Altered brain functional connectivity in APOE E4 carriers relative to noncarriers has also been found in older adults undergoing surgery, independent of serum biomarkers of AD neuropathology [49]. In both cases, APOE-influenced differences were observed in known dementia-risk brain regions (e.g., mesial temporal lobe, posterior cingulate, and angular gyrus). Additional volumetric differences have been detected in young adults in parahippocampal gyrus and anterior cingulate regions, suggesting that greater cortical thickness and volume in AD risk regions are associated with better cognitive performance in young APOE E4 carriers [50, 51]. These structural and functional imaging differences contribute to evidence of possible early-life antagonistic pleiotropy of the *APOE* gene, but whether these pleiotropic effects hold for individuals with congenital brain malformations is unknown.

2 | Methods

2.1 | Participants

Young adults with SB were recruited from the Duke SB multidisciplinary clinic for this prospective observation cohort study.

A clinical nurse, a research coordinator, or the study principal investigator (J.M.J.) identified individuals meeting study inclusion criteria. Participants were included if they had a diagnosis of SB with hydrocephalus, were ≥ 18 years of age, were proficient in English, and had either a current right-sided ventricular shunt or history of ventricular shunt. Study procedures conformed to the World Medical Association's Declaration of Helsinki ethical principles. The Duke University Medical Center Institutional Review Board provided study oversight and approval.

2.2 | APOE Genotyping

Four buccal swabs per participant were collected using commercially available genotyping kits (Labcorp; Burlington, North Carolina, the United States). All participant samples were collected in the clinic under the instruction and observation of the research coordinator. Buccal swab kits were processed via PCR (Labcorp test 504040—APOE Alzheimer Disease Risk), which resulted in the detection of all possible APOE epsilon allelic combinations. Participants were offered to obtain their results and discuss the significance of this result with the PI.

2.3 | Neuroimaging Protocol and Procedures

Neuroimaging data were acquired using a 48-channel head coil on a 3-Tesla General Electric MR750 magnetic resonance scanner with a gradient strength of 100 mT/m and slew rate of 250 T/m/s provided by the Brain Imaging and Analysis Center at Duke University Medical Center. Participants were screened for possible MRI-incompatible implants or devices, as well as possible claustrophobia-related anxiety, which would preclude reliable imaging data collection.

Structural neuroimaging data were acquired using a high-resolution, T1-weighted, fast spoiled gradient (FSPGR) sequence. T1-weighted FSPGR data were acquired with a 2244 ms repetition time (TR), 2.9 ms echo time (TE), 900 ms inversion time (TI), and an 8° flip angle. The T1 acquisition matrix was set at $256 \times 256 \times 176$ with a field of view set at $240 \times 240 \times 176$ mm and a voxel size of $0.937 \times 0.937 \times 1.0$ mm.

2.4 | Neurocognitive Assessment Procedures

A battery of standardized neuropsychological assessment measures was constructed for this study to sensitize for working memory and visuospatial abilities, while still covering memory, executive, and psychomotor assessment domains. Greater emphasis was placed on visuospatial and working memory performance sampling, given known common neurocognitive deficits in these domains in persons with SB [1–3, 6, 7]. The Montreal Cognitive Assessment (MoCA) [52] screen was used as a single point estimate of overall cognitive abilities. Auditory-verbal learning, recall, and recognition were assessed using the Rey Auditory Verbal Learning Test (RAVLT) [53], and similar memory components were assessed for visual information using the Brief Visuospatial Memory Test—Revised (BVMT-R) [54]. Complex mental rotation and pattern completion abilities were

assessed using the Wechsler Adult Intelligence Scale—fourth revision (WAIS-IV) Visual Puzzles subtest [55], while more basic visuospatial abilities were assessed using the Judgment of Line Orientation (JOLO) [56]. Selective attention was assessed using the National Institutes of Health (NIH) Toolbox—Flanker Task [57], and complex attention (i.e., working memory) was evaluated using the List Sorting Working Memory Task from the NIH Toolbox [58]. Upper-extremity bilateral manual dexterity abilities were assessed using the Grooved Pegboard Test [59]. The test order was structured to mitigate proactive interference effects during testing (i.e., JOLO, Grooved Pegboard, and NIH Toolbox—Flanker Task measures were administered between the initial learning and delayed recall of the RAVLT and BVMT-R memory measures). All neurocognitive assessment procedures and testing were carried out by trained research personnel under the supervision of a licensed clinical neuropsychologist (J.N.B.). Participants were offered to review their neurocognitive results after testing.

3 | Analyses

3.1 | A Priori Neuroimaging Data Analyses

T1-weighted volumetric data were compared between SB APOE E4 carriers and noncarriers on 10 a priori LOAD-risk regions-of-interest (ROIs), which covered the left and right mesial temporal lobe (hippocampus, parahippocampal gyrus, and amygdala), angular/supramarginal gyrus region, and posterior cingulate cortices. The LOAD-risk ROIs were generated using atlas-based segmentation using the 84 region-defined Illinois Institute of Technology (IIT) young adult segmentation atlas (v.5.0) [60]. The IIT brain atlas was brought to individual participant space using the small animal multivariate brain analysis (SAMBA) pipeline [61]. Volumetric data were evaluated using an ANCOVA model, controlling for age, sex, and total intracranial volume (TIV). IIT atlas-derived regional volume differences between APOE carrier and noncarrier groups were considered statistically significant with an FDR-adjusted p value < 0.05 , corrected for multiple ROI comparisons.

3.2 | Neurocognitive Assessment Data Analyses

Participant neurocognitive assessment data were converted to demographically adjusted standard scores (z -scores) based on normative data comparison. Nonparametric group comparisons were conducted to examine differences between persons with SB who are APOE E4 carriers relative to noncarriers. Group comparisons were conducted using Mann–Whitney U test with statistical significance set to $p < 0.05$ (corrected for multiple variable comparisons). The effect sizes of any neurocognitive variable group differences were determined using nonparametric rank biserial correlation.

3.3 | Exploratory Neuroimaging Analyses

A Food and Drug Administration–approved, turnkey, and data-driven brain segmentation approach was undertaken after the

APOE group LOAD-risk region analyses to evaluate general SB-associated volumetric differences relative to normative neuroimaging data. NeuroQuant (Cortechs Labs Inc., San Diego, the United States) atrophy report metrics were collected on all participants and processed from the same 3-Tesla MRI T1-weighted FSPGR anatomical sequence data as was used in the LOAD-risk ROI analysis. This exploratory NeuroQuant segmentation approach allows for regional volumetric comparison of young adult SB participants to age- and sex-matched normative volume ranges, derived from a normative neuroimaging database of more than 4000 individuals (ages 3–100 years). The NeuroQuant atrophy report derives absolute volumes (cm^3), as well as intracranial volume corrected age-/sex-adjusted comparison percentiles, for a combination of cortical and ventricular volumes [62, 63]. Whole-brain volume and total cortical gray and total white matter volumes are reported with superior and inferior lateral ventricle volumes, the latter of which factor into a NeuroQuant-derived hippocampal occupancy composite metric. The metric reflects the ratio of a hippocampal volume to the combination of hippocampal and inferior ventricle volumes (see Table 4) and is helpful in interpreting ex vacuo dilatation patterns associated with normal aging and LOAD-related neurodegenerative changes. Aside from additional entorhinal cortex volumes, the remainder of the NeuroQuant atrophy report metrics reflect large regional volumes and age and sex normative-adjusted percentiles for those volumes in the posterior and anterior cingulate cortices and temporal, parietal, occipital, and frontal lobes.

Participants' normative-adjusted NeuroQuant atrophy report percentile metrics were examined for either extreme volume enlargement ($\geq 95\%$) or volume reduction ($\leq 5\%$), and the frequency counts of any extreme volume differences were compared between young adult APOE carrier and noncarrier groups with SB using the nonparametric Fisher exact test. A nonparametric binomial test procedure was used to assess the statistical probability of volumetric extremes in both SB groups combined relative to age-/sex-adjusted NeuroQuant normative neuroimaging data. Statistical significance for both nonparametric analyses was Bonferroni multiple comparison-corrected for the total number of NeuroQuant atrophy report metrics (i.e., 14 metrics; $p < 0.00357$).

4 | Results

4.1 | Participant Demographic and Clinical Characteristics

A summary of participant cohort characteristics is presented in Table 1. Of the 11 participants (4 were E4 allele heterozygous), 8 were female (mean age of 26.1 ± 7.4 years) and mean years of education was 13.7 ± 2.4 years (range 12–18 years), all but one participant was right upper-extremity dominant, and the bulk of the cohort was White. Three participants had a history of prematurity, nine had a history of shunt malfunction, three had a history of seizures, and four were non-ambulatory. None of the participants had a history of central nervous system infection.

There were no statistically significant differences in the demographic and clinical variables between APOE groups (see Table 1).

TABLE 1 | Demographic and general clinical characteristics of APOE E4^{-/+} adult participants with spina bifida.

Demographic variables	Total cohort		APOE E4 ⁻ participants (n = 7)		APOE E4 ⁺ participants (n = 4)		p value		
	Mean (SD)	Count or range	Median	[IQR]	Count or range	Median [IQR]		Count or range	
Age (years)	26.1 (7.4)	27	27.0	4.0	26	22.5 3.5	5	0.11 ¹	
Education (years)	13.7 (2.4)	6–12 years 1–13 years 1–14 years 1–16 years 2–18 years	14.0	5.0	3–12 years 1–14 years 1–16 years 2–18 years	12.0	0.3	3–12 years 1–13 years	0.22 ¹
Sex (female)	—	8	—	—	4	—	4	0.24 ²	
Race	—	10—White 1—Black	—	—	6—White 1—Black	—	4—White	0.99 ²	
Hand dominance (right)	—	10	—	—	6	—	4	0.99 ²	
Hx of prematurity	—	3	—	—	2	—	1	0.99 ²	
Hx of shunt malfunction	—	9	—	—	6	—	3	0.99 ²	
Hx of seizures	—	3	—	—	3	—	0	0.24 ²	
Ambulatory motor function	—	7	—	—	5	—	2	0.58 ²	

Note: Summary statistics presented as mean and standard deviation (SD) for the total study cohort; median, interquartile range (IQR), and simple counts are provided for APOE group-based comparisons. Total cohort N = 11 (n = 7 E4⁻, n = 4 E4⁺). Hx, history.

¹Mann–Whitney U test.

²Fisher exact test.

There was a 4.5-year median age difference between APOE E4⁻ relative to E4⁺ allele carriers (i.e., E4⁻ 27 years, E4⁺ 22.5 years; $p = 0.11$). A total of 55% of participants were unemployed, but some were students. One participant in each APOE group had no insurance coverage.

4.2 | A Priori Neuroimaging Data Results

Of the 10 a priori neuropathological risk ROIs (e.g., bilateral mesial temporal lobe [hippocampus, parahippocampal gyrus, and amygdala], angular/supramarginal gyrus region, and posterior cingulate cortices), TIV-adjusted left amygdalar volumes were significantly smaller (FDR-adjusted $p = 0.04$; see Figure 1) in E4⁺ carriers relative to young adult SB noncarriers. Mean TIV-adjusted left amygdala proportional volumes (cm³) were 0.08 (0.01 SD) in E4⁺ carriers and 0.11 (0.01 SD) in noncarriers. On average, right amygdala TIV-adjusted proportional volumes were also smaller in E4⁺ carriers relative to noncarriers (0.10 [0.01 SD] versus 0.11 [0.01 SD], respectively), but these differences did not exceed statistical significance (FDR-adjusted $p = 0.42$). No other differences were observed for the remaining eight a priori neurodegeneration-linked ROIs. See Table 2.

4.3 | Secondary Analyses

4.3.1 | Neurocognitive Data Results

Group comparison of summary cognitive domain and individual neuropsychological assessment measure variables failed to

yield any statistically significant results, though there may be a trend in greater psychomotor impairment in the young adult SB E4⁺ allele carriers ($p = 0.07$). While statistical significance was not obtained between APOE groups, mean performance was consistently lower in E4⁺ carriers for individual test variables and cognitive domains (see Table 3 and Figure 2). Among the cognitive domains, psychomotor, attention, and visual memory demonstrated the greatest effect magnitude between APOE groups (rank biserial corr. 0.62, 0.60, and 0.52, respectively).

4.3.2 | Exploratory Analysis Results

NeuroQuant segmentation was successful in 10 of the 11 young adult SB participants. Gross structural abnormalities in one participant exceeded NeuroQuant's "goodness of fit" quality assurance metrics. For the remaining 10 participants (7 E4⁻/3 E4⁺), none of the NeuroQuant TIV-adjusted ROIs were statistically significant, though there may be a trend toward greater relative parietal lobe volumes in E4⁺ carriers ($p = 0.07$).

An examination of the probability of significant volume deviations (> 95% and < 5%) in all young adults with SB combined relative to age-/sex-adjusted normative volumetric estimates revealed notable volume abnormalities of the anterior cingulate in 100% of the SB cases ($p = 0.001$). All SB participants, save one with notable anterior cingulate volume reduction (1%), were in the 99% relative to NeuroQuant age-/sex-adjusted normative volumetric estimates. All three of the SB APOE E4⁺ carriers were subsumed in the larger grouping of participants with enlarged anterior cingulate volumes. Additionally, 80% of the

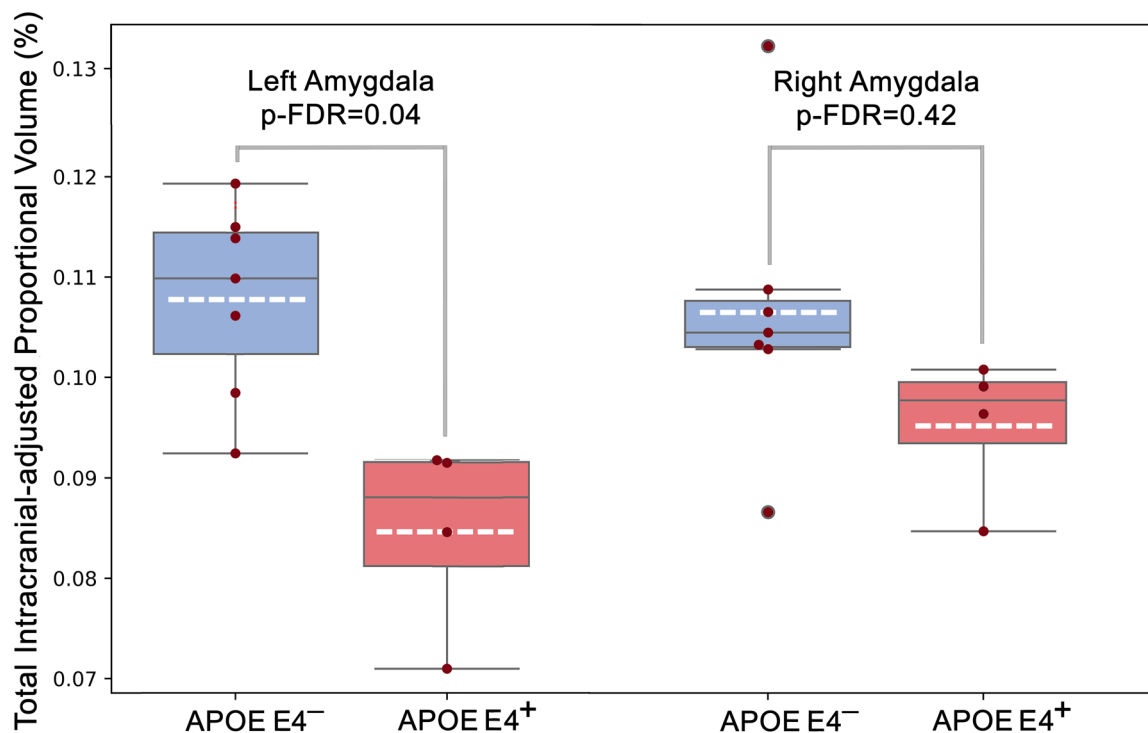


FIGURE 1 | APOE E4⁺ carriers ($n = 4$) demonstrated smaller left amygdala volumes relative to noncarriers ($n = 7$). The only region that survived FDR correction was the left amygdala ($F(10,1) = 14.7$; FDR-adjusted p value 0.04). Boxplots indicate mean value (dashed white line), median value (solid black line), quartiles (box limits), and minimum/maximum values.

TABLE 2 | Proportional volumes of neurodegeneration risk ROIs in APOE E4^{-/+} adult participants with spina bifida.

A priori neurodegeneration risk regions-of-interest (ROIs)	APOE E4 ⁻ participants ($n = 7$)		APOE E4 ⁺ participants ($n = 4$)		FDR-adjusted p value ¹	Effect size ²
	Mean	(SD)	Mean	(SD)		
Left amygdala	0.11	0.01	0.08	0.01	0.04	0.77
Right amygdala	0.11	0.01	0.10	0.01	0.42	0.33
Left hippocampus	0.36	0.03	0.40	0.04	0.42	0.35
Right hippocampus	0.33	0.04	0.35	0.02	0.65	0.07
Left parahippocampal gyrus	0.20	0.05	0.20	0.04	0.98	0.00
Right parahippocampal gyrus	0.19	0.03	0.17	0.02	0.49	0.22
Left supramarginal gyrus	0.80	0.04	0.82	0.06	0.65	0.11
Right supramarginal gyrus	0.75	0.12	0.80	0.17	0.65	0.07
Left posterior cingulate cortex	0.30	0.04	0.34	0.03	0.42	0.33
Right posterior cingulate cortex	0.27	0.05	0.31	0.04	0.42	0.29

Note: Means and standard deviations (SD) reflect ROI proportional volumes relative to total intracranial volume for each study participant. Total cohort $N = 11$ ($n = 7$ E4⁻, $n = 4$ E4⁺).

¹FDR-adjusted p values from group-wise comparison ANCOVA model, controlling for age, sex, and total intracranial volume.

² η^2 (partial).

young adult cohort with SB demonstrated small hippocampal volumes (< 5%) relative to age-/sex-adjusted normative volumetric data estimates. All the SB participants with reduced hippocampal volumes demonstrated comorbid frontal lobe volumetric enlargement (> 95%), while 90% demonstrated comorbid hippocampal reduction and volumetric enlargement of the frontal lobe, including the anterior cingulate cortex and entorhinal cortices (70% incidence) (Table 4).

5 | Discussion

SB is the most common and most serious congenital anomaly of the human nervous system that is compatible with long-term survival [64]. Fortunately, individuals with SB are living longer. However, with increased survival, there are more adults than children alive with SB, and there is a growing need for ongoing research to understand the impact of aging on this congenital

TABLE 3 | Age-adjusted neurocognitive domain summary and individual test scores in APOE E4⁻/+ adult participants with spina bifida.

Cognitive domain summary scores and individual age-adjusted test scores	APOE E4 ⁻ participants (n = 7)		APOE E4 ⁺ participants (n = 3)		p value ¹	Effect size ²
	Mean (SD)	[Median]	Mean (SD)	[Median]		
Visual memory domain	-1.18 (1.84)	[-0.63]	-2.44 (1.39)	[-3.25]	0.25	0.52
BVMT-R Trial 1	-1.59 (1.48)	[-1.90]	-3.23 (1.33)	[-4.00]	0.25	0.52
BVMT-R Total Recall	-1.87 (2.08)	[-1.87]	-3.20 (1.39)	[-4.00]	0.33	0.43
BVMT-R Learning Curve	0.10 (2.03)	[-0.40]	-0.40 (1.04)	[-1.00]	0.99	0.05
BVMT-R Delayed Recall	-1.36 (2.55)	[-1.36]	-4.00 (0.00)	[-4.00]	0.26	0.57
Verbal memory domain	-0.56 (1.43)	[0.18]	-0.64 (0.31)	[-0.54]	0.55	0.33
RAVLT Trial 1	-0.74 (0.80)	[-0.75]	-1.09 (0.58)	[-1.11]	0.52	0.33
RAVLT Total Recall	-1.07 (1.84)	[-0.25]	-1.24 (0.90)	[-1.38]	0.71	0.22
RAVLT Learning Curve	0.03 (1.50)	[0.21]	0.67 (0.58)	[1.00]	0.70	0.22
RAVLT Delayed Recall	-0.58 (2.16)	[0.32]	-0.87 (0.80)	[-0.77]	0.79	0.20
Working memory/executive	-0.43 (1.01)	[-0.37]	-0.68 (0.36)	[0.66]	0.36	0.43
NIH Toolbox List Sorting*	-0.38 (0.99)	[-0.10]	-1.40 (1.51)	[-0.90]	0.25	0.60
RAVLT Proactive Interference	-0.69 (0.95)	[-0.92]	-0.86 (0.02)	[-0.85]	0.99	0.00
RAVLT Retroactive Interference	-0.17 (1.23)	[0.30]	-0.91 (0.65)	[-1.00]	0.23	0.60
Attention	-0.32 (1.20)	[-0.20]	-1.67 (1.32)	[-1.40]	0.25	0.60
NIH Toolbox Flanker*	-0.32 (1.20)	-0.20	-1.67 (1.32)	-1.40	0.25	0.60
Visuospatial processing	-0.57 (1.87)	[0.30]	-1.60 (1.85)	[-0.82]	0.38	0.43
JOLO	-0.89 (2.32)	[0.39]	-1.75 (2.86)	[-0.63]	0.30	0.48
WAIS-IV Visual Puzzles	-0.24 (1.39)	[0.00]	-1.44 (0.51)	[-1.33]	0.30	0.48
Psychomotor	-2.12 (1.08)	[-1.95]	-3.67 (1.08)	[-3.05]	0.18	0.62
Pegboard—dominant	-2.10 (0.92)	[-1.70]	-3.87 (0.98)	[-3.40]	0.07	0.81
Pegboard—nondominant	-2.14 (1.28)	[-2.10]	-3.47 (1.34)	[-2.90]	0.17	0.62
Global performance	-1.02 (1.39)	[-0.46]	-1.73 (0.85)	[-1.59]	0.52	0.33

Note: Summary statistics presented as mean, standard deviation (SD), and range for individual tests and cognitive summary scores. All values are expressed as normative age-adjusted z-scores. N = 10 (n = 7 E4⁻, n = 3 E4⁺).

¹Mann-Whitney U test.

²Rank biserial correlation.

*Missing in two E4⁻ participants.

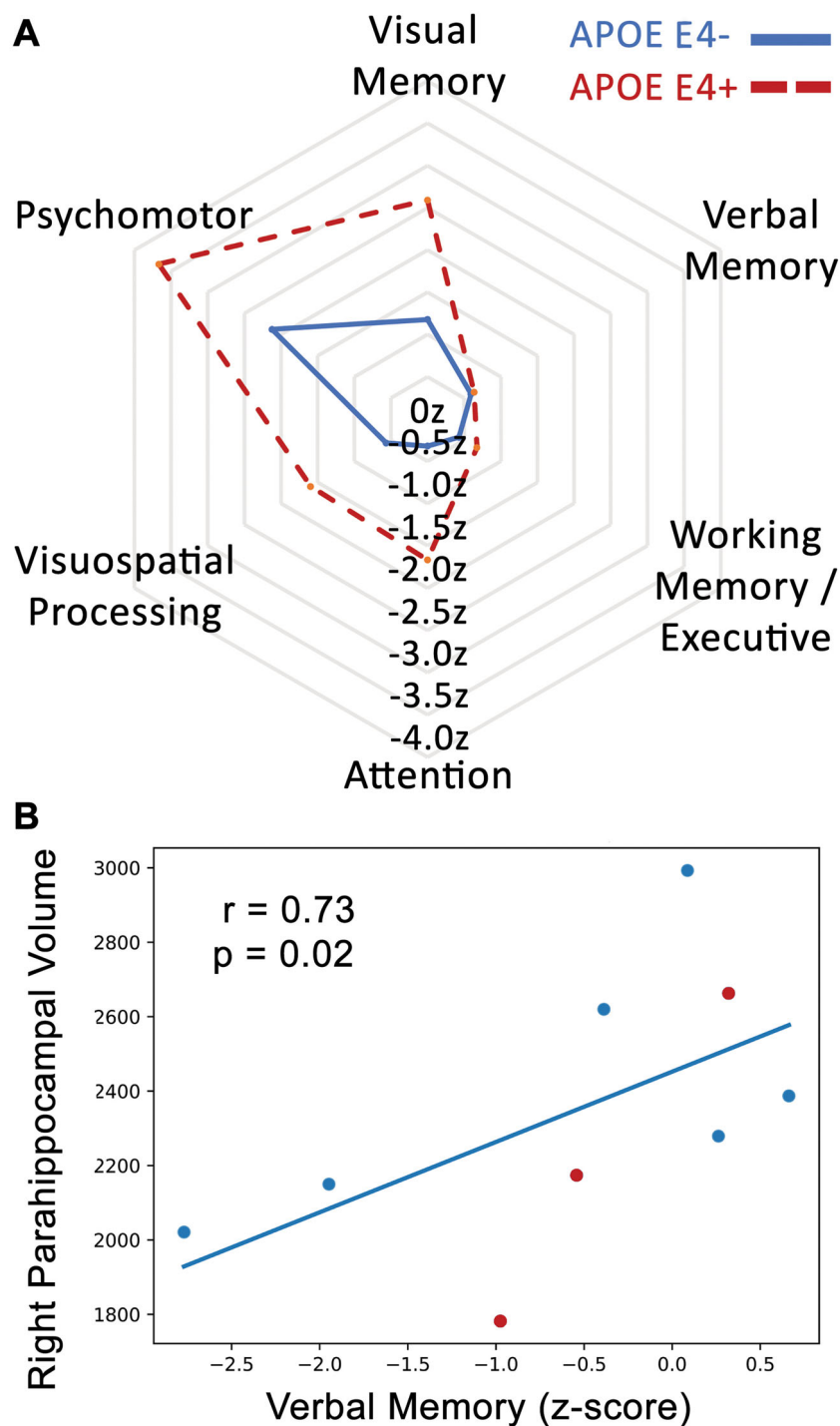


FIGURE 2 | Neurocognitive performance abilities in APOE E4⁺ carriers and noncarriers. (A) Age-adjusted standard scores (z-score) for cognitive domain performances in APOE E4⁺ individuals (red dashed line) and noncarriers (solid blue line). In APOE E4⁺ carriers, greater than 1.5 SD impairment relative to normative age cohort estimates was observed for tasks of attention, visuospatial processing, visual memory, and psychomotor abilities, whereas greater than 1.5 SD impairment deficits in psychomotor abilities were only observed in noncarriers. Values are z-scores (mean 0; SD ± 1). (B) There were significant positive correlations between mean right parahippocampal region volume and verbal memory performance (Spearman $r = 0.73$, $p = 0.02$) and for this region and psychomotor performance (Spearman $r = 0.70$, $p = 0.02$).

disorder [65]. A better understanding of SB brain characteristics from young adulthood, as assessed in the current study, to old age and any potential LOAD-risk genetic variation associated with those brain characteristics may help optimize earlier detection strategies and improve quality of life and advanced planning considerations.

In the a priori primary analysis, our finding of the smaller left amygdala volume shows that there are changes that can be detected even when neurocognitive differences are not identified. This provides a compelling justification for further study to investigate the significance of this amygdala finding over time to find out if it could be a useful biomarker. In addition, this

TABLE 4 | Exploratory differences in NeuroQuant-derived regional brain volumes between SB APOE E4 groups and the combined incidence of significant volumetric abnormalities in young adult SB individuals relative to age-/sex-matched normative peer volumes.

NeuroQuant variables	APOE E4 ⁻ participants (n = 7)		APOE E4 ⁺ participants (n = 3)		p value ¹	Effect size ²	Frequency of abnormality ³	p value ⁴
	Mean (SD)	[Median]	Mean (SD)	[Median]				
Total intracranial volume (TIV)	1068.14 (95.42)	[1025.00]	1241.67 (90.39)	[1192.00]	0.17	0.62	40%	0.83
Total gray matter volume	0.39 (0.02)	[0.39]	0.40 (0.02)	[0.40]	0.67	0.24	30%	0.94
Total white matter volume	0.32 (0.02)	[0.32]	0.32 (0.02)	[0.31]	0.67	-0.24	30%	0.94
Hippocampal volume	0.004 (0.001)	[0.004]	0.004 (0.001)	[0.004]	0.99	0.05	80%	0.06
Entorhinal volume	0.004 (0.001)	[0.005]	0.005 (0.001)	[0.005]	0.18	0.62	70%	0.17
Hippocampal occupancy ⁵	0.87 (0.05)	[0.88]	0.90 (0.03)	[0.91]	0.42	0.38	10%	0.99
Superior lateral ventricles	0.02 (0.02)	[0.02]	0.03 (0.02)	[0.03]	0.52	0.33	40%	0.83
Inferior lateral ventricles	0.001 (0.000)	[0.001]	0.000 (0.000)	[0.000]	0.38	-0.43	30%	0.94
Temporal cortex	0.09 (0.005)	[0.10]	0.10 (0.002)	[0.10]	0.83	0.14	0%	0.99
Parietal cortex	0.08 (0.005)	[0.08]	0.09 (0.002)	[0.09]	0.07	0.81	10%	0.99
Frontal cortex	0.16 (0.008)	[0.16]	0.15 (0.013)	[0.15]	0.67	-0.24	90%	0.01
Occipital cortex	0.04 (0.005)	[0.04]	0.04 (0.003)	[0.04]	0.83	-0.14	20%	0.98
Anterior cingulate cortex	0.008 (0.002)	[0.008]	0.009 (0.000)	[0.009]	0.12	0.71	100%	0.001
Posterior cingulate cortex	0.005 (0.000)	[0.005]	0.006 (0.001)	[0.005]	0.18	0.62	10%	0.99

¹Mann-Whitney U test.

²Rank biserial correlation.

³Percentage of participants with SB, APOE groups-combined with significant hypertrophy (> 95%) or atrophy (< 5%), relative to NeuroQuant normative age- and sex-adjusted volumetric estimates.

⁴Proportion test (H_0 is the proportion of participants with significant abnormality > 0.5).

⁵Hippocampal occupancy score is a ratio of hippocampal volume to the sum of the hippocampal and inferior lateral ventricular volumes in each hemisphere separately ((left hippocampal volume/[left hippocampal volume and left inferior ventricles volume]) + (right hippocampal volume/[right hippocampal volume + inferior lateral ventricular volume]))/2.0.

finding advocates for the need to target follow-up neurocognitive testing of individuals with SB related to functions that are affected by the left amygdala. Our small sample size limited our ability to investigate all confounding factors and comorbidities that could impact this result; there were, however, no statistically significant differences in the demographic and clinical variables of shunt malfunctions, seizures, and prematurity between APOE groups. All participants had hydrocephalus (none had CNS infections), so it is possible that this pathology impacted our a priori finding, but it did not have a differential effect based on APOE status.

The reduced left hemispheric amygdala volumes in SB APOE E4 carriers relative to noncarriers are consistent with reduced volumetrics in non-SB APOE E4 carriers with mild cognitive impairment (MCI) and/or AD in the general population [66, 67]. Hashimoto and colleagues [68] investigated the *APOE* E4 gene “dose effect” on regional brain volumes in 138 older individuals with AD (69.5 ± 6.0 years). When the effects of age, sex, education, disease duration, and Mini Mental Status Exam scores were accounted for, amygdala and hippocampal volumes were decreased with increasing APOE E4 allelic burden [68]. Additionally, in the general non-SB population, asymmetric left hippocampal volume reduction has been observed in older MCI APOE E4 carriers compared with noncarriers who subsequently converted to AD within 3 years of neuroimaging evaluation [66]. In contrast, non-SB APOE E4 carriers relative to noncarriers with AD (ages 65–72) have demonstrated right, but not left, amygdala volume reduction [67].

The amygdalae regulate emotion and memory and are associated with the brain’s reward system, stress, and the “fight-or-flight” response when someone perceives a threat. The latter function is expressed phenomenologically through functional brain networks that include the amygdalae, as well as the anterior cingulate cortices (i.e., salience network), which are thought to help identify emotionally salient information, prompting attentional shifts from internal, reflective thought to active outward task engagement (or noxious stimulus avoidance) [69, 70]. The left amygdala has been implicated in mediating longitudinal associations between exposure to threat and psychiatric symptomatology in youth [71] and has been associated with sustained emotional and verbal processing, whereas the right amygdala tends to be associated with visual and dynamic emotional analysis [72].

Given possible issues in spatial cognition in persons with SB [73], our finding of more notable left amygdalar volume reduction relative to right amygdala volumes in this small cohort is somewhat surprising. One would posit that, given SB-associated developmental challenges in spatial cognition, a reversal of this asymmetric amygdalar pattern might be more likely. In our secondary analyses, this is particularly so when considering our findings of relatively more severe visual memory and attention impairment in our cohort (see Table 3 and Figure 2). In addition, the greatest effect size differences were observed for these two cognitive domains, as well as psychomotor abilities, in our E4 young adult APOE E4 carriers with SB relative to peer noncarriers. It is possible that the greater psychomotor deficits observed in the young adult APOE E4 carriers with SB may have influenced their subsequent

performances on visual memory and attention. However, greater deficits in visuospatial processing were observed in the SB APOE E4 carriers on standardized measures that did not involve task-related upper- or lower-extremity motor demands (e.g., JOLO and WAIS-IV Visual Puzzles) [56, 74].

Visual processing and memory task performances have been found to be worse in older adult APOE E4 allele carriers in the general population without evidence of dementia (> 60 years) relative to noncarrier controls [75]. Parasuraman and colleagues [76] attribute deficits in spatial attention and working memory in APOE E4 carrier middle-aged adults without dementia to the modulating effect APOE has on cholinergic neurotransmission to regions in the posterior parietal cortex. The same underlying alterations in cholinergic neurotransmission may underlie the observed neurocognitive issues in our young adult cohort with SB.

Memory impairment is common in individuals with SB of all ages and has been partly attributed to hippocampal reduction found in children [18, 19] and adults [5] with SB. Smaller hippocampal volumes without ex vacuo dilatation in younger individuals are thought to more likely reflect congenital issues than atrophic change. Our cohort with SB similarly had significantly smaller hippocampi relative to age-/sex-adjusted normative volume estimates, but the observed smaller mesial temporal lobe volumes were coincident with enlarged entorhinal, frontal, and anterior cingulate cortices. Frontal cortical thickness has been described [77], but our findings of enlarged anterior cingulate cortices have not been previously reported. Kulesz and colleagues [29] hypothesized that attentional control and conflict resolution would correlate with the size of the anterior cingulate cortex in children with SB ($n = 54$; mean age 12 years). However, they discovered that only poorer conflict resolution, but not attentional control, was correlated with aberrant anterior cingulate volumes.

Perhaps our finding of enlarged anterior cingulate and entorhinal cortices in young adults with SB reflects developmental compensation for chronic hydrocephalic processes. Regional compensatory parenchymal enlargement has been postulated by other investigators who have found enlarged frontal, yet smaller posterior parietal, volumes in individuals with SB [2, 29]. It is possible that the regional enlargements observed in our cohort with SB could simply be due to congenital anatomical brain differences without compensation, but these regional volume aberrations have not been described in the literature. Adults without SB who develop idiopathic normal pressure hydrocephalus (iNPH) have been found to have reduced volumes in the same regions that appear to be enlarged in our cohort of young adults with SB [78]. While not confirmatory, the possible polar differences in frontal, mesial temporal, and cingulate cortex regional volumes between individuals with iNPH and individuals with SB suggest that our findings reflect a possible combination of neurodevelopmental morphological and hydrocephalic compensation.

Our findings, while illustrative of possible APOE-mediated morphological differences in amygdala volumes in young adults with SB and overall volumetric aberrations relative to age-/sex-adjusted normative volume estimates, are limited due to

sample size. Neurocognitive and neuroimaging procedures in individuals with SB present challenges that limit larger-scale recruitment and population-wide evaluation. However, we hope that the findings presented here provide possible clues for more targeted investigation. The observed volumetric findings provide possible guidance for future investigations of APOE-associated morphological and functional brain differences using diffusion tensor and functional MRI technologies. We recommend more detailed neuroimaging examination of mesial temporal lobe and frontal and anterior cingulate cortex regions in young adults with SB, which may provide additional insights into possible APOE-related pleiotropic differences in individuals with SB and provide a mechanistic bridge between observed neuroimaging and neurocognitive deficits. We also recommend that subsequent investigations consider differential expressions of any APOE-related pleiotropisms by race and ethnic backgrounds in participant cohorts with SB.

In conclusion, this pilot investigation identified a possible significant difference in amygdalar volumes between young adult SB persons with and without APOE E4 positivity. Neurocognitive impairment was ubiquitous in our cohort with SB, but there were more severe deficits in visual memory, attention, visuospatial processing, and psychomotor performance in those young adults with SB who were E4 positive. The worse cognitive task performances in E4 carriers are likely due, in part, to psychomotor limitations, but overall, there is little in the observed neuropsychological assessment results to suggest a neurocognitive pleiotropic benefit of APOE in our young adult cohort. Post hoc examination of regional NeuroQuant volumes in this cohort, collapsing across APOE carrier groups, revealed possible broader regional morphological aberration in young adults with SB relative to age-/sex-adjusted normative volume estimates. While limited by overall sample size in this patient population, this study provides clues for further investigation into the interplay between SB-associated developmental morphological differences in brain regions and neurocognitive functions known to be impacted in APOE E4 carriers.

Author Contributions

Joan M. Jasien: conceptualization, investigation, funding acquisition, writing – original draft, writing – review and editing, formal analysis, project administration, supervision, resources, methodology. **Jacques A. Stout:** methodology, validation, visualization, formal analysis, data curation, software, writing – review and editing. **Mohamad A Mikati:** writing – review and editing, supervision, conceptualization, resources. **Robert J. Anderson:** writing – review and editing, formal analysis, data curation, software, visualization. **Brittany G. Nave:** writing – review and editing, project administration, data curation. **Herbert E. Fuchs:** writing – review and editing, data curation, supervision, resources. **Brian Smith:** writing – review and editing, formal analysis, software, data curation. **Alexandra Badea:** conceptualization, methodology, validation, visualization, writing – review and editing, formal analysis, supervision. **Jeffrey N. Browndyke:** conceptualization, investigation, writing – review and editing, visualization, validation, methodology, formal analysis, data curation, supervision.

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Ethics Statement

Study procedures conformed to the World Medical Association's Declaration of Helsinki ethical principles. The Duke University Medical Center Institutional Review Board provided study oversight and approval.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data is available upon request to the authors.

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