The Effect of the Relationship of APOE Polymorphisms and Cerebral Vasospasm on Functional Outcomes in Children With Traumatic Brain Injury

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Abstract

Background: Pediatric traumatic brain injury (TBI) is a leading cause of death and disability. Polymorphisms in the apolipoprotein E (APOE) gene have been linked to cerebral vasospasm (CV) and poor outcomes in adults with TBI, yet these associations remain poorly defined in children. **Objective:** We examined the effect of the relationship between APOE polymorphisms and CV on functional outcomes in children with a TBI. **Method:** This prospective, descriptive study examined 60 children (aged 10 days to 15 years) with a TBI. Data included demographic information, genetic sampling for the APOE gene and single-nucleotide polymorphisms (SNPs; rs405509, rs429358, rs7412), and daily transcranial Doppler ultrasounds to evaluate for CV. We examined Glasgow Outcome Scale–Extended Pediatrics (GOS-E Peds) scores at the time of discharge and 4–6 weeks after discharge. **Results:** More than half (56.7%) of the 60 children ($M_{\text{age}} = 5.9$ years) were male. Twenty-six participants (43.3%) experienced an occurrence of CV. There were significant differences in injury mechanism (unadjusted $p = .048$) and age (unadjusted $p = .02$) between those with and without CV. Also, the noncoding promoter SNP rs405509 T/T, when considered with injury severity, appeared to modify the relationship of APOE genotype to CV. The relationship between APOE and CV had no significant effect on GOS-E Peds scores. **Conclusion:** Injury severity and the APOE noncoding promoter SNP rs405509 may modify the relationship between APOE and CV in children with TBI. More studies are needed to understand the role of APOE polymorphisms in outcomes in children with TBI.

Keywords

apolipoprotein E (APOE), cerebral vasospasm, functional outcome, pediatric, traumatic brain injury

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality in children. The estimated annual number of TBI-related emergency department visits, hospitalizations, and deaths for children up to age 14 in the United States, between 2007 and 2013, was 511,257 (Taylor, Bell, Breiding, & Xu, 2017). Of the estimated 1.7 million TBIs that occur each year, approximately 30% happen in children aged 0–14 years (Coronado et al., 2011). TBI severity is classified as mild, moderate, or severe (Thurman, 2016). The primary mechanism of TBI in children varies by age, with the most common causes by age-group being falls at 0–4 years (72.8%), being struck by or against an object and falls at 5–14 years (34.9% and 35.1%, respectively), and motor vehicle collisions at 15–24 years (22%; Centers for Disease Control and Prevention, 2016).

Following a TBI, there are two distinct phases that may result in tissue injury and worsen functional outcome. The first phase occurs at the moment of impact as a result of mechanical forces that directly upset the main functional components of the brain. The second phase involves a cascade of metabolic,

cellular, and biochemical derangements, which are influenced by exogenous factors such as hypoxia and hypotension that may exacerbate secondary tissue injury (Adelson et al., 1997). Post-traumatic cerebral vasospasm (CV), a phenomenon found in the second phase of injury, results in diminished cerebral blood flow and can lead to ischemia in pediatric TBI patients (O'Brien, Maa, & Yeates, 2015). CV in pediatric TBI

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has been associated with poorer functional outcomes (O'Brien et al., 2015).

Although genetic studies in pediatric TBI are still in their infancy, there is evidence that genetic factors may exist, such as the potential role of the apolipoprotein E (*APOE*) gene in TBI outcome (Bennett, Reuter-Rice, & Laskowitz, 2016; Kurowski, Martin, & Wade, 2012; Reuter-Rice, Eads, Berndt, & Bennett, 2015). A more complete understanding of the relationship of CV to the mechanism by which *APOE* polymorphisms influence functional outcome in this setting may have therapeutic significance. For instance, a therapeutic peptide derived from the *APOE* binding region has been found to improve TBI outcomes by reducing the luminal narrowing of the middle cerebral artery caused by CV in the setting of experimental subarachnoid hemorrhage (SAH) in a murine model (J. Gao et al., 2006; Laskowitz et al., 2001; Mesis et al., 2006). In the present study, we aimed to explore the effect of the relationship of *APOE* genotype and CV on functional outcomes in children following an acute TBI.

Background

APOE and TBI

APOE is a protein-coding gene with three common protein isoforms designated apoE2, apoE3, and apoE4. *APOE* single-nucleotide polymorphisms (SNPs) rs429358 and rs7412 result in an arginine (C) or cysteine (T) substitution and define the protein isoform. Additional SNPs have been identified in the noncoding promoter region that may influence gene expression. One of these, SNP rs405509, results in a G/T nucleotide change at position -219 and modifies *APOE* gene expression (Bennett et al., 2016).

Recently, genetic associations with functional and neurocognitive outcomes following pediatric TBIs have gained significant attention. Polymorphisms within the *APOE* gene are among those genetic markers researchers are studying due to their role in modifying central nervous system responses to acute and chronic neurologic disease (W. Gao, Lu, Kochanek, & Berger, 2014; Kurowski et al., 2012). *APOE* is produced in the brain where it exerts neurotrophic (i.e., membrane repairing and neuroplasticity-related) and neuroprotective (i.e., antioxidant and anti-inflammatory) effects (Bennett et al., 2016). In addition, the protein has been associated with susceptibility to neurologic and vascular diseases, coronary artery disease, oxidative stress, Alzheimer's disease, and modulation of endothelin 1-mediated vasoconstriction in an isoform-specific manner (Bennett et al., 2016; Khurana, Fox, Meissner, Meyer, & Spetzler, 2006).

CV and TBI

CV refers to a functional narrowing of the central lumen of the cerebral vessel. The exact incidence and mechanism by which CV occurs following TBI is poorly defined, although it is thought to result from a disturbed balance between cerebral vasoconstrictive and vasodilatory factors. Direct stretching or

mechanical irritation of cerebral arteries may enhance the early development of CV (Arutiunov, Baron, & Majorova, 1974). The arterial narrowing of CV results in decreased cerebral blood flow, which can lead to cerebral ischemia and/or infarction. CV has been primarily studied in the context of aneurysmal SAH and TBI in adult patients (Lee et al., 1997; Oertel et al., 2005). However, in the last 10 years, there has been an increased interest in examining CV in pediatric TBI. The prevalence of CV in children with TBI is 21% in the anterior circulation and 12% in the posterior circulation (O'Brien, Reuter-Rice, Khanna, Peterson, & Quinto, 2010). In a follow-up study, using age-specific cerebral flow velocities to define CV, O'Brien, Maa, and Yeates (2015) found that children with moderate and severe TBI had CV prevalence as high as 33% in the anterior circulation and 21% in the posterior circulation. CV in pediatric TBI has also been associated with areas of ischemia on computerized tomography and has been linked to poorer outcomes (O'Brien et al., 2015).

APOE and CV

Little information exists on the correlation between *APOE* polymorphisms and CV in pediatric TBIs. However, studies have suggested an association between *APOE* polymorphisms and CV in SAH, with independent effects of *APOE* polymorphisms and CV on functional outcome. Although TBI and SAH have different clinical etiologies, the limited research on CV and *APOE* in TBI, particularly in children, led us to hypothesize that the same biopathologic mechanisms of CV that occur in SAH may also occur in TBI.

In a murine model of SAH, W. Gao, Lu, Kochanek, and Berger (2014) found that targeted replacement mice expressing human *APOE4* had more severe CV than *APOE3* mice when they assessed them 72 hr after injury. Moreover, they observed that the presence of the *APOE4* human transgene was associated with poorer functional outcome, increased mortality, and more severe CV after experimental SAH. These preclinical study findings in mice were inconsistently borne out in clinical populations. For example, Alexander et al. (2008) found that there was no relationship between the *APOE4* allele and CV (angiographic or symptomatic) after SAH, though they did suggest that lower *APOE* protein levels might be associated with CV following experimental SAH. On the other hand, after adjusting for various confounding variables, Wu et al. (2010) demonstrated in a study comprising 185 adults with SAH that the presence of the *APOE4* allele was a risk factor for CV.

APOE and Functional Outcomes in TBI

An epidemiologic review of *APOE* genotype and TBI in children suggested that the *APOE4* allele might exert a protective effect in children, whereas research has shown the converse to be the case in adults (Blackman, Worley, & Strittmatter, 2005). Few studies have described *APOE* polymorphisms and functional outcomes in pediatric TBI, but those that do report results that conflict with the conclusions of Blackman and

colleagues (Brichtová & Kozák, 2008; Moran et al., 2009). In 70 children with mild to severe TBI, Brichtová and Kozák (2008) found an association between presence of *APOE4* and poorer outcome. In their study, children with the *APOE4* allele were more likely to have severe clinical symptoms and poor neurological outcomes following TBI. In a meta-analysis, Zhou and colleagues (2008) identified 14 adult and pediatric studies of the *APOE4* allele and outcomes after TBI and found that the allele ($n = 736$) was significantly associated with poor outcomes 6 months after a TBI (Relative Risk = 1.36; 95% confidence interval (CI) [1.04, 1.78]). Of note, the *APOE4* allele frequency was higher (29%) among their study population than has been observed in the general population (15%). In a recent study, Treble-Barna et al. (2016) found that the *APOE4* allele was associated with worse functional outcome in children after TBI. In a meta-analysis and meta-regression examining the relationship of *APOE4* and outcomes in children with TBI, Kassam, Gagnon, and Cusimano (2016) analyzed six studies: Two measured outcomes at multiple time points (3–36 months after TBI) and four measured outcomes at a single time point (6 or 12 months after TBI). Findings revealed that children with at least one *APOE4* allele had a 2.36-fold increase in the likelihood of poor outcome following TBI when compared to those without the allele (95% CI [1.26, 4.42], $p = .007$). In addition, they found that children with *APOE4* had an incremental trend of outcomes worsening by 7% per month (95% CI [−9, 25], $p = .359$) in children. These findings suggest that the association of *APOE4* allele with outcomes maybe important to the prognosis in pediatric TBI because the effect of the *APOE4* allele over time differs in children versus adults with TBI.

In contrast, Teasdale, Murra, and Nichols (2005) evaluated 1,094 TBI patients aged 0–93 years and found that overall, the *APOE4* allele was not associated with unfavorable outcomes. Interestingly, when the researchers adjusted for age, they found that the association of the *APOE4* allele and unfavorable neurological outcomes was strongest for those aged 0–15 years. In another study among 99 children with mild TBI, Moran et al. (2009) found that the presence of *APOE4* was associated with lower Glasgow Coma Scale (GCS) scores, indicating a more severe brain injury, but was not consistently related to poorer outcomes.

Polymorphisms in the *APOE* gene have been linked to CV and poor outcomes in adults with TBI, yet these associations remain poorly defined in the pediatric TBI population. In the present study, we explored the association of *APOE4* with CV incidence and subsequently tested the ability of this association to predict functional outcomes in children admitted to hospital with TBI. Our central hypothesis was that the relationship between *APOE4* polymorphism and CV would be associated with poor functional outcomes over two time points in children admitted for TBI.

Method

Design and Participants

In this descriptive study with a correlational design, we examined 60 children (aged 10 days to 15 years) who sustained a

TBI. The study is part of a funded 3-year exploratory prospective cohort study to measure CV, *APOE* genotype, physiologic symptoms, and functional outcomes in pediatric TBI patients (National Institute of Nursing Research [NIH], 1P30-NR014139; Robert Wood Johnson Foundation, 71244). We obtained institutional review board approval prior to study enrollment. We prospectively enrolled participants from December 2012 through August 2015 upon their admission to a tertiary care Level 1 trauma center and following parental consent. All participants were previously healthy with no known prior history of TBI and were enrolled into the study within 24 hr of admission. Enrollment criteria included (1) admission to hospital, (2) age of 5 days to 15 years, (3) a diagnosis of TBI, (4) ability to undergo adequate transcranial Doppler ultrasound examination (TCD), and (5) English or Spanish speaking. Children were excluded from participating if there was a previously diagnosed significant neurodevelopmental delay or a diagnosis of nontraumatic intracranial hemorrhage. Our goal was to exclude children whose baseline neurocognitive function would impair their ability to cooperate with neurodevelopmental testing and those whose mechanism of injury and intracranial pathophysiology might alter cerebral hemodynamics in a way that differed from that of the desired study population.

Data Collection and Measures

We collected data by accessing each participant's electronic health record (EHR) for demographic data, performing serial daily TCD, collecting buccal swabs for *APOE* genotype upon enrollment, and extracting from the EHR functional outcome data at the time of discharge from the hospital and first follow-up visit at 4–6 weeks after discharge.

Pediatric TBI severity is clinically evaluated using the GCS for infants and children. The GCS score determines severity of injury, which determines clinical care. The GCS (Teasdale & Jennett, 1974) is the most commonly used standardized measure of primary injury in prehospital and acute care for grading TBI severity. This clinical tool assesses three dimensions on a 15-point scale: eye opening, best verbal response, and best motor response. We used traditional GCS thresholds to categorize the degree of brain injury: 13–15 = *mild*, 10–12 = *moderate*, and 3–8 = *severe* (Centers for Disease Control and Prevention, 2015; Teasdale & Jennett, 1974).

TCD ultrasound. A trained sonographer from the department of neurodiagnostic studies used a commercially available TCD ultrasonography unit with a 2-MHz pulsed probe (Sonara Digital TCD; CareFusion, Middleton, WI) to perform a TCD ultrasound at the participant's bedside within 24 hr of admission to the hospital for a TBI. Anterior circulation was defined as the measurement of the right and left middle cerebral arteries (MCA), and posterior circulation was defined as the basilar artery (BA). Our protocol followed the method described by Aaslid, Markwalder, and Nornes (1982) and Lindegaard, Nornes, Bakke, Sorteberg, and Nakstad (1989) where MCA,

BA, and extracranial internal carotid arteries (EC-ICAs) were insonated at 2–5 mm intervals (LaRovere & O'Brien, 2015; Reuter-Rice, 2017). Participants underwent a baseline TCD within 24 hr of enrollment in the study and daily TCD examinations through hospital Day 8 or discharge/death. If CV was present and persisted beyond hospital Day 8, we extended daily TCDs until we noted resolution of CV. The health-care team was blind to the TCD results and did not use the TCDs to direct clinical care in study participants. TCDs were not standard of care; therefore, no set number of missed TCDs disqualified participants from the study. A neurodiagnostician with expertise in TCD interpretation determined the absence or presence of CV.

We used the following criteria to define anterior or posterior CV. The criteria which are based on expert consensus and have been used in previous studies to diagnose abnormal flow velocities in children (Bode & Eden, 1989; Vavilala et al., 2005). The mean flow velocity measure has not been defined for infants 0–11.9 months old (Bode & Wais, 1988). Therefore, we used the definition of the mean flow velocity for 1- to 2.9-year-olds for these younger infants in our study. In the anterior circulation, a Lindegaard ratio is required to determine CV. We calculated a Lindegaard ratio of flow velocity in the MCA to flow velocity in the EC-ICA. Ratios less than 3 are most consistent with hyperemia as a cause of elevated flow velocities, whereas values equal to or greater than 3 are most consistent with CV (Lindegaard, Nornes, Bakke, Sorteberg, & Nakstad, 1989; O'Brien, 2015).

APOE genotyping. Following enrollment of study participants, we collected duplicate samples of buccal cells using cytobrushes (EpiCentre Mini Amp swabs, EpiCentre Co., Madison, WI) by gently brushing and twirling the brushes on the inner cheek for 15 s and then repeating this on the opposite cheek with another brush. We air-dried the brushes at room temperature, placed them into their individual packaging sleeves, and delivered them to the brain injury translational research laboratory at the medical facility for DNA extraction and genetic analysis. Prior to DNA extraction, the brushes were securely maintained at room temperature in a locked cabinet. To isolate DNA, the brushes were first separated from the sticks with scissors, transferred to a microcentrifuge tube, and extracted using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's specifications. The DNA concentration in each sample was determined using a NanoDropND[®]-1000 (Thermo-Scientific), and DNA was maintained at –20°C. Genotyping was performed by the DNA Analysis Facility using Applied Biosystems TaqMan SNP genotyping assays (10 ng DNA/assay) and a 7500 Fast Real-Time polymerase chain reaction (PCR) system according to the manufacturer's instructions. Data analysis was performed using Applied Biosystems 7500 software Version 2.0.4.

Functional outcome measure. To measure functional outcomes, we used the Glasgow Outcome Scale—Extended, Pediatric (GOS-E Peds). The GOS-E Peds Scale scores are used in TBI

research to quantify a participant's functional outcomes, and they were not used in the clinical management of participants in the present study. The GOS-E Peds Scale, which is validated and reliable, categorizes TBIs based on the quality of an injured child's functional outcome using the following 8-point numerical scale: 8 = *death*, 7 = *vegetative state*, 6 = *lower severe disability*, 5 = *upper severe disability*, 4 = *lower moderate disability*, 3 = *upper moderate disability*, 2 = *lower good recovery*, and 1 = *upper good recovery* (Beers et al., 2012). The outcome score is determined using a questionnaire that accounts for the child's independence in and outside the home, ability to function in a school environment, participation in leisure and social activities, and maintenance of healthy friendships and relationships. The questionnaire may be administered by telephone interview, in-person assessment, or data extraction from the participant's EHR. In the present study, we completed the GOS-E Peds using the patient's EHR data.

Statistical Analysis

In preparation for analysis, we processed the data in the following manner. Based on O'Brien (2015), we defined high flow velocity in the MCA as a velocity ≥ 2 SD above the sex–age mean flow velocity. Anterior circulation CV was defined by a high MCA flow velocity and a Lindegaard ratio ≥ 3 . Posterior circulation CV was defined by a BA flow velocity of ≥ 2 SD above the sex–age mean for BA flow velocity.

We grouped the GOS-E Peds categories to reflect severity of functional outcome: good recovery (1 and 2), moderate disability (3 and 4), and severe disability through death (5–8). We grouped the GCS score categories to reflect the severity of injury—moderate to severe (3–12) and mild (13–15)—for regression models due to the scarcity of information in the severe group.

We used descriptive statistics (mean, SD, count, and percentage) to summarize participant characteristics. We used a two-sided two-sample *t* test with unequal variances and the Welch modification to the degrees of freedom to compare participants who had CV with those who did not based on TCD values. Because the sample size was small and we had low expected frequency counts, we used the Fisher–Freeman–Halton exact test (Fisher exact test for a 2-by-2 table) for categorical variables.

We used binary logistic regression to characterize the association between the *APOE* genotype and SNPs and CV occurrence. We used ordinal logistic regression (i.e., proportional odds model) to test the associations among *APOE* genotype (SNPs analyzed individually), incidence of CV, and functional outcomes (measured by GOS-E Peds score) at both measurement time points, discharge and follow-up. For these models, the referent *APOE* genotype was E3E3. The referent for SNP rs405509 was the G/G homozygote. The referent for CV was no occurrence. The referent group for the GCS was Levels 13–15 (mild TBI); for GOS-E Peds, the referent was the group with Levels 1 and 2 (good recovery).

For the logistic regression models, binary and ordinal, we did not include the SNPs rs429358 and rs7412 for the following

Table 1. Clinical Characteristics Related to Traumatic Brain Injury and Cerebral Vasospasm.

Characteristic	Levels	No CV ^a	CV ^a	Total (% Sample) ^b
		<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
Imaging diagnosis of brain injury				
Subdural	Yes	13 (48)	14 (52)	27 (45)
Epidural	Yes	6 (46)	7 (54)	13 (21.7)
Intracerebral	Yes	1 (50)	1 (50)	2 (3.3)
Intraventricular	Yes	2 (100)	0 (0)	2 (3.3)
Subarachnoid	Yes	6 (46)	7 (54)	13 (21.7)
Diffuse axonal	Yes	1 (100)	0 (0)	1 (1.7)
Diffuse cerebral	Yes	1 (33)	2 (67)	3 (5)
Extra axial hematoma	Yes	5 (56)	4 (44)	9 (15)
Fracture	Yes	15 (47)	17 (53)	32 (53.3)
Infarction	Yes	1 (33)	2 (67)	3 (5)
CSF leak	Yes	1 (50)	1 (50)	2 (3.3)
Contusion	Yes	3 (43)	4 (57)	7 (11.7)
Concussion	Yes	7 (100)	0 (0)	7 (11.7)
GCS category	<i>n</i> (%)	Mean (SD)	Median	Minimum, Maximum
Mild (13–15)	41 (68.3)	2.3 (1.7)	2.0	0, 8
Moderate (9–12)	3 (5.0)	5.3 (2.3)	4.0	4, 8
Severe (3–8)	16 (26.7)	6.8 (4.3)	6.5	2, 21
High flow velocities or CV by TCD	Number of CV Events		Unique Participants <i>n</i> (%)	
Right MCA high flow velocity	47		18 (30.0)	
Left MCA high flow velocity	48		18 (30.0)	
Right MCA CV	18		4 (6.7)	
Left MCA CV	17		3 (6.0)	
Basilar artery vasospasm	52		25 (41.7)	

Note. N = 60. CSF = cerebrospinal fluid; CV = cerebral vasospasm; GCS = Glasgow Coma Scale; MCA = middle cerebral artery; SD = standard deviation; TCD = transcranial Doppler ultrasound.

^aCerebral vasospasm occurrence percentages are conditional on imaging diagnosis. ^bTotal percentage is for the sample.

reasons. When we included them as covariates, either individually or collectively, the regression models failed to converge, had insufficient information for parameter estimation, or yielded parameter estimates indicating potential instability in parameter estimation. There was much concordance among the *APOE* genotypes and the SNPs rs429358 and rs7412 (i.e., redundant information), hence the algorithmic concerns and instability concerns previously stated. Additionally, there was a single participant with the *APOE4/E4* genotype and a participant with an undefined SNP rs405509; we did not include these participants in the relevant regression analyses. Of note, no participants had the E2/E2 genotype. We assessed models for adherence to assumptions and model quality. For all statistical tests, we used two-sided tests against the null hypothesis of no difference. We used the *R* software environment for statistical computing and graphics (<http://www.R-project.org/>) for all statistical analyses.

Results

There were 269 points of observation distributed among 60 participants. The sample comprised 26 female (43.33%) and 34 male (56.67%) participants. The ages of participants ranged from 10 days to 15 years, with a median age of 4.3 years and a

mean of 5.9 years ($SD = 5.5$). The largest age-group comprised children under 1 year of age ($n = 18$, 30.0%), with six being approximately 2.5 months of age and five who were approximately 3.5 months old. The mean weight of participants was 28.7 kg ($SD = 26.72$). The majority of the participants or their parents/guardians self-reported as non-Hispanic or non-Latino ethnicity ($n = 53$, 88.3%); 61.7% ($n = 37$) self-reported as White/Caucasian, 33.3% ($n = 20$) as Black/African American, and the remaining three individuals as Multirace, Asian, or American Indian/Alaskan. Participants had been admitted to either the pediatric intensive care unit ($n = 40$, 67%) or the pediatric step-down unit ($n = 20$, 33%). Clinical characteristics of study participants, including TCD findings, appear in Table 1.

A total of 26 participants (43.3%) experienced an occurrence of CV (anterior or posterior circulation), among which we identified 62 CV events. A total of five individuals (8.3%) experienced an episode of CV in the anterior circulation, among which we identified 22 events of CV. For CV in the posterior circulation, 25 individuals (41.7%) experienced at least one with a total of 52 events recorded.

Unless otherwise indicated, all *p* values presented are unadjusted for covariates. We found no evidence of unadjusted differences in distribution across *APOE* genotypes ($p =$

.649), SNPs (rs429358 $p = .926$, rs7412 $p = .180$, rs405509 $p = .502$), GOS-E Peds score at discharge (original scale $p = .352$, coarsened scale $p = .396$), GOS-E Peds score at follow-up (original scale $p = .649$; coarsened scale $p = .591$), or injury severity ($p = .405$) between those who had no CV with those who experienced CV (Table 2). There is evidence, however, for associations between both injury mechanism ($p = .048$) and age ($p = .02$) and occurrence of CV.

Injury severity and the noncoding promoter SNP rs405509 may modify the relationship between *APOE* polymorphism and CV (Table 3). The inclusion of SNP rs405509 results in a 30.8% increase in the regression coefficient when compared with the model that does not include SNP rs405509. Additionally, there appears to be a slight trend for increased risk of CV in homozygous T/T carriers of the SNP rs405509 (odds ratio [OR] = 4.37, $p = .119$). This finding suggests that SNP rs405509 may play a confounding or conditional modification role in this relationship.

Table 4 shows the distribution of *APOE* (genotypes and SNPs), CV occurrence, and injury severity within the aggregated GOS-E Peds outcome groups at discharge and at follow-up. These unadjusted results suggest a potential relationship between *APOE* genotype and GOS-E Peds at discharge but not at follow-up (discharge $p = .092$, follow-up $p = .303$). Similarly, we observed a statistical trend for associations between SNP rs7412 variation and discharge GOS-E Peds score ($p = .057$) and follow-up GOS-E Peds score ($p = .133$). We saw no evidence for association of GOS-E Peds score with either the other protein-coding SNP rs429358 (discharge $p = .463$, follow-up $p = .281$) or the noncoding promoter SNP rs405509 (discharge $p = .640$, follow-up $p = .724$). CV occurrence showed no association with GOS-E Peds outcome points (discharge $p = .396$, follow-up $p = .591$). However, there is strong evidence for an association between injury severity and GOS-E Peds at both time points (discharge and follow-up $ps < .001$). The unadjusted associations in Table 3 do not suggest strictly monotonic trends for GOS-E Peds outcome distribution by variable.

Evidence (Table 3) suggests an association between the noncoding promoter SNP rs405509 G/T and the GOS-E Peds score at discharge, after adjustment for injury type and genotype ($p = .093$). When comparing the carrier status of the SNP rs405509 G/T against that of G/G, we found a 4-fold increase in the odds of the participant having a GOS-E Peds score in the lower categories versus the higher categories. There is a potential trend, though we found no statistical significance, toward association between genotype and the discharge GOS-E Peds score (E2/E3 $p = .184$, E3/E4 $p = .134$). We observed the anticipated association between injury severity and GOS-E Peds score at both discharge ($p = .000$) and follow-up ($p < .001$). The similarity of OR between genotypes E2/E3 and E3/E4 at both discharge and follow-up suggests that participants may have similar adjusted probabilities across the three GOS-E Peds groups. We wish to note that the probabilities for all E3/E3 SNP rs405509 combinations are lower for GOS-E Peds groups [1,2] and [3,4] than for the [5,8] group (data not presented), thus translating to higher probabilities of a poor

Table 2. Participant Characteristics by Presence or Absence of Cerebral Vasospasm.

Characteristic	No CV	CV	<i>p</i> value
	<i>n</i> (%)	<i>n</i> (%)	
Sex, female	14 (41.2)	12 (46.2)	.795
GCS			.532
Mild (13–15)	25 (61.0)	16 (39.0)	
Moderate (9–12)	7 (43.8)	9 (56.2)	
Severe (3–8)	2 (66.7)	1 (33.3)	
Age group (years)			.020
0–1	14 (41.2)	4 (15.4)	
1–3	2 (5.9)	6 (23.1)	
3–6	2 (5.9)	7 (26.9)	
6–12	7 (20.69)	5 (19.2)	
12+	9 (26.5)	4 (15.4)	
Mechanism of injury			.048
Pedestrian versus auto	1 (2.9)	0 (0)	
MVC	4 (11.8)	1 (3.9)	
Fall	12 (35.3)	11 (42.3)	
Abusive head trauma	10 (29.4)	8 (30.8)	
Recreational activity	7 (20.69)	1 (3.9)	
Other	0 (0)	5 (19.2)	
<i>APOE</i> genotype			.649
E2E3	3 (8.8)	5 (19.2)	
E3E3	23 (67.7)	16 (61.5)	
E3E4	7 (20.6)	5 (19.2)	
E4E4	1 (2.9)	0 (0)	
<i>APOE</i> SNP rs429358			.926
CC	1 (2.9)	0 (0)	
CT	7 (20.6)	4 (15.4)	
TC	1 (2.9)	1 (3.9)	
TT	25 (73.5)	21 (80.8)	
<i>APOE</i> SNP rs4712			.18
CC	31 (91.2)	21 (80.8)	
CT	3 (8.8)	2 (7.7)	
TC	0 (0)	3 (11.5)	
<i>APOE</i> SNP rs405509			.502
GG	16 (47.1)	9 (34.6)	
GT	14 (41.2)	11 (42.3)	
TT	4 (11.8)	5 (19.2)	
Und	0 (0)	1 (3.9)	
GOS-E Peds at discharge			.352
1	11 (32.4)	8 (32)	
2	9 (26.5)	6 (24)	
3	5 (14.7)	3 (12)	
4	0 (0)	4 (16)	
5	2 (5.9)	1 (4)	
6	6 (17.6)	2 (8)	
8	1 (2.9)	1 (4)	
GOS-E Peds at follow-up			.649
1	13 (44.8)	9 (39.1)	
2	5 (17.2)	8 (34.8)	
3	4 (13.8)	2 (8.7)	
4	0 (0)	1 (4.4)	
5	2 (6.9)	1 (4.4)	
6	4 (13.8)	1 (4.4)	
8	1 (3.4)	1 (4.4)	
GOS-E Peds at discharge by group			.396
[1,2]	20 (58.8)	14 (56)	
[3,4]	5 (14.7)	7 (28)	
[5,8]	9 (26.7)	4 (16)	

(continued)

Table 2. (continued)

Characteristic	No CV	CV	<i>p</i> value
	<i>n</i> (%)	<i>n</i> (%)	
GOS-E Peds at follow-up by group			.591
[1,2]	18 (62.1)	17 (73.9)	
[3,4]	4 (13.8)	3 (13.0)	
[5,8]	7 (24.1)	3 (13.0)	

Note. *N* = 60. *APOE* = apolipoprotein E gene; CV = cerebral vasospasm; GCS = Glasgow Coma Scale; GOS-E Peds = Glasgow Outcome Scale–Extended Pediatrics; MVC = motor vehicle collision; SNP = single nucleotide polymorphism; Und = undefined.

Table 3. *APOE* Genotype, SNP rs405509, and Injury Severity Association With Cerebral Vasospasm Occurrence (Logistic Regression) and With GOS-E Peds Outcome at Discharge and Follow-Up (Proportional Odds).

Variable	OR	95% CI	<i>p</i> value
Cerebral vasospasm (logistic regression)			
<i>APOE</i> genotype E2E3	2.46	[0.44, 13.65]	.302
<i>APOE</i> genotype E3E4	0.49	[0.10, 2.46]	.384
<i>APOE</i> SNP rs405509 GT	1.54	[0.44, 5.42]	.498
<i>APOE</i> SNP rs405509 TT	4.37	[0.69, 27.8]	.119
GCS [3,12]	2.02	[0.59, 6.89]	.262
GOS-E Peds (proportional odds)			
Discharge			
<i>APOE</i> genotype E2E3	4.25	[0.58, 41.31]	.184
<i>APOE</i> genotype E3E4	5.56	[0.73, 65.42]	.134
<i>APOE</i> SNP rs405509 GT	4.00	[0.89, 22.67]	.093
<i>APOE</i> SNP rs405509 TT	2.35	[0.24, 38.94]	.496
GCS [3,12]	0.01	[0.00, 0.04]	.000
Follow-up			
<i>APOE</i> genotype E2E3	1.78	[0.28, 13.16]	.555
<i>APOE</i> genotype E3E4	1.79	[0.21, 21.51]	.617
<i>APOE</i> SNP rs405509 GT	1.28	[0.29, 5.89]	.743
<i>APOE</i> SNP rs405509 TT	2.54	[0.21, 78.9]	.518
GCS [3,12]	0.05	[0.01, 0.24]	.001

Note. *APOE* = apolipoprotein E gene; GCS = Glasgow Coma Scale; GOS-E Peds = Glasgow Outcome Scale–Extended Pediatrics; SNP = single nucleotide polymorphism; OR = odds ratio; CI = confidence interval.

GOS-E Peds outcome for individuals with the E3/E3 genotype. In participants with a GCS score of 13–15, an analogous differentiation between the E3/E3 and other genotypes occurs. Overall, these results suggest that E3/E3 G/G and T/T may have differential trend patterns and have the lowest probabilities of a better outcome independent of injury severity (Table 4). At follow-up, there is a lack of evidence for associations between genotype or SNP rs405509 with GOS-E Peds score (Table 4). As with discharge, there is an association between injury severity and GOS-E Peds score at follow-up ($p = .001$).

Discussion

Our study revealed that children and adolescents with TBI (of mixed injury severities) experience CV in both the anterior and

posterior circulation. However, we found no evidence for differences between participants with and without the *APOE4* allele with regard to the incidence of anterior or posterior CV. Therefore, larger studies are needed to determine whether there is a significant *APOE4* association with location of CV. It is possible that the conflicting results from previous studies regarding the effects of the *APOE4* allele on CV may be due to differences in the physiologic mechanisms of CV (W. Gao et al., 2014; Wu et al., 2010).

It is feasible that there are physiologic mechanisms for CV that we have yet to determine. If that were true, then it might be that the *APOE4* allele affects one or more of the physiologic pathways leading to CV but not others (Ladner, Zuckerman, & Mocco, 2013). The *APOE4* allele has already been implicated in several pathways leading to CV, including endothelin 1-mediated vasoconstriction and increased oxidative stress of vascular cells, which results from the inability of *APOE4* to scavenge free radicals as efficiently as other *APOE* polymorphisms (Lantern et al., 2005). Further evidence is needed to discern the different CV pathways that might be associated with *APOE4* and *APOE4*'s impact on endothelin 1. Clinical implications could include pharmacogenomic therapies targeting endothelin 1 for the prevention of CV in TBI (Laskowitz et al., 2010).

We observed that the noncoding promoter SNP rs405509 T/T was associated with incidence of CV when we adjusted for injury severity. This finding suggests that rs405509 T/T may have a mediating role in CV in pediatric TBI (Table 3; $OR = 4.37$, $p = .119$). Further exploration is needed to better understand this relationship.

Effect of *APOE4* Allele on Outcome

In the present study, the distribution of the *APOE4* risk allele did not differ by participant outcome; the distribution for patients with this allele who had upper good recovery was comparable to the distribution for participants with this allele who had poor functional outcomes. However, a significantly greater proportion of participants without the *APOE4* allele had poor functional outcomes (GOS-E Peds of 3–8) when compared to those with the allele at discharge and 30 days after discharge from hospital. This finding contradicts previous reports that the *APOE4* allele is associated with poorer outcomes in children with TBI (Brichtová & Kozák, 2008; Teasdale, Murra, & Nichols, 2005). However, we adjusted our analysis for injury severity (mild, moderate, and severe), which may explain some of the differences between our findings and those of the prior studies. Among most other studies, the severity of TBI was often not categorized accordingly or covaried as mild, moderate, or severe. Interestingly, the one patient in our study who was homozygous for the *APOE4* allele had GOS-E Peds scores indicating severe disability both at discharge and 30 days after discharge. In a more recent preliminary study examining *APOE* in children followed for outcome at hospital discharge and after discharge, researchers found that the *APOE4* allele was detrimental for long-term adaptive

Table 4. Glasgow Outcome Scale–Extended Pediatrics Patient Outcomes at Discharge and Follow-Up.

Variable	GOS-E:[1,2] n (%)	GOS-E:[3,4] n (%)	GOS- E:[5,8] n (%)	p value
Discharge				
CV				.396
No	20 (58.8)	5 (41.7)	9 (69.2)	
Yes	14 (41.2)	7 (58.3)	4 (30.8)	
GCS				<.001
[13,15]	33 (97.1)	6 (50.0)	1 (7.7)	
[3,12]	1 (2.9)	6 (50.0)	12 (92.3)	
APOE genotype				.092
E2E3	2 (5.9)	4 (33.3)	2 (15.4)	
E3E3	23 (67.7)	6 (50.0)	9 (69.2)	
E3E4	9 (26.5)	2 (16.7)	1 (7.7)	
E4E4	0 (0)	0 (0)	1 (7.7)	
APOE SNP				.463
rs429358				
CC	0 (0)	0 (0)	1 (7.7)	
CT	8 (23.5)	1 (8.3)	2 (15.4)	
TC	1 (2.9)	1 (8.3)	0 (0)	
TT	25 (73.5)	10 (83.3)	10 (76.9)	
APOE SNP				.057
rs7412				
CC	32 (94.1)	8 (66.7)	11 (84.6)	
CT	1 (2.9)	2 (16.7)	2 (15.4)	
TC	1 (2.9)	2 (16.7)	0 (0)	
APOE SNP				.640
rs405509				
GG	12 (35.3)	6 (50.0)	7 (53.9)	
GT	15 (44.1)	4 (33.3)	6 (46.1)	
TT	6 (17.6)	2 (16.7)	0 (0)	
Und	1 (2.9)	0 (0)	0 (0)	
Follow-up				
CV				.591
No	18 (51.4)	4 (57.1)	7 (70)	
Yes	17 (48.6)	3 (42.9)	3 (30)	
GCS				<.001
[13,15]	30 (85.7)	4 (57.1)	1 (10)	
[3,12]	5 (14.3)	3 (42.9)	9 (90)	
APOE genotype				.303
E2E3	3 (8.6)	2 (28.6)	2 (20)	
E3E3	24 (68.6)	4 (57.1)	6 (60)	
E3E4	8 (22.9)	1 (14.3)	1 (10)	
E4E4	0 (0)	0 (0)	1 (10)	
APOE SNP				.281
rs429358				
CC	0 (0)	0 (0)	1 (10)	
CT	7 (20)	0 (0)	2 (20)	
TC	1 (2.9)	1 (14.3)	0 (0)	
TT	27 (77.1)	6 (85.7)	7 (70)	
APOE SNP				.133
rs7412				
CC	32 (91.4)	5 (71.4)	8 (80.0)	
CT	1 (2.9)	1 (14.3)	2 (20.0)	
TC	2 (5.7)	1 (14.3)	0 (0)	

(continued)

Table 4. (continued)

Variable	GOS-E:[1,2] n (%)	GOS-E:[3,4] n (%)	GOS- E:[5,8] n (%)	p value
APOE SNP				.724
rs405509				
GG	13 (37.1)	4 (57.1)	5 (50)	
GT	14 (40)	2 (28.6)	5 (50)	
TT	7 (20)	1 (14.3)	0 (0)	
Und	1 (2.9)	0 (0)	0 (0)	

Note. APOE = apolipoprotein E gene; CV = cerebral vasospasm; GCS = Glasgow Coma Scale; GOS-E Peds = Glasgow Outcome Scale–Extended Pediatrics; SNP = single nucleotide polymorphism; Und = undefined.

functioning in the context of positive parenting, whereas in less optimal parenting contexts, being a noncarrier was detrimental (Treble-Barna et al., 2016). Hence, the *APOE4* allele may play an important role in long-term functional outcomes in children with TBI.

In a study of 71 children with TBI who required inpatient rehabilitation, Blackman, Worley, and Strittmatter (2005) reported that the *APOE4* allele ($n = 4$) was associated with improved outcome at discharge; however, the E4 allele frequency (2.8%) was well below that observed in the population at large (15%). Despite the differences, data from other studies examining the role of *APOE4* and outcomes in pediatric TBI found that children with TBI who are carriers of the *APOE4* allele have 2.44 times greater odds (95% CI [1.25, 4.80]) of a poor outcome compared to noncarriers (Bennett et al., 2016). In a more recent meta-analysis, Kassam and colleagues (2016) found evidence that the *APOE4* allele has a time-dependent action that leads to poor outcomes in pediatric TBI, which may explain the differences in outcomes in children with TBI.

Injury Mechanism, Age, and CV

In the present study, we found significant interactions among injury mechanism, age, and CV. The cause for these interactions is likely multifactorial. The most prevalent injury mechanisms in our participants who experienced CV were falls and abusive head trauma. These two mechanisms are most commonly reported in the 0- to 4-year-old population (CDC, 2015). In younger children, these injury mechanisms are also associated with more severe injury (Taylor et al., 2017). Severe TBI has been associated with enhanced neuroinflammation with increased secondary neuronal injury (Simon et al., 2017). Furthermore, both neuroinflammation and secondary neuronal injury have been associated with the *APOE4* allele (Laskowitz et al., 2017). Research has shown that the absence of endogenous APOE (presumably due to reduced protein translation as a result of *APOE* genetic variation) exacerbates postinjury neuroinflammation and development of cerebral

edema and CV (Lynch et al., 2002; Miller, Turan, Chau, & Pradilla, 2014). Miller, Turan, Chau, and Pradilla (2014) report that pro-inflammatory markers are responsible for CV in SAH (Miller et al., 2014). It is possible that in children, particularly the very young, these neuroinflammatory biophysiological responses to injury increase vulnerability to CV.

Implications for Research

Future work should address Type I error concerns with the small sample sizes and event rates common to pediatric TBI research. Additionally, other yet to be identified factors involved in functional physiologic differences may contribute to the effects of the relationship between *APOE4* and CV on functional outcomes. To better understand the inconsistent results in the pediatric *APOE* literature, a next step should include exploring the potential influence of other gene variants (including microRNAs) and differential *APOE* transcription or protein translation effects on the relation between CV and outcomes in pediatric TBI.

Limitations

In this small, single-site study, we identified associations between *APOE4* and various functional outcomes in pediatric TBI as well as potential moderating and interacting factors that may help to refine these associations. Our sample is sufficient in size for stable parameter estimation, which can provide useful effect size information, directions for further investigation, and potential clinical practice insights (Peduzzi, Concato, Kemper, Holford, & Feinstein, 1996). As is frequently the case in pediatric TBI studies, the heterogeneity of our participants and the small sample limit generalization. We also appreciate that a single daily measure for CV limits the opportunity to capture CV during unmonitored times; thus, it is possible that we underestimated the number of participants with CV or the number of CV events. As there is no validated reference for cerebral flow velocities in the young infant, we used the reference for the velocities for 1- to 2.9-year-olds for the younger group, which might have led to under- or overestimation of the flow velocities in these participants. The use of the GOS-E Peds in moderate to severe TBI has been well established in the research literature; however, its use in mild acute TBI patients is less prominent. Finally, there are many noncoding polymorphisms of *APOE* that could affect expression; we did not examine these in the present study.

Conclusion

In the present study, we describe potential effects of the relationships between *APOE* polymorphisms and CV on functional outcomes in pediatric TBI. Larger studies of children with TBI would aid in the determination of scientifically important and meaningful relationships between *APOE4* risk allele carrier status and the incidence of CV and their combined effects on functional outcomes. Exploring the effects of *APOE* genotype

on the presence of CV and functional outcomes in children with TBI may one day lead to the development of pharmacogenomic targets that allow for the personalization of treatments and subsequent improvement in outcomes.

Author Note

This manuscript has not been previously presented.

Author Contribution

Karin Reuter-Rice contributed to conception and design; contributed to acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Michael Regier contributed to acquisition and analysis, drafted the manuscript, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Ellen Bennett contributed to conception and design, contributed to interpretation, drafted the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Daniel Laskowitz contributed to conception and design, contributed to interpretation, drafted the manuscript, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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