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State of the Science of Pediatric Traumatic Brain Injury: Biomarkers and Gene Association Studies

Karin Reuter-Rice, PhD, CPNP-AC, FCCM¹, Julia K. Eads, BA¹, Suzanna Boyce Berndt, BA, MPA¹, and Ellen Bennett, PhD²

¹Duke University, School of Nursing 307 Trent Drive, DUMC 3322 Durham, NC 27710

²Duke University, School of Medicine Dept. of Neurology Bryan Research Bldg. Durham, NC 27710

Abstract

Objectives—Our objective is to review the most widely used biomarkers and gene studies reported in pediatric traumatic brain injury (TBI) literature, to describe their findings, and to discuss the discoveries and gaps that advance the understanding of brain injury and its associated outcomes. Ultimately, we aim to inform the science for future research priorities.

Data sources—We searched PubMed, MEDLINE, CINAHL, and the Cochrane Database of Systematic Reviews for published English language studies conducted in the last 10 years to identify reviews and completed studies of biomarkers and gene associations in pediatric TBI. Of the 131 biomarker articles, only 16 were specific to pediatric TBI patients, whereas of the gene association studies in children with TBI, only four were included in this review.

Conclusion—Biomarker and gene attributes are grossly understudied in pediatric TBI in comparison to adults. Although recent advances recognize the importance of biomarkers in the study of brain injury, the limited number of studies and genomic associations in the injured brain has shown the need for common data elements, larger sample sizes, heterogeneity, and common collection methods that allow for greater understanding of the injured pediatric brain. By building on to the consortium of interprofessional scientists, continued research priorities would lead to improved outcome prediction and treatment strategies for children who experience a TBI.

Implications for nursing research—Understanding recent advances in biomarker and genomic studies in pediatric TBI is important because these advances may guide future research, collaborations, and interventions. It is also important to ensure that nursing is a part of this evolving science to promote improved outcomes in children with TBIs.

Keywords

Biomarkers; Child Abuse; Children; Genomics; Head Injury; Traumatic Brain Injury

INTRODUCTION

Traumatic brain injury (TBI) is a major health concern and affects persons of all ages, races, ethnicities, and incomes. It is responsible for approximately one-third of all injury-related deaths in the United States and is the leading cause of morbidity and mortality in children and adolescents, with 7,400 children under the age of 19 dying annually from a TBI (Coronado et al., 2011; Center for Disease Control and Prevention, n.d.). Death rates are highest among children 0–4 years of age, boys, and minority populations (Coronado et al., 2011). The incidence of TBI is 35% higher in African Americans than in Whites and other races, and African Americans also have the highest death rate (Bruns & Hauser, 2003; Jager, Weiss, Coben, & Pepe, 2000). Acute and rehabilitative costs associated with TBI because of accidents and child abuse are estimated at \$60 billion annually, with average lifetime costs ranging from \$600,000 to \$1,875,000 (Corso, Finkelstein, Miller, Fiebelkorn, & Zaloshnja, 2006; Schneier, Shields, Hostetler, Xiang, & Smith, 2006). In 2012, the second edition of the Guidelines for the Acute Medical Management of Severe Traumatic Brain Injury in Infants, Children and Adolescents was published to reflect once again the lack of Class I evidence to support the therapeutic interventions recommended (Kochanek et al., 2012). The role of biomarkers and gene association studies are important in the diagnosis of injury and inform future treatment strategies in pediatric TBI. However, there remain limitations with their use in children who have sustained a TBI. Thus, the financial, personal, and societal costs of pediatric TBI are immense, and the limited therapeutic interventions make a comprehensive research agenda a compelling need.

Injury as a result of head trauma in children occurs in two distinct phases. The *primary injury* phase occurs on impact and results from mechanical forces that cause direct disruption of the brain parenchyma. This injury phase may be preventable, which is why there is a heavy emphasis on education for injury prevention, such as protective headgear and seat belt use. Once the first irreversible injury is sustained, a *secondary injury* follows. Here, endogenous factors, such as metabolic, cellular, cytotoxic, and vasogenic edema, and biochemical derangements as well as exogenous factors, such as hypoxia and hypotension, lead to neuronal cell degeneration and, ultimately, neuronal death (Kochanek et al., 2000). The goal in the acute injury phase is to improve cerebral perfusion and to stabilize the injured brain while promoting neuroprotection strategies to optimize functional outcomes (Anderson, Brown, & Newitt, 2010; Coronado, et al., 2011; Langlois, Rutland-Brown, & Thomas, 2004; Oh & Seo, 2009; Faul, Xu, Wald, & Coronado, 2010). This acute phase may occur for days to weeks in the injured brain, and it is within this time frame that the majority of the biomarker study research has occurred, because associating a biomarker with cascading protein activity may lead to identifying outcomes (Papa et al., 2013).

Biomarkers are defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (Biomarkers Definitions Working Group, 2001, p. 91). The identification of biomarkers in injury and diseases has led others to consider their genetic association. Gene association studies are designed either as candidate gene approaches or as genome-wide association studies. In the candidate gene approach, the identification is of a single nucleotide polymorphism (SNP). SNPs are the most common type of genetic

variations in a single base pair of deoxyribonucleic acid (DNA), and if found in an important gene, it can change the protein or biomarker structure, which may ultimately change an illness or injury or response (Kurowski, Martin & Wade, 2012). To date, the only published gene studied in children with head injury has been a candidate gene association by evaluating for the presence of the apolipoprotein E (APOE) gene, specifically allele 4 (APOE4). APOE4 has been associated with poor neurocognitive outcomes in adult patients with TBI, but has demonstrated mixed findings in children with a TBI (Blackman, Worley, & Strittmatter, 2005; Brichtova, & Kozak, 2008; Moran et al., 2009).

LITERATURE REVIEW

A systematic review was conducted in May 2014 of English language–published literature on pediatric TBIs, which included biomarker and genetic studies using PubMed, MEDLINE, CINAHL, and Cochrane Database of Systematic Reviews. The articles were narrowed down using the following MeSH search terms: pediatrics, children, traumatic brain injury, head injury, biomarkers, gene, genomics, and polymorphism. Studies were limited to the last 10 years and to children aged 18 years or younger who were hospitalized. Those studies that did not have pediatric TBI and biomarkers or a specific gene identified as their primary focus were excluded. Of the 131 biomarker articles, only 16 were specific to pediatric TBI patients, whereas of the gene association studies in children with TBI, only four were included in this review (Tables 6.1 and 6.2). The bibliographies and reference lists of all articles were also reviewed for potentially relevant articles, which were independently reviewed by the three authors for further biomarker and genetic studies in association with pediatric TBI. No additional studies were identified or included; however, published literature reviews on the science of biomarkers and gene studies in pediatric TBI were included because they informed the state of the science and offered an additional historical foundation. There were no published biomarker or gene association research studies in pediatric TBI found in journals with nursing titles.

Biomarker Studies

The presence of biomarkers in the blood, cerebral spinal fluid (CSF), and/or urine can indicate a mediated cellular response to an injury (Feala et al., 2013). Although the number of biomarker studies were relatively few in children, as opposed to studies of adult TBI, there were six published comprehensive review articles since 2006 that specifically addressed the use of biomarkers in pediatric TBI (Berger, 2006; Daoud et al., 2014; Kochanek et al., 2008; Kovesdi et al., 2010; Sandelr, Figaji, & Adelson, 2010; Papa et al., 2013). The reviews addressed biomarkers and their use, prognostic value, clinical implications and applications, research utility, and outcome prediction. They included comprehensive tables that illustrated the most widely studied biomarkers, their time frame for discovery, values of measure, and referenced studies.

Although the majority of the biomarker research has only been within the last 15 years in children with head injury, the work of one group from the University of Pittsburgh has largely driven the focus on TBI (Kochanek et al., 2013). Along with colleagues from a number of institutions, the collaborative research led to the identification of a number of candidate molecular biomarkers (Table 6.3). Pediatric TBI biomarker research has reported

the detection of viable candidate markers and their association with outcome prediction (Berger, 2006; Berger, Beers, Papa, & Bell, 2012; Kochanek et al., 2000; Kochanek et al., 2008). Having said that, only a few studies have reported a significant correlation between the biomarkers and an outcome measure, whereas others have determined those biomarkers to have poor prognostication qualities (Babcock et al., 2013; Bandyopadhyay et al., 2005; Berger & Kochanek, 2006; Chiaretti et al., 2008; Daoud et al., 2014; Geyer, Ulrich, Grafe, Stach, & Till, 2009). However, several biomarkers, such as S100B, neuron-specific enolase, and myelin basic protein, have been reported in some studies to have a correlative relationship with outcomes in children with TBI, while in others, they have been called into question.

The heterogeneity of TBI may bear on this conflicting evidence. Pediatric TBI classification (mild, moderate, and severe) does not accurately represent the actual injury type (noninflicted vs. inflicted) or the structural damage and biomechanical and biochemical responses. These biochemical responses differ significantly based on the evolution of the secondary injury phase and the treatment delivered. Although there are standardized TBI guidelines for the treatment of severe head injury, the guidelines' singular pathway for approach may not appropriately address the different injuries and may also explain the contradiction in the biomarker findings (Bell & Kochanek, 2013; Kochanek, Bell, & Bayir, 2010; Kochanek, et al., 2012). The lack of clinical validation of defined biomarkers may contribute in part to the fact that the Food and Drug Administration has yet to approve any specific biomarkers for clinical evaluation in children with TBI (Berger et al, 2011; Papa et al., 2013).

Inconsistencies within the published research findings exist with respect to the relationships among study outcome measures, age groups, inclusion criteria, injury severity, and type of injury (accidental vs. nonaccidental/inflicted TBI [iTBI]). Reports of cognitive and functional outcomes have been limited by the absence or paucity of measures for children. Researchers are challenged by limited outcome assessment instruments designed for pediatric TBI, small study participant numbers and heterogeneous patients, dormant injury sequelae presenting after the injury occurrence, and outcome evaluation (Kövesdi et al., 2010). For example, one of the largest pediatric TBI studies suggested that biomarkers were associated with outcomes, when the Glasgow Outcome Scale-Extended Pediatrics (GOS-E Peds) was used as the outcome measure instead of the Glasgow Outcome Scale (GOS; Beers et al., 2012). Most other studies show different results because their outcomes are based either on survival or on a GOS score. The difference between these two measures is that the GOS-E Peds is specific to children with TBI and is considered the "gold" standard of measure. It has demonstrated validity in the pediatric TBI population (Beers et al., 2012), and yet, most studies still report outcomes based on survival or the GOS (Berger et al., 2011).

Although the prevalence of biomarker research in pediatric TBI has increased over the last decade, variable study methods have limited the accuracy of prediction and generalization (Berger et al., 2012; Papa et al., 2013). Analytic limitations in the published studies included small sample sizes, leading to underpowered studies, which prevent obtaining a direct association between the biomarker and the outcome measure (Daoud et al., 2014). Sample

collection variability, including both the type of sample and the time of sample collection, has confounded the interpretation of biomarker effects, thereby creating increased complexity in attributing a biomarker change with an injury or predictive outcome. Some biomarkers, such as S100B, are dependent on age and time of injury (Filippidis, Papadopoulos, Kapsalaki, & Fountas, 2010). Additionally, depending on the severity of the injury, repeated collection of a biomarker may be inconsistent, thereby preventing repeated statistical measures. Other studies that collected biomarkers over time reported results by initial, mean, and/or peak concentration. Consistent reporting of concentrations has provided opportunities for comparison across studies, lending support to the finding that worse patient outcomes were associated with increasing biomarker concentrations (Berger et al., 2010).

An emerging science in pediatric TBI studies has focused on degradation products, specifically spectrin breakdown products (SBDPs), cleaved tau (c-tau), and amyloid- β 1–42 (Kövesdi et al., 2010). This growing field of interest has also been subject to the limitations of the biomarker research in pediatric TBI, that is, small samples sizes, collection variation, and mixed severity of the injury.

Another new area of biomarker research is “rehabilomics,” where biomarkers are collected to study the patient’s biological and functional treatment response to rehabilitation (Berger et al., 2011; Kobeissy et al., 2011). This evolving field of biomarker discovery is rich for children with a TBI and opens the doors to promoting long-term, outcome-based treatments and research. It also supports the argument for pediatric neurocritical care and neurorehabilitation units (Pineda et al., 2013). These care units can promote health services and create environments that promote brain injury management and recovery by a standardized approach to TBI or neurologic insult (Kochanek et al., 2010).

Gene Association Studies

Studies of the gene association, specifically candidate gene identification in TBI in children are extremely limited. Kurowski and colleagues (2012) published a comprehensive literature review of APOE in children with TBI. Five of the studies described in that review focused on hospitalized children with TBI (Tables 6.2 and 6.3). The current review excluded one of these (Quinn et al., 2004), which focused on the presence of APOE in postmortem tissue samples. Although APOE4 was associated with younger age and unfavorable outcomes, the fact that all participants had died appears to have favored the outcome (Quinn et al., 2004).

The apolipoprotein gene, mapped to chromosome 19, consists of four exons and three introns, totaling 3597 base pairs. Three major isoforms of APOE are APOE2 (cys112, cys158), APOE3 (cys112, arg158), and APOE4 (arg112, arg158; Ghebranious, Ivacic, Mallum, & Dokken, 2005). Because the isoforms differ from each other, they change the APOE structure and function. Secreted locally by macrophages after a peripheral nerve injury and by astrocytes and oligodendrocytes after central nervous system insults, APOE polymorphisms have been associated with changes in neurocognitive and functional outcomes (Jofre-Monseny, Minihane, & Rimbach, 2008). The presence of the APOE genotype is associated with increased biochemical surrogates of inflammation. Evidence shows that the multifunctional nature of the APOE genotype may be in large part due to an

impact on its oxidative status or the immunomodulatory/anti-inflammatory properties (Jofre-Monseny et al., 2008).

Studies examining APOE found that the possession of APOE4 allele led to poorer recovery outcomes in children with TBI. There were significant differences between the trauma severity and outcome for E2/E3 and E3/E3 genotype allele carriers. The presence of APOE4 was found to be associated with lower Glasgow Coma Scale and with more severe injury. Studies also found that there was little evidence to support that APOE4 has a greater impact on injury severity or functional outcome, whereas there was evidence to suggest that APOE4 may be associated with negative early response to injury. The presence of APOE4 in a child who happens to sustain a TBI may set him/her up for an exaggerated inflammatory process that maybe in part responsible for worse outcomes after injury. APOE4 has been associated with poor GOS scores (Blackman et al., 2005; Brichtová, & Kozák, 2008; Lo et al., 2009; Moran et al., 2009).

RESEARCH PRIORITIES

Common Data Elements

In 2009, the National Institute on Disability and Rehabilitation Research and the National Institute of Neurological Disorders and Stroke (NINDS) called for a review of the original TBI common data element (CDE) recommendations to ensure they were relevant to pediatric populations. An interprofessional work group composed of pediatric TBI experts developed and then revised the recommendations in 2012 (Papa et al., 2013). The work group published three descriptive articles to inform future study development (Adelson et al., 2012; Berger et al., 2012; Miller, Odenkirchen, Duhaime, & Hicks, 2012). At the University of Pittsburgh, Drs. Bell and Wisniewski received a \$16.5 million grant from the NIH to lead the international Approaches and Decisions for Acute Pediatric TBI (ADAPT) trial (www.adapttrial.org). Funded for 5 years (2013–2017) as a comparative effectiveness study, CDEs will be collected from 1,000 children with severe TBI. The study will estimate the impact of strategies to lower intracranial pressure and treat secondary injuries and strategies to determine the delivery of nutrient treatments on outcomes up to 1 year after the injury, thereby determining what approach to clinical management works best. There is an opportunity for this body of work to include biomarkers and gene association studies. It may also be the needed step to defining predictive markers and polymorphism with this injury.

Research Methodologies

There is growing consensus that research conducted in pediatric TBI should have comparable methodologies. Small sample sizes and heterogeneous populations prevent collected biomarkers from being optimally sensitive and specific. The day-to-day variability of the patient's injury can be captured better by time-course collection of biomarkers, which has a higher specificity than point estimates, and can influence clinical validity and inform future treatment and recovery (Berger et al., 2011). Therefore, research may be served better if biomarkers that have demonstrated a positive relationship with patient outcomes are optimized in larger sample sizes to demonstrate their usefulness in informing the course of treatment. Additionally, developing programs of research that incorporate and more clearly

elucidate the roles of degradation products in pediatric TBI may guide future treatment strategies.

Improving workgroups and multisite research communication will advance the field and ensure study designs and methodologies that can statistically support the research hypotheses. An example of this is the Pediatric Neurocritical Care Research Group (PNCRG). This group is dedicated to performing clinical and preclinical studies aimed at optimizing functional outcomes for critically ill children with neurological conditions. Its membership is international and inter-professional, comprising those who share a common passion for research that will ultimately lead to improved neurocritical care for children. Current research includes biomarkers and quality of life in children with TBI; determining the prevalence of acute critical neurologic disease in children; and computer tomography classification to guide therapeutic intervention and outcome prediction in children with severe TBI (www.pncrg.org).

As with biomarkers, gene association studies in TBI would benefit by being included in all TBI studies to promote comparison and combined analysis across studies. Kurowski and colleagues (2012) support gene association studies that would provide an opportunity to evaluate comprehensive and tailored outcomes. They suggest that pediatric TBI genetic research should include demographic, environmental, and genotypic characteristics to better contrast and compare patients and their outcomes (Kurowski et al., 2012). Multisite studies would also promote large sample sizes, thereby affecting the magnitude of the genetic polymorphism identification. The limited focus on gene association and polymorphism studies in children with TBI may be influenced in the future by a recent increase in such studies among adult TBI patients, as researchers have begun to recognize the importance of the interrelationships of genetics, recovery, rehabilitation, and promotion of functional outcomes. Improving the understanding of how genes are associated with phases of injury and how polymorphism influences outcomes is increasingly recognized as an important next step in pediatric TBI research.

Although there is more uniformity in preclinical (experimental) research than clinical research, researchers have developed animal models that explain some effects of injury such as impact, rotational, and repeat injury (Kochanek et al., 2010). Replications of developed injury models used in preclinical models have demonstrated utility in defining responses of the injured brain. However, limitations exist in the developing human brain, human biomarker response and identification, as well as gene associations studies.

To minimize the difficulty with tissue and biologic sample collection, Feala and colleagues (2013) suggest an important role for systems biology in TBI biomarkers. In this emerging science, systems biology researchers examine the interwoven molecular pathways and networks by computational modeling of diverse datasets. Sophisticated methods that create and measure protein-binding interaction (PPI) networks lead researchers to move heterogenous information from high-throughput molecular data sets of complex molecular TBI responses into testable hypotheses (Feala et al., 2013). By using protein/proteomic data sets for TBI, researchers can make new discoveries of biomarkers, and scientists would gain

understanding into the underlying molecular mechanism of TBI as a promising frontier for improving TBI outcomes.

Biorepository

In 2010, an interagency TBI CDE Biospecimens Workgroup developed Common Data Elements for TBI Research: Pediatric Considerations, which presented recommendations for the best practice of the collection, processing, and storing of biological specimens collected in a child with TBI. The report stressed the importance of adopting standard practices for sample collection, processing, and storage, and for recording biomarker-specific data in all future pediatric biomarker studies (Berger et al., 2012; Papa et al., 2013). Highlights from these guidelines included the following: biospecimen collection should maintain a consistent volume collected at each sample time point and from the sample origin, appreciating that samples may be collected over a period of hours to months; if the TBI prevents assessment of time of injury, such as may occur in an iTBI, a consistent approach to timing should occur with all subjects; and internal review board approval should be sought for collection and storage; risk for hemolysis of samples, especially in younger children and infants, in whom hemolysis has been demonstrated to affect some biomarkers results. Collection of CSF collection may occur from existing drains; however, specialized attention should be taken with indwelling catheters. Additionally, lumbar puncture is not indicated unless for use with control subjects; processing of samples should include aliquots to avoid refreezing of samples; storage should ensure the protection of samples and rights of donors; and consent for sample storage must be revisited when the donor turns 18. Overall, the technologic and biotechnologic advances occurring daily strongly support the storage and development of biologic specimens so that future markers and gene associations may be defined in children with TBI.

IMPLICATIONS FOR NURSING RESEARCH

Understanding recent advances in biomarker and genomic studies in pediatric TBI is important because these advances may guide future research, collaborations, and interventions. It is also important to ensure that nursing is a part of this evolving science to promote improved outcomes in children with TBIs. Nurse scientists have been active in biologic and genetic work in adult TBI research. Many are fellows of the Summer Genetics Institute, National Institute of Nursing Research and have been integral in the development of the International Society of Nurses in Genetics (ISONG), which supports dissemination of biomarkers and gene association studies by nurses (www.isong.org). Nursing colleagues who study adult TBI have contributed widely, from the identification of mitochondrial polymorphism in severe TBI to the discovery of novel targets in human TBI by gene expression profiling (Barr, Alexander, & Conley, 2011; Conley et al., 2014). Others have evaluated the role of APOE4 in functional outcomes after severe TBI (Alexander et al., 2007). The opportunities are plenty for nurse researchers who are trained and eager to participate in the important discoveries to be made in pediatric TBI research.

CONCLUSION

The future is bright for pediatric TBI biomarker and gene association research. In order to improve the clinical utility of research, specific gaps must be closed and, most importantly, consistent outcomes must be demonstrated. This critical analysis of study designs revealed that large, homogeneous patient populations and more consistent use of similar injury and outcome measures may enable future studies to improve upon suspected associations (Kövesdi et al., 2010). The increased attention to the area can provide the impetus for a strong community of researchers to collaborate toward legitimizing biomarkers in pediatric TBI. As this subspecialty of research grows, groups such as ISONG and PNCRG provide opportunities to promote education, coordination, and dissemination, while multisite studies such as the ADAPT trial can foster consistent sample collection methods and ultimately more biomarker and gene association data. Equally important to the success of biomarker and gene association research is the continuation of training of the nurse fellows of the Summer Genetics Institute and the ongoing support of research strategies by the National Institute of Nursing Research, National Institutes of Health. Continued exploration and validation of biomarkers and gene association studies for pediatric TBI should include robust research methodologies, CDEs, and biologic repositories. Biomarkers and gene association studies can contribute to illuminating the complexities of the brain injury process in children.

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TABLE 6.1

TBI Biomarkers Studies That Focused on Children 18 Years and Younger

Year/Author	Biomarker	Population (n)	Severity	Sample Collection Method	Outcome
Babcock et al. (2013)	S100B	n = 76; age range 5–18 years	Mild (GCS > 13)	Serum; samples were collected upon ED admission	<ul style="list-style-type: none"> • 28 children presented with PCS; mean (SD) S100B level 0.092 (0.376) $\mu\text{g/L}$, median was 0.008 $\mu\text{g/L}$ (range 0–2.00 $\mu\text{g/L}$) • For patients with no PCS (n = 48), the mean (SD) S100B level was 0.022 (0.3756) $\mu\text{g/L}$ and median was 0.012 $\mu\text{g/L}$ (range 0–0.141 $\mu\text{g/L}$) • No association was seen between initial S100B levels measured in the ED and development of PCS or severity of PCS symptoms
Piazza et al. (2007)	S100B	n = 15; age range 1–15 years	Mild to severe (GCS 3–15)	Serum; samples were collected upon ED admission and after 48 hours	<ul style="list-style-type: none"> • 9 patients with mild TBI, 2 with moderate TBI, and 4 with severe TBI • S100B levels were higher in severe than in mild TBI patients • Correlation was seen between brain damage severity and serum S100B increase • No correlation was seen between serum S100B levels and outcome (GOSe = 8) • All patients had good 6-month neurological outcomes
Berger and Kochanek (2006)	S100B	n = 29 (9 HBI and 6 iTBI) and 14 healthy controls; age >17 years	Mild to severe (GCS 3–15)	Serum and urine; for TBI, initial level taken as quickly as possible after injury and then every 12 h for 3 days; for control, one serum and urine sample was collected	<ul style="list-style-type: none"> • Urinary S100B concentrations were detectable in 80% of patients with increased serum S100B; in 0% of controls • Increased urinary S100B was found in majority of TBI patients with abnormal serum S100B • Peak urinary S100B occurred later than peak serum S100B • No relationship was found between GCS and GOS or between GCS and initial or peak urinary S100B • Patients with undetectable urinary S100B and a normal serum S100B, independently, are more likely to have good outcome
Spinella et al. (2003)	S100B	n = 163 (136 healthy and 27 TBI); age range <18 years	Mild to severe (GCS 3–15)	Serum; TBI samples were collected within 12 h after injury; control samples collected preoperatively from outpatient surgery	<ul style="list-style-type: none"> • S-100B levels in healthy children had a mean of 0.3 $\mu\text{g/L}$ and inversely correlated with age • In TBI children, 6 months after injury, outcome inversely correlated with GOS score and S-100B levels; comparing good outcome vs. poor outcome, median admission GOS scores (range) were 8 (3–15) and 3 (3–7), and median S-100B levels (range) were 0.85 $\mu\text{g/L}$ (0.08–4.8 $\mu\text{g/L}$) and 3.6 $\mu\text{g/L}$ (1.4–20 $\mu\text{g/L}$) • A serum S-100B level of >2.0 $\mu\text{g/L}$ was associated with poor outcome • After TBI in children, the acute assessment of serum S-100B levels associated with outcome
Berger et al. (2002)	S100B NSE	n = 15 (10 TBI [5 nTBI and 5 iTBI] and 5 meningitis LPs for comparison group); age range 0.1–9 years	Severe (GCS < 8)	CSF; for TBI, samples were collected at the time the catheter was placed and intermittently until catheter removal	<ul style="list-style-type: none"> • CSF S100B concentration/CSF NSE concentration increased in TBI versus the median value of control • 8/10 patients' S100B concentrations had a single peak with rapid decline; could be a correlation between early increases in S100B concentrations and primary BI at or near impact time • iTBI patients had initial peak in NSE concentration on Day 1 after injury followed by a second, higher peak sustained for up to 8 days; second peak may reflect delayed neuronal death • The mean and peak S100B concentration and time of peak were not associated with mechanism of injury • Mean S100B was associated with GCS • S100B concentrations for TBI were higher in patients (GCS > 4); could be a Type I error

Year/Author	Biomarker	Population (n)	Severity	Sample Collection Method	Outcome
Chiaretti et al. (2009)	NSE	n = 64 (32 TBI and 32 lumbar puncture controls); age range 1.3–15.6 years	Severe (GCS = 8)	CSF; samples were collected at 2 h and 48 h after admission to PICU	<ul style="list-style-type: none"> • CSF concentrations of NSE measured 2 h after admission dramatically increased in TBI patients versus controls • At 48 h after injury there was a sustained and delayed peak in NSE concentrations • An early and late peak suggests that there may be two waves of neuronal death, the second wave potentially representing neuronal apoptosis • Significant association was seen between increased NSE expression and poor outcome; this suggests that the production of NSE may indicate the extent of neuronal damage in patients with severe TBI
Bandyopadhyay et al. (2005)	NSE	n = 86; age range 11 months to 18 years	Mild to severe (GCS 3–15)	Serum; sample was collected at ED evaluation and within 24 h of injury (average time interval = 3.8 h).	<ul style="list-style-type: none"> • The mean ($\pm$SD) NSE level was significantly higher with poor outcome (GOS $\pm$ 5). 46.4 $\pm$ 12.7 ng/mL, versus good outcome (GOS = 5), 19.5 $\pm$ 1.4 ng/mL. • Mean NSE levels (ng/mL; mean $\pm$ SE) were significantly higher in abnormal CT scan patients (26.9 $\pm$ 3.0) versus normal CT (16.8 $\pm$ 1.1). • Levels (mean $\pm$ SE) were notably elevated in those with abnormal GCS score ($\pm$ 15), 31.1 $\pm$ 3.6, than normal GCS ($\pm$ 15), 16.7 $\pm$ 1.2. • NSE levels had suitable ability to predict good versus poor outcome. • NSE levels were poor predictors of abnormal CT scans.
Lo et al. (2009)	S100B NSE IL-6 IL-8	n = 28; age range 0.3–14 years	Mild to severe (GCS 3–13)	Serum; samples were collected exactly 24 h after injury	<ul style="list-style-type: none"> • Patients with unfavorable outcomes had significantly higher S100b, NSE, IL-6, and IL-8 concentrations. • Combinations using brain-specific proteins, S100B or NSE, as “screening markers” had higher predictive values for unfavorable outcome. • It was demonstrated that Day 1 serum levels of inflammatory mediators had higher prognostic values than brain-specific proteins, but best outcome predictive value was achieved with combinations of two biomarkers from different mediator families.
Chiaretti et al. (2008)	IL-6	n = 60 (29 TBI and 31 lumbar puncture controls); age range 1–16 years	Severe (GCS < 8)	CSF; samples were collected at 2 h and 48 h after injury	<ul style="list-style-type: none"> • NGF and IL-6 concentrations were significantly higher in TBI than controls 2 h after injury (T₁). • From 2 to 48 h (T₂), IL-6 concentrations declined. • At T₁, no correlation was found between GCS score and IL-6 (–0.31). • T₁ IL-6 levels were not significantly lower in patients with better outcomes; at T₂, IL-6 was significantly higher in those with good outcome. • Correlation between the early upregulation of IL-6 and better outcomes may reflect internal effort at neuroprotection in response to TBI.
Chiaretti et al. (2008)	IL-6	n = 48 (27 TBI and 21 bacterial meningitis LP controls); age range 1.3–15.6 years	Severe (GCS < 8)	CSF; samples were collected at 2 h and 48 h after injury	<ul style="list-style-type: none"> • 2 h after injury (T₁), CSF IL-6 concentration in TBI patients was 10-fold higher than the control group. • There was a decrease in IL-6 concentration from T₁ to 48 h after injury • No significant correlation was found between GCS score and IL-6 concentration. • The stronger the IL-6 upregulation early after initial injury, the better the outcome was for the TBI patient, confirming the neuroprotective role of IL-6 following TBI.
Whalen et al. (2000)	IL-8	n = 58 (27 TBI, 7 bacterial meningitis LP controls, and 24 normal LP controls); age range 0.1–16 years	Severe (GCS = 8)	CSF; samples were collected within 12 h of injury and every 12 h until catheter removed.	<ul style="list-style-type: none"> • CSF IL-8 concentration in TBI patients 0–12 h after injury was much greater than concentration found in control group. • The median IL-8 level for TBI was 4,452.5 pg/mL (range 0–20,000); the median for control group was 14.5 pg/mL (range 0–250). • Median TBI IL-8 was similar to IL-8 in meningitis patients (median 5,300 pg/mL; range 1,510–22,000). • Strong correlation was seen between CSF IL-8 concentration and mortality

Year/Author	Biomarker	Population (n)	Severity	Sample Collection Method	Outcome
Fraser et al. (2011)	GFAP	n = 27; age range 2–17 years	Severe (GCS < 8)	CSF and serum; samples were taken daily until arterial catheter was removed in PICU.	<ul style="list-style-type: none"> • GFAP was markedly elevated in CSF and serum after pediatric TBI. • Serum GFAP measured on PICU Day 1 correlated with functional outcome at 6 months. • Hypothermia therapy did not alter serum GFAP levels compared with normothermia after severe pediatric TBI. • Serum GFAP concentration, together with other biomarkers, may have prognostic value after pediatric TBI.
Su et al. (2012)	MBP	n = 84 (27 TBI and 57 LP controls); age range 8 days to 16 years	Severe (GCS score 8)	CSF; samples were collected daily for first 5 days after injury or until the EVD stopped draining and/or was removed.	<ul style="list-style-type: none"> • Mean CSF MBP concentration in TBI patients (50.49 ± 6.97 ng/mL) from all 5 days after injury was significantly greater than mean in controls (0.11 ± 0.01 ng/mL). • Mean CSF MBP concentration increased versus controls on the first day after injury (43.02 ± 15.34 vs. 0.11 ± 0.01 ng/mL) and concentration sustained through the first 5 days. • Correlation was found between age and CSF MBP concentrations in TBI patients; TBI patients 1 year old had higher mean than those who were younger (60.22 ± 8.26 vs. 19.18 ± 1.67 ng/mL).
Berger et al. (2010)	S100B NSE MBP	n = 100 (72 TBI and 28 HIE); age <17 years	Mild to severe (GCS 3–15)	Serum; samples were collected as soon as possible after injury and then every 12 h for 120 h.	<ul style="list-style-type: none"> • 43% of the subjects had poor outcome 3 months after injury; there was no difference with regard to outcome by sex, race, or injury mechanism (HIE vs. TBI); the mean age $\pm$ SD of poor outcome was lower than a good outcome (2.3 ± 3.4 vs. 4.7 ± 4.6 years). • For each biomarker, the study validated 2-, 3-, and 4-group models for outcome prediction, using sensitivity/specificity; for S100B, the 3-group model predicted poor outcome 59%/100%; NSE, the 3-group model predicted poor outcome 48%/98%; and for MBP, the 3-group model predicted poor outcome 73%/61%. • When the models predicted a poor outcome, there was a very high probability of a poor outcome; conversely, 17% of subjects with a poor outcome were predicted to have good outcome by all 3 biomarker trajectories. • Data suggests trajectory analysis of biomarker data may be a useful approach for predicting outcome after pediatric BI.
Berger et al. (2007)	S100B NSE MBP	n = 152; age range 0.1–12.5 years	Mild to severe (GCS 3–15)	Serum; for mild and moderate TBI, sample was collected as soon as possible after injury and again 12–24 h after injury; for severe TBI, additional samples were collected approximately every 12 h for up to 5 days; 1 sample was collected from control.	<ul style="list-style-type: none"> • In all biomarkers, at any time point, higher concentration was associated with worse outcome. • The number of hours that NSE concentration was in abnormal range had highest correlation with 0–3 months GOS score. • Initial/peak NSE concentrations and initial MBP concentrations were more strongly correlated to outcome in 4-year-old TBI patients than in >4-year-old TBI patients. • NSE, S100B, and MBP concentrations obtained at the time of TBI may be useful in predicting outcome.

Year/Author	Biomarker	Population (n)	Severity	Sample Collection Method	Outcome
Berger et al. (2005)	S100B NSE MBP	n = 164 (100 TBI [56 nTBI and 44 iTBI] and 64 controls); age range 0.01–13 years	Mild to severe (GCS 3–15)	Serum; for mild and moderate TBI, sample was collected as soon as possible after injury and again 12–24 h after injury; for severe TBI, additional samples were collected approximately every 12 h for up to 5 days; 1 sample was collected from control.	<ul style="list-style-type: none">• The initial median serum NSE/serum S100B concentrations were higher in TBI than in controls (24.29 ng/mL compared with 10.15 ng/mL)/(0.026 ng/mL compared with 0.016 ng/mL); no difference was seen in initial median MBP concentration in TBI compared with controls.• No difference was found in initial or peak NSE, S100B, and MBP concentrations between nTBI and iTBI.• The differences in the time course of all three for nTBI compared with iTBI suggests differences in the pathophysiology of the injuries; this may provide insight into the observed differences in outcome between children with nTBI and children with iTBI.• No relationship was seen between initial or peak NSE, S100B, and MBP concentrations and GCS score.

Note. BI = brain injury; CSF = cerebrospinal fluid; ED = emergency department; EVD = extraventricular drain; GCS = Glasgow Coma Scale; GFAP = glial fibrillary acidic protein; GOS = Glasgow Outcome Scale; GOSe = Extended Glasgow Outcome Scale; h = hour; HBI = hypoxic ischemic brain injury; HIE = hypoxic ischemic encephalopathy; IL = interleukin; iTBI = inflicted traumatic brain injury; LP = lumbar puncture; MBP = myelin basic protein; NGF = nerve growth factor; NSE = neuron specific enolase; nTBI = noninflicted traumatic brain injury; PCS = postconcussion symptoms; PICU = pediatric intensive care unit; TBI = traumatic brain injury; T₁ = time 1; T₂ = time 2.

TABLE 6.2

TBI Gene Association Studies That Focused on Children 18 Years and Younger

Year/Author	Gene	Population	Severity	Sample Collection Method	Outcome
Lo et al. (2009)	APOE	n = 225 (65 TBI and 160 healthy controls); age range undeclared	2 groups: "regained consciousness" (GCS>8) and "delayed return of consciousness" (GCS 8 or less)	Buccal smears	<ul style="list-style-type: none"> The CPP insult level among E4 carriers with poor outcome was significantly less than non-E4 carriers. Homozygotic E3 with good outcome did so with 26× more CPP insult than non-E3 homozygous. 46 TBI children "regained consciousness" and 9 were in coma at discharge; at 6 months after injury 8 had "poor outcome" and only 1 "regained consciousness" at discharge. 3/38 of E3 homozygous had "poor outcome" at 6 months after injury; 3/16 of E2 carriers and 3/14 of E4 had "poor outcome." The trend was for the E2 allele carriers to stay in coma at discharge.
Moran et al. (2009)	APOE	n = 99; age range 8–15 years	Mild (GCS 13–14)	Buccal swabs at 2 weeks after injury and 12 months after injury	<ul style="list-style-type: none"> 28 children had APOE4 gene and 71 did not. Presence of APOE4 was associated with lower GCS and with more severe injury. There was little evidence to support that APOE4 has a great impact on injury severity or functional outcome. There was evidence to suggest that APOE4 may be associated with negative early response to injury.
Brichová and Kozák (2008)	APOE	n = 70; age range 1 month to 17 years	Mild to severe (GCS 3–15)	Serum	<ul style="list-style-type: none"> 4 groups based on APOE genotype: E2/E3 (7 patients); E3/E3 (52); E2/E4 (2); E3/E4 (9). There was a significant difference between the trauma severity and outcome for E2/E3 and E3/E3 genotype; no statistical difference in groups with the APOE4 allele. Significant difference was seen between GCS and GOS in E3/E3 genotype groups and E2/E3 genotype.
Teasdale et al. (2005)	APOE	n = 1,094; age range 0–93 years; subset (n = 215 children <18)	Mild to severe (GCS 3–15)	Buccal swab; sample was collected at acute stage of injury	<ul style="list-style-type: none"> Of 984 viable patients, 324 carriers of APOE4 allele; 660 non-APOE4 carriers. No association was seen between having APOE4 allele and GOS. There was no association between having APOE4 and unfavorable outcome: 118/324 (36%) of APOE4 carriers had unfavorable outcome versus 215/660 (33%) of noncarriers. Association between unfavorable outcome and APOE4 carriers was strongest in patients aged 0–15 years; the adverse effect of being a APOE4 carrier decreased gradually with age and defused by age 55–60 years.

Note. APOE = apolipoprotein E; APOE4 = apolipoprotein E4; CPP = cerebral perfusion pressure; GCS = Glasgow Coma Scale; GOS = Glasgow Outcome Scale; TBI = traumatic brain injury.

TABLE 6.3

Pediatric Traumatic Brain Injury Biomarkers

Biomarker	Mechanism of Action	CNS Source	Serum CSF Levels		Preclinical Work	Clinical Development	Knowledge Gap
			Mild TBI	Severe TBI			
GFAP	This protein is released upon cellular injury into the extracellular space. ^a	Glia	>0.033 µg/L increased ^a	>15.04 µg/L unfavorable outcome (death) ^a	May be useful for identifying various types of brain damage and is explicitly linked to CNS injury/not located outside CNS. ^{b,c}	Useful measurement immediately following TBI of predicted mortality and severity of injury ^b	Research is limited, as it is tough to apply this measure to peds due to its developmental chemical expression. ^d
IL-6	Injury induces release of T-cells and microphages, stimulating immune response, leading to inflammation. ^e	Neuron, astrocytes, endothelial, glia	No data	No data	May help stimulate NGF, which can indicate severity of head trauma ^e	IL-6 and NGF increase can repair damaged tissue and may lead to more positive outcomes in recovery. ^e	Studies are limited, which give contradictory results—upregulation of NGF and IL-6 has led to poor outcomes after TBI, or no relationship whatsoever. ^e
IL-8	Cooperates with G-protein-coupled receptors on neutrophils to induce chemotaxis and inflammation ^f	Endothelium, epithelium, leukocytes, and glia	No data	No data	Very elevated in peds TBI for first 108 h, indicating an acute inflammatory element in TBI ^f	May be involved in secondary injury. Also, inflammation could be a target of anti-inflammatory treatment. ^f	Limited research provides contradictory results—helpful versus hurtful to recovery ^f
MBP	Damaged myelin sheath releases elements of the sheath (MBP) ^g	Myelin sheath	No data	No data	Increases in MBP in CSF follow TBI ^g	Significantly elevated increases of MBP immediately following TBI were associated with poor outcomes ^g	There is a lack of study of mild TBI cases and MBP as it is most visible in CSF; age affects concentration levels in the control population (not as elevated). ^g
NSE	Emerges after damage to neuronal brain cells ^a	Neuron	>7–10 µg/L increased ^a	Mean 12.8 µg/L (Serum), mean 7.8 µg/L ^a (CSF)	Indicator of intracranial injury as the enzyme appears after cellular damage within 6 h of injury ^h	May be used as predictor of short-term, physical disability in peds ^h	Focus is needed on long-term outcomes as well. ^h
S-100 beta (S100B)	Stimulates neuronal growth and enhances the survival of neurons after injury ⁱ	Glia	>0.25 µg/L increased ^a	>2–2.5 µg/L unfavorable outcome ^a	Served as markers of neuronal damage after TBI ⁱ	Increases in S100B serum concentrations were associated with brain damage severity. ⁱ	Variety of results makes it hard to conclusively use data for prognostic purposes. ⁱ
APOE	Found on chromosome 19. Alleles E2, E3, E4;	Neuron, glia	No data ^a	Mean 3.7 mg/L ^a	Possession of E4 allele in adults is shown to	Possession of E4 allele may lead to poorer recovery	Very few research studies, which have

Biomarker	Mechanism of Action	Serum CSF Levels		Preclinical Work	Clinical Development	Knowledge Gap
		CNS Source	Mild TBI			
	associated with increased biochemical surrogates of inflammation. associated with increased biochemical surrogates of inflammation. associated with increased biochemical surrogates of inflammation.			be related to poorer outcomes following brain injury. ^k	outcomes in children with TBI. ^k	resulted in contradictory evidence, and even fewer pediatric studies ^k

Note. APOE = apolipoprotein E; CNS = central nervous system; CSF = cerebrospinal fluid; h = hour; GFAP = glial fibrillary acidic protein; IL = interleukin; MBP = myelin basic protein; NGF = nerve growth factor; NSE = neuron-specific enolase; peds = pediatrics; TBI = traumatic brain injury.

- ^a Kövesdi and colleagues (2010).
- ^b Pelinka and colleagues (2004).
- ^c Mondello and colleagues (2011).
- ^d Fraser and colleagues (2011)
- ^e Chiaretti and colleagues (2008)
- ^f Whalen and colleagues (2000)
- ^g Su and colleagues (2012)
- ^h Bandyopadhyay and colleagues (2005)
- ⁱ Piazza and colleagues (2007)
- ^j Berger and colleagues (2002)
- ^k Moran and colleagues (2009).