THE EPIDEMIOLOGY OF POST-TRAUMATIC SEIZURES FOLLOWING MODERATE TO SEVERE TRAUMATIC BRAIN INJURY

by

Anne Connelly Ritter

BA, Gettysburg College, 2009

MPH, University of Pittsburgh, 2010

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This dissertation was presented

by

Anne Connelly Ritter

It was defended on

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and approved by

Dissertation Advisor:
Caterina Rosano, MD, MPH
Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Committee Members:
Maria M Brooks, PhD
Professor and Vice Chair of Education
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Anthony Fabio, PhD, MPH
Assistant Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Amy K Wagner, MD
Associate Professor and Endowed Research Chair
Department of Physical Medicine & Rehabilitation
School of Medicine
University of Pittsburgh

Caterina Rosano, MD, MPH

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Anne Connelly Ritter, DrPH

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ABSTRACT

Though death rates due to traumatic brain injury (TBI) are decreasing in the United Statues, TBI remains a significant public health problem. Individuals who survive moderate and severe TBI become at risk of developing secondary complications, including post-traumatic seizures (PTS). PTS are well-recognized sequelae of TBI. Despite previous research, there remains a high degree of variability in who will develop PTS and no approved prophylactic medications to prevent late PTS exist. Late PTS is associated with significant morbidity and worse outcomes following TBI. Therefore, it is of public health importance to understand the characteristics of individuals with PTS, identify factors to improve prognostication, and explore novel risk factors to support a personalized medicine approach.

Using the Traumatic Brain Injury Model Systems, we examined the incidence of immediate (<24hours), early (1–7 days), and late (>7 days post-injury) PTS. Incidence of new onset seizures was highest immediately (8.9%) and one-year (9.2%) post-injury. Late PTS prevalence surpassed 20% at five-years post-injury. Incidence was stratified by potential risk factors and relative risk calculated. Individuals with immediate but not early seizures had a significantly greater incidence of late PTS compared to individuals not seizing during acute hospitalization.

We then developed and internally validated prognostic models for PTS during acute hospitalization, at one-year, and two-years post-TBI. We identified multiple variables, including

novel factors such as pre-injury mental health conditions, predictive of PTS. Year one and two models showed fair-to-good ability to discriminate PTS, supporting the idea that more accurate prognostication of late PTS can be accomplished.

Lastly, we examined genetic variation in neuronal glutamate transporter genes as risk factors for PTS. We identified genetic variants significantly associated with increased PTS risk, after controlling for known risk factors. The relative effect size of the genetic markers suggests these variants may be significant predictors of PTS and may improve prognostic model reliability and validity.

Classifying subpopulations at high-risk for PTS could facilitate research regarding the effectiveness of tiered prophylaxis and novel pharmacological interventions, improving prevention and treatment. Together, findings from the current work may affect future research and programmatic decisions, positively impacting those at risk for PTS.

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PREFACE

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1.0 INTRODUCTION

Traumatic brain injury (TBI) is a significant public health concern and is a major cause of morbidity and mortality, especially to those under age 45, in the United States [1; 2]. TBI is extremely heterogeneous regarding mechanism of injury, injury severity, and possible outcome. Individuals may experience many different complications and comorbidities associated with TBI that may continue chronically, persisting for many years or even throughout the lifetime following injury[3-5].

Individuals with more severe injury are particularly affected by chronic conditions associated with TBI. Among individuals who survive a severe TBI, disability rates are estimated as high as 77% [6]. Chronic complications include, but are not limited to, poor functional outcome, decreased cognitive function, psychosocial and/or behavioral problems, decreased general health, and other neurological sequelae. These complications contribute substantially to the cost of care associated with TBI, which is estimated at more than $60 billion annually in the United States [7]. Lifetime total cost of care is estimated to exceed $1.8 million for an individual [7]. In addition to medical cost incurred, TBI may significantly increase years of potential life lost and decrease quality of life.

Post-traumatic seizures (PTS) are a well-recognized sequela of traumatic brain. PTS have been documented as a common complication of TBI for decades. Incidence of PTS varies drastically throughout the literature and is dependent on many factors including study design and

characteristics of the study population. As incidence of TBI increases and death due to TBI decreases, more individuals will be at risk of developing and living with chronic complications. It can also be expected that PTS incidence will increase. Previously, seizure prophylaxis has been shown to be effective in reducing the incidence of PTS in the first week after injury, but has no long-term benefit (Temkin, 1990). Current recommendations from the American Academy of Neurology and the Brain Trauma Foundation include delivery of phenytoin for seizure prophylaxis during the first seven days post-TBI [8; 9]. Yet, despite decades of research, there are no effective pharmacological interventions to prevent post-traumatic seizures that develop after seven days post-injury and, it does not appear that rates of PTS are decreasing. Increasingly, novel risk factors for PTS and mechanisms of epileptogenesis following TBI are being investigated to identify potential new targets for therapeutic treatment.

1.1 TRAUMATIC BRAIN INJURY

1.1.1 Definitions

Traumatic brain injury is caused by an impact to the head from an external force that disrupts physiological function. External forces can include direct mechanical impact (i.e. blunt trauma), acceleration or deceleration associated injury (i.e. whiplash), blast injury caused by a pressure wave (i.e. explosion), or penetrating injury (i.e. gunshot).

Severity of TBI can be classified multiple ways. Originally developed to classify levels of consciousness [10], the Glasgow Coma Scale (GCS) has become the most widely used tool to describe TBI severity. The GCS score ranges from 3 to 15: 3-8 indicating severe, 9-12 indicating

moderate, and 13-15 indicating mild TBI [11]. Alternative criteria based on loss of consciousness and post-traumatic amnesia are also used [12]. Many organizations, including the Department of Defense (DoD), Veterans Affairs (VA), and Centers for Disease Control and Prevention (CDC), continue to utilize a combination of clinical variables to assess TBI severity (Table 1).

Table 1. Classification of Traumatic Brain Injury

Criteria	Mild	Moderate	Severe
Structural Imaging[1]	Normal	Normal or abnormal	Normal or abnormal
Loss of Consciousness	0 - 30 min	> 30 min but < 24 hours	> 24 hours
Alteration of Consciousness[2]	0 - 24 hours	> 24 hours	
Post-Traumatic Amnesia	≤ 24 hours	24 hours – 7 days	> 7 days
Glasgow Coma Scale[3]	13 – 15	9 – 12	3 - 8

[1]Abnormalities not related to trauma may be present with mild injury;
[2]Classification of severe injury not made based on alteration of consciousness alone;
[3]Typically based on best GCS score is first 24 hours post-injury
**adapted from Silver, McAllister, & Yudofsky. Textbook of Traumatic Brain Injury, Second Edition*

In addition to severity, TBI is often classified as closed-head or penetrating brain injury. Many studies within the existing literature categorize TBI into one of four classifications: mild, moderate, severe, or penetrating. Despite the fact that it is possible to obtain a GCS score on an individual with a penetrating TBI (pTBI), researchers and clinicians often refer to pTBI as a distinct category.

As medical care has evolved, and computed topography (CT) became a part of standard care, neuroradiological findings have increasingly been used to differentiate and define TBI. Abnormalities detected via CT are categorized based on location and type of injury [i.e. which hemisphere, brain region, and pathology present (Table 2)]. Advances in neuroradiological imaging through tools such as magnetic resonance imaging (MRI), positron emission topography

(PET), and magnetic resonance spectroscopy (MRS), may provide additional ways to classify and differentiate brain injury. Some definitions regarding severity of TBI include positive evidence of abnormal pathology via CT imaging as a distinguishing characteristic.

Table 2. Abnormal Pathologies Identified via Computed Tomography

Variable	Definition
Intracranial Hemorrhage (ICH)	Bleeding within the skull (cranium)
Contusion	Area(s) of bleeding on the surface of the brain, most commonly along the undersurface and poles of the frontal and temporal lobes
Subdural Hematoma (SDH)	Presence of extra-axial blood clot or collection within sub-dural space, between surface of the brain and dura matter
Subarachnoid Hemorrhage (SAH)	Bleeding within the subarachnoid space, between the surface of the brain and arachnoid layer. May include blood in ambient, basal, interpenduncular cisterns or cisterna magna, or along falx or tentorium
Intra-ventricular Hemorrhage (IVH)	Blood documented within intra-ventricular space
Epidural Hematoma (EDH)	Presence of extra-axial collection within epidural space, between skull and dura matter
Penetrating TBI (pTBI)	Typically defined by penetration of the dura matter. May include bone, metal, or other foreign bodies present within the parenchyma, skull fractures displaced or depressed > 2mm, or “through and through” injuries penetrating the dura

1.1.2 Epidemiology

The incidence of traumatic brain injury and its sequelae documented in the existing literature is heavily influenced by many factors, most importantly injury severity and type. In the United States, approximately 2.5 million traumatic brain injuries occur annually[3]. Of these, approximately 284,000 (11%) result in hospitalization, and 53,000 (2%) in death[3]. However, these figures are limited by their reliance on national surveillance data and cannot account for individuals with TBI that do not seek care, or for those who seek care from a primary care

physician only. Therefore, it is likely that these data are an underestimate of the incidence of TBI, particularly mild TBI, in the United States.

Despite this limitation, national surveillance data show trends of increasing overall TBI incidence and emergency department visits related to TBI from 2001 to 2010 (Figure 1). The observed increase may be influenced by recent public health campaigns to increase awareness of mild TBI (mTBI), leading to an increase in health care utilization associated with mTBI.

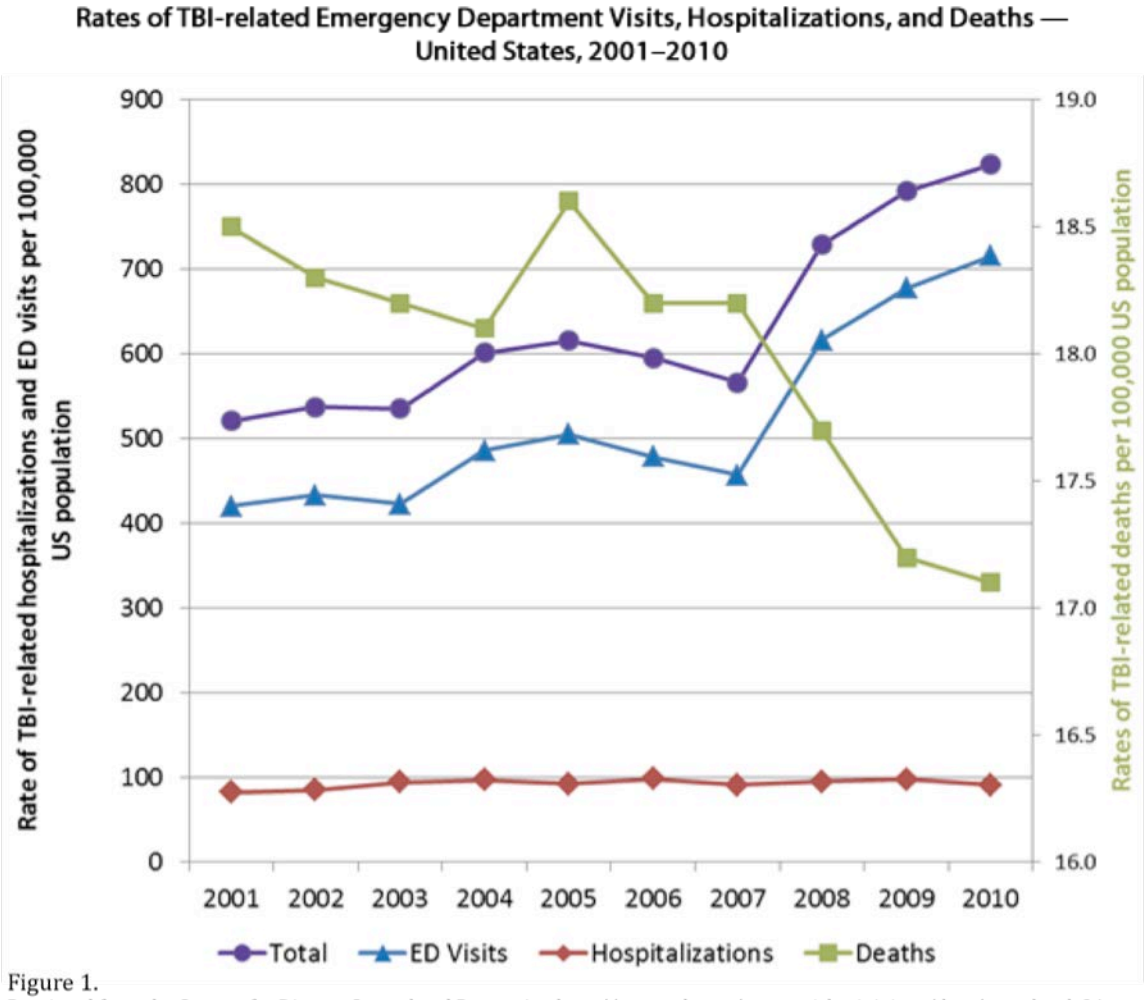

Figure 1.
Reprinted from the Centers for Disease Control and Prevention http://www.cdc.gov/traumaticbraininjury/data/rates.html. 24 Feb 2014.

Figure 1. Rates of TBI Related Emergency Department Visits, Hospitalizations, and Deaths - United States, 2001-2010

This theory is supported by the relatively constant incidence of TBI hospitalizations throughout the same timeframe, suggesting the frequency of moderate and severe TBI requiring hospitalization is not increasing and the resulting overall increase is likely attributable to more mild injuries. Also within the last decade, rates of TBI-related death have decreased in the United States [13].

Across all injury severities, as classified by the GCS, males have consistently higher rates of TBI compared to females [14; 15]. There are also significant differences in risk of TBI across the lifespan. Individuals ages 0 to 4, 15 to 19, and greater than 65 years old are at a significantly increased risk of TBI [14; 16; 17]. Of these age groups, individuals greater than 75 years old have the highest rates of hospitalization and death [13; 15; 17].

Differences in TBI rates across race and ethnicity are also documented. Annual TBI rates, including emergency department utilization and hospitalizations, are higher for black individuals than white and those of other racial backgrounds. However, annual average mortality rates are lower for black individuals compared to whites [17].

1.1.3 Primary and Secondary Injury

The highly heterogeneous nature of traumatic brain injury makes it difficult to establish standards of care across the spectrum of injury. There are currently no therapeutic interventions proven to be effective across a broad range of clinical presentations of TBI. Similarly, outcomes vary greatly and are difficult to predict based solely on injury severity. Differences in neurobiological factors, such as those involved in secondary injury, likely contribute substantially to differences in outcome.

Mechanistically, traumatic brain injury can be thought of as two events occurring successively. The first event, the primary injury, refers to the moment of impact of external forces causing the TBI. The primary injury is a discrete event.

Unlike the primary injury, the secondary injury is often thought of as a cascade, initiated by the primary injury and evolving over time. The secondary injury is not discrete; it is composed of multiple pathophysiological processes that may occur simultaneously or sequentially and are not consistent across individuals. Different primary injury types (i.e. diffuse axonal injury, intra-cerebral hemorrhage, or penetrating injury) may initiate different secondary injury cascades [18]. However, this too may differ across individuals. It is necessary to understand the pathological processes that encompass secondary injuries and their impact on TBI complications, especially development of PTS.

Briefly, the secondary injury cascade has been described as a two-stage process, with multiple pathological processes occurring [19]. Among the first pathological processes to take place are direct tissue damage, abnormalities in cerebral blood flow, and deregulation and functional impairment of cerebral metabolism [19]. Damage to brain tissue including disruption and/or destruction of cerebral blood vessels can result in cerebral ischemia. Multiple studies have documented both focal and global episodes of cerebral ischemia following TBI [20-22].

Adding to the cerebral ischemic state, damage from the primary injury to neuronal, glial, and endothelial cells can disrupt the brain's autoregulatory pathways. Vasoconstriction and vasodilation are well-documented examples of autoregulatory pathways that may be impaired, or completely decimated, following TBI [23-25]. Autoregulatory dysfunction can also cause hypotension and hypoperfusion, progressively leading to metabolic dysfunction and inability to

meet glucose demands [19; 26; 27]. Despite documentation of disrupted pathways, there are no consistent findings regarding the timing of impairment or loss of autoregulatory.

Precise control of cerebral metabolism is vital to maintain proper neurological function [22]. Failure to maintain cerebral metabolism and energy demands leads to mitochondrial dysfunction, decreased ATP production, and chemical and ionic imbalances within cells [19; 28]. Each of these events can cause cell death, contributing to poor outcome following TBI.

Traumatic brain injury can also induce depolarization of the neuron and excessive release of excitatory neurotransmitters, such as glutamate [6; 29]. The excitotoxicity pathway is further enhanced by impairment of glutamate uptake and glutamate receptors following injury [30]. Inability to compensate for excessive glutamate release can cause subsequent breakdown of the blood brain barrier, further disrupting ionic balance [31; 32]. Trying to restore proper neurotransmitter and ion levels increases metabolic demands on tissues that may already be suffering from metabolic dysfunction due to pathological processes described above [33]. Inability to break this cycle and restore balance can lead to cell death.

Oxidative stress, the production and release of reactive oxygen species, often occurs in response to excitoxicity following primary TBI [34-36]. Oxidative stress can then induce additional pathological processes leading to immediate cell death [36], as well as activating inflammatory processes [34].

Inflammatory processes can occur immediately in response to primary injury and tissue damage, or in response to secondary injury cascades. The inflammatory process following TBI is extremely complex and can persist well into the chronic phase [37]. As part of the inflammatory response, cytokines are released, activating subsequent proteins such as chemokines and adhesion molecules that are responsible for activating glia, importantly microglia, and other

immune cells [38]. Activated microglia and immune cells adhere to damaged and potentially non-damaged cells surrounding the damaged tissue, ultimately leading to cell destruction [19; 39]. This process can spread across tissues, continue for sustained periods of time post-injury, and activate astrocytes to produce glial scarring in the effected regions [40].

Many of the same pathological processes taking place in response to TBI can be found during epileptogenesis. Thus, TBI primes the brain for ictogenic activity, a condition that can persist decades following the primary injury.

1.2 POST TRAUMATIC SEIZURES

1.2.1 Definitions and Classification

Simplistically, post-traumatic seizures (PTS) refers to an incident seizure following head trauma. The definitions and classification systems for PTS vary throughout the literature and have changed across time. These differences make it difficult to compare findings across multiple studies and to aggregate data for use in meta-analyses.

Post-traumatic seizures are classified based on time of seizure: immediate (<24 hours), early (1 to 7 days), and late (>7 days) post-injury[41] (Table 3). Immediate and early seizures are considered provoked and decrease seizure threshold only temporarily after TBI [42]. Recently, the term acute symptomatic has been used to describe provoked seizures[43; 44]. Cut-points for PTS classification are based on hypothesized differences in causal pathology and epileptogenic potential [18; 31; 45; 46].

Late PTS and post-traumatic epilepsy (PTE) are often used interchangeably [47; 48]. However, some studies make clear distinctions between PTS, late PTS, and PTE. The distinction typically lies in the intricacies involved with the definition of epilepsy, which has changed over time, and in the timeframe post-injury in which the first seizure occurs. Some studies use time of first seizure to delineate PTS from PTE. In these instances, PTS is used to refer to seizures occurring early, up to seven days post-injury; PTE is then used to refer to seizures that occur after seven days [49; 50]. Other previous research has previously differentiated PTS from PTE based on the number of seizures that occurred. Earlier definitions of epilepsy required the occurrence of two or more unprovoked seizures >24 hours apart. Using this information, many researchers defined PTE as more than one seizure occurring after 7 days post-injury; some studies then used late PTS to describe a single seizure occurring after 7 days post-injury [18; 51-54].

Recently, the International League Against Epilepsy (ILEA) made the recommendation to revise and operationalize the definition of epilepsy. The ILEA concluded that requiring two unprovoked seizures to diagnose epilepsy was no longer adequate to accurately capture the clinical variability across epilepsy disorders [55]. Therefore, the recommendation was made to revise the definition of epilepsy and include conditions where an individual has a single unprovoked seizure and their risk of a recurrent seizure is similar to, or greater than, the risk of seizure recurrence after two unprovoked seizures occurring ≥24hrs apart (≥60%)[42]. These recommendations were adopted as the official position by the ILAE in December 2013, thus changing the definition of epilepsy [42].

Table 3. Overview of Current and Past Definitions for Classification of Seizures Occurring after Traumatic Brain Injury

	Classification	Definition
Current Definition	Immediate PTS[1]	Seizure occurring <24 hours post-injury
	Early PTS[1]	Seizure occurring 1 – 7 days post-injury
	Late PTS[2]	Seizure occurring >7 days post-injury
	PTE	Seizure occurring >7 days post-injury; synonymous with late PTS (not used in current work)
Past Definitions	PTS	Single seizure occurring post-injury
	PTE	Two or more seizures occurring >7 days post-injury
	PTS	Seizures occurring ≤7 days post-injury
	PTE	Seizure occurring >7 days post-injury
	Late PTS	Single seizure occurring >7 days post-injury

[1]*Also referred to as acute symptomatic*
[2]*Individuals with immediate and early PTS remain at risk of developing late PTS*

Previous research on seizure recurrence following a single, unprovoked seizure >7d post-TBI documents risk of seizure recurrence is high enough to consider late PTS as an epileptic condition[42; 56]. Haltiner and colleagues determined, of individuals with a single late post-traumatic seizure, 86% will have a second seizure within two years [56]. Following with the most current clinical definitions of epilepsy, PTE would be defined as *one* or more seizures occurring after 7 days post-injury. Therefore, late PTS and PTE are equivalent. For the purposes of this work, PTS including immediate, early, or late classification will be used to refer to post-traumatic seizure activity. Importantly, individuals with immediate or early PTS who have a subsequent late seizure can be classified as having late PTS (i.e. PTE).

1.2.2 Mechanisms

Post-traumatic seizures may arise from multiple pathological mechanisms initiated by traumatic brain injury. While it is hypothesized that acute symptomatic and late seizures result from different pathological mechanisms [18], multiple epileptogenic processes can occur within one individual.

Epileptogenesis refers to the process through which a healthy, normally functioning brain transforms into a brain characterized by a predisposition toward seizure activity [31]. Inherent to epileptogenesis is a latent time period before the initial epileptic seizure occurs during which cellular and molecular changes are taking place. Following TBI, these cellular and molecular changes can occur as a part of secondary injury cascades. Because the primary injury is an acute event, TBI allows the prospective investigation of epileptogenic processes, which is not feasible with epilepsies of non-traumatic etiology.

Excitotoxicity is one such mechanism that may predispose the brain to epileptic seizure activity and can occur as a result of TBI via secondary injury cascades as discussed above (Section 1.1.3). Immediately following injury, there can be a substantially large release of excitatory neurotransmitters, particularly glutamate[57; 58]; this release may cause excitotoxicity, triggering seizures and other excitotoxic injury [29; 31; 59]. These immediate seizures, in direct response to release of excitatory neurotransmitters would be considered provoked, and therefore would not meet the definition of epileptic seizure activity [31; 55]. However, excitotoxicity can lead to neuronal and astrocytic swelling, mitochondrial damage, cell death, and immediate/early PTS[60]. Seizures can cause over-activation of excitatory amino acid receptors, inducing calcium dependent production of nitric oxide and reactive oxygen species and free radical damage to DNA and cellular membranes [18]. These observations suggest decreased glutamate clearance, and

low-level excitoxicity, is an ongoing mechanism of TBI pathology and contributor to epileptogenesis. Antecedent immediate/early seizure activity may, along with altered glutamate transporter expression, perpetuate excitoxicity and cell death and contribute to epileptogenesis [6; 61].

Glutamate levels must be carefully regulated via release and reuptake to prevent excitotoxic injury. There are five glutamate transporters with distinct cellular, synaptic, and regional distributions within the human brain, each encoded by a different gene [62]. It is possible that dysfunction of glutamate transporters, from injury or genetic predisposition toward a reduced function, could potentially increase seizure susceptibility through excitoxicity, decreased antioxidant reserves, or decreased inhibitory neurotransmission.

Additional epileptogenic processes related to inflammation and glial activation have been identified. As previously described (Section 1.1.3), the inflammatory response can begin immediately following TBI and persist chronically[37]. Activated glia adhere to damaged cells and initiate a feedback loop with immune cells and pro-inflammatory factors[38]. This feedback loop can cause neuronal injury, promote glial scar formation, and decrease glutamate re-uptake, all of which contribute to epileptogenesis [40; 62]. The occurrence of epileptic seizure activity can also maintain this cycle of glutamate release, inflammation, neuronal injury, and glial activation [62].

Ultimately, there are numerous mechanisms that can contribute to epileptogenesis, many of which are initiated by secondary injury cascades. Increasing research regarding biomarkers for PTS may help to delineate pathological processes taking place within sub-groups of individuals. If possible, knowledge of specific epileptogenic processes occurring within individuals could also provide new targets for prevention of PTS, particularly late PTS.

1.2.3 Epidemiology

The incidence of PTS varies widely within the literature and is dependent upon many factors including study design, population characteristics, and how PTS is defined. To date, few large epidemiological studies of PTS have been conducted. The exact percentage of individuals with TBI who will develop PTS, including late PTS (PTE), remains unknown [54].

The incidence of acute symptomatic seizures has not been well described. Of studies that document early PTS, most do not differentiate immediate from early seizures, nor do they specify if immediate and early seizures are considered concurrently. In a population based study from Rochester County Minnesota, Annegers and colleagues reported 2.1% of individuals with TBI (all severities) developed early PTS[63], and of these, approximately 76% of individuals seized within the first 24 hours post-injury. This study included all ages and TBI severity ranges, but when individuals with severe TBI only were considered, the documented incidence of early PTS increased to 10.3%[63]. Smaller studies that do not differentiate immediate from early seizures document early PTS incidence to range from approximately 2% to 17% [12; 45; 47; 64; 65]. Although, incidence rates as high as 25% [66] and as low as 0.9%[67] have been reported in a cohort of brain injury rehabilitation patients in Finland and a cohort of hospitalized Chinese patients, respectively. Within a military cohort, incidence of early PTS falls within the reported range (5%) [68]. The wide range of early PTS incidence may be due to differences in seizure classification and ascertainment methods (i.e. continuous EEG monitoring) or population characteristics (i.e. greater proportion of children, pTBI).

Using the same population from Rochester County Minnesota, Annegers and colleagues conducted one of the first and largest population-based studies of late PTS, investigating individuals injured between 1935 and 1989. They documented an overall standardized incidence

ratio (SIR) of 3.1 for late PTS over the course of full follow-up compared to a demographically similar, non-injured population [12]. Among those with severe TBI, the five-year cumulative probability of late PTS was 10.0%, and increased to 16.7% at 30 years post-injury[12]. Importantly, Annegers and colleagues determined that individuals with severe TBI remained at a significantly greater risk of unprovoked seizure throughout the entire study duration compared to expected epilepsy rates in the general population. However, this study population was racially and socioeconomically homogeneous and results may not generalize to a more diverse population.

A second, more recent, population-based study in the United States examining a more representative sample concluded the incidence of late PTS following TBI of all severities, presenting to a participating emergency department, was 2.2 per 100 persons in the first year post-injury [69]. Incidence increased to 4.1 and 3.1 cases per 100 persons in year two and year three, respectively [69]. Among individuals with severe TBI, the cumulative incidence of late PTS was 13.6 per 100 persons over the first three years post-injury[69].

In a study of individuals with moderate to severe TBI admitted to hospital and requiring inpatient rehabilitation, the cumulative probability of late PTS was 13.2% at two years post-injury [47]. Of individuals with late PTS, 80.3% developed late PTS in the first year, and 92.4% of cases occurred within the first 18 months following injury [47]. Additional smaller studies, in primarily adult populations, have reported the prevalence of late PTS to range from approximately 5% to 19%[66; 67; 70; 71].

Reported prevalence of late PTS is even higher in military populations, surpassing 53%[72; 73]. Compared to studies in primarily civilian populations, military populations have greater incidence and prevalence of late PTS. In the longest follow-up study of TBI in a United States military cohort, 23% of individuals had new onset seizures in the first year post-injury.

Prevalence of late PTS increased to 29% in year two and 53% in year 15; at 35 years post-injury, prevalence was estimated at 43.7%[68; 72; 73]. Eleven (5.5% of study cohort) of the individuals followed up to 35 years post-injury reported new onset seizures between the 15 and 35 year follow-up interviews [72].

In an independent cohort of Service Members and Veterans from the Korean War, 7.1% of individuals developed PTS in the first week post-injury and 22.7% developed late PTS [45]. However, this is likely an underestimate of late PTS since it is unclear if individuals with early PTS developed seizures after one week post-injury, qualifying them for late PTS (i.e. PTE). The majority of late PTS cases (54.2%) within the study cohort developed within one year post-injury and 18.1% of cases developed during the second year [45]. However, new onset cases continued to be ascertained out to 11 years post-injury.

One very important limitation of the majority of late PTS studies thus far is inherent to all epilepsy research: misclassification bias. Seizure activity can present in many different ways. Any physiological brain function can manifest during a seizure and it may not be evident to the individual that what they are experiencing is in fact, seizure activity. Similarly, not all seizures are clinically evident. Subclinical seizure incidence is reported to be higher than incidence of clinically recognized seizures and has been reported to be even higher for individuals with penetrating brain injury [74]. In one study of moderate and severe TBI utilizing continuous EEG (cEEG) monitoring up to 14 days post-injury, 22% of individuals were found to have early PTS; of individuals with early PTS, 57% of seizures were non-convulsive and only detected as a result of continuous monitoring [75; 76]. Therefore, incidence rates of immediate and early PTS reported in the literature, which are largely based on medical record and billing review, are likely underestimates of PTS. For studies of PTS following moderate to severe TBI, where individuals

are in a hospital setting and cEEG monitoring is possible, rates may be less biased. But, the risk for bias remains, especially if cEEG monitoring is only used on subsets of individuals for clinical care, such as those with suspected seizure activity.

1.2.4 Risk Factors of PTS

1.2.4.1 PTS at all Time Points

Few risk factors have been identified for PTS across all time-points (immediate, early, and late). Injury severity is the most commonly examined risk factor for PTS. Various algorithms have been used to define injury severity (see Table 1, Section 1.1.1). Within the literature, there is general agreement that greater severity is associated with increased risk of PTS in all timeframes [12; 50; 63; 66; 67; 69-71; 77-79].

Where there are slight differences in results regarding severity as a risk factor, the method used to categorize severity may be responsible. Englander and colleagues found the highest cumulative probability of late PTS among individuals with moderate TBI, classified by GCS (GCS 9-12), over the first two years post-injury [47]. However, this finding highlights the construct of survival bias, particularly for late PTS. Individuals with severe TBI are more likely to expire within the first week post-injury, excluding them from being at risk for development of late PTS.

Further complicating the effect of injury severity on PTS, specifically immediate and early PTS, when continuous EEG monitoring is used to ascertain cases, there is no significant difference in injury severity, as measured by the GCS, between individuals who seize and those who do not [76]. As such, increasing injury severity may correlate with increasing risk of late PTS,

limited to clinically apparent seizures. Using consistent metrics to classify injury and to ascertain PTS status would increase comparability of findings across studies.

Injury characteristics have been extensively studied as risk factors for PTS. As CT imaging became common practice following head trauma, specific pathology types were also examined. Presence of intra-cerebral blood, including intracerebral hemorrhage and subdural hematoma, has been identified as a risk factor for early and late PTS, increasing risk up to 30% [12; 47; 48; 50; 63; 71; 78; 80; 81]. While pTBI is often associated with late PTS, depressed skull fracture has been specifically documented as a risk factor for both early and late PTS [12; 50; 63; 66; 78; 82].

In addition to injury severity, age at injury is consistently cited as a risk factor for PTS. Children are at an increased risk of PTS at all time-points post-injury, but are particularly prone to immediate and early seizures [12; 63; 66; 77; 83]. The effect of age within adult populations is less clear. The Rochester Epidemiology Project found age greater than or equal to 65 was associated with a late PTS rate ratio of 2.5, which remained significant even after correction for other risk factors including early PTS and depressed skull fracture [12]. Asikainen et al concluded increasing age among adults is correlated with increasing risk of late PTS [66]. Yet, other studies found no association between age and risk of late PTS [69]. Additional research is needed to more thoroughly investigate the effect of age on late PTS among adults.

1.2.4.2 Early PTS

Characteristics of individuals who develop immediate and early PTS are more highly variable. In addition to risk factors for PTS during all timeframes discussed above, few risk factors are specific to immediate and/or early PTS. This is likely attributable to the fact that immediate and early are considered acute symptomatic seizures, direct responses to the head trauma. Therefore, research has not extensively examined risk factors for early PTS outside of injury severity. As

more research is conducted, novel risk factors specific to immediate and/or early seizures may be identified.

1.2.4.3 Late PTS

Penetrating TBI is often defined by dura penetration and may include the presence of bone and/or foreign fragments (e.g. shrapnel). In both civilian and military cohorts, pTBI is one of the most prominent risk factors for late PTS [46; 47; 66; 68; 72; 73; 78; 84; 85]. Salazar and Grafman reviewed pTBI in both military and civilian cohorts and documented approximately 34 to 63% of individuals with pTBI develop late PTS [86]. Similarly, the highest probability of developing PTE in a civilian population, 62.5% in two years post-injury, was associated with bone or metal fragments and a relative risk of 3.94 compared to those with no dura penetration[47]. Penetrating TBI is much more common in military cohorts compared to civilian cohorts [56; 72] and may explain the reported differences in rates of late PTS.

Among individuals with late PTS, Weiss and Caveness found no statistically significant differences in seizure frequency for those with penetrating compared to non-penetrating TBI [45]. Interestingly, a penetrating wound greater than 3 cm deep was highly associated with increased seizure frequency [45], suggesting deep brain penetration may initiate different pathophysiological pathways leading to increased seizure.

As CT technology became increasingly available and used as part of the standard of care to diagnose TBI, abnormal neuroradiological findings were identified as risk factors for late PTS. Englander and colleagues found cisternal compression and midline shift, often associated with presence of intracranial bleeding and elevated intracranial pressure (ICP), were significantly associated with late PTS in a cohort of individuals hospitalized for moderate to severe TBI [47]. In addition to intracerebral blood collection (i.e. SDH, ICH), multiple studies have documented

contusion as a significant risk factor for late PTS [12; 47; 64; 67; 78; 87; 88]. In a large population-based study, brain contusion and subdural hematoma remained significantly associated with late PTS after adjusting for additional risk factors including linear skull fracture, depressed skull fracture, and early seizure [rate ratios (95% CI); SDH: 6.3 (2.2-18.0), contusion: 5.0 (2.5-10.0)] [12]. Risk of late PTS has also been shown to vary based on specific location of contusion (e.g. temporal) [47; 70], likely related to certain brain regions/structures being more susceptible to seizure activity independent of TBI.

While few previous studies have specifically examined risk factors for early PTS, many studies document seizures occurring in the first week post-injury as a significant risk factor for late PTS. In univariate models, early seizures were shown to approximately double the probability of late PTS [47; 78]. Angeleri et al determined the relative risk of late PTS was approximately 8.6 (95%CI: 2.9-25.6) for individuals with at least one early seizure compared to those with no early seizure activity [70]. Early seizures were also found to significantly increase risk for late PTS in multivariable models [66; 69]. Contrary to these findings, early seizures were not determined to be significantly associated with late PTS in the Rochester Epidemiology Project cohort after controlling for other known risk factors [12]. Despite the majority of evidence indicating early seizures increase risk of late PTS, it remains unknown if immediate and early seizures have different effects on risk of lat PTS.

With advances in medicine and neuro-critical care, neurosurgical procedures have become common interventions following severe TBI. Neurosurgical procedures may include ventriculostomy, craniotomy, and craniectomy. Previous research indicates that neurosurgical interventions increase risk of late PTS [47; 78]. However, craniotomy and craniectomy are implicated as risk factors for seizure, even when used to address non-traumatic CNS pathologies

[89]. Thus, its possible post-operative seizures within a certain timeframe may be considered acute symptomatic and not late PTS (i.e. PTE). Further research is needed to more comprehensively describe temporal trends of seizure activity post-surgery.

In addition to injury related characteristics, recent studies have investigated the effect of pre-morbid personal and medical history on late PTS. In a population-based sample of individuals hospitalized for TBI, pre-morbid history of depression [adjusted risk ratio (95% CI): 1.85 (1.16-2.94)] was significantly associated with increased risk for late PTS in multivariable analysis [69]. In earlier studies, pre-morbid chronic alcoholism was also documented to be associated with development of late PTS [71; 80]. Although, seizures may also be caused by alcohol withdrawal and the duration of time between alcohol cessation and seizure development should be carefully inspected to ensure acute symptomatic seizures secondary to withdrawal[44] are not indicated as late PTS. Moreover, there is a bidirectional relationship between alcohol use/misuse and epilepsy [88; 90] and further research is needed to more thoroughly examine the effect of alcoholism on late PTS.

1.2.4.4 Genetic Variance

Few studies have examined genetic variance and possible associations with PTS. Of those that have, a candidate gene approach, as opposed to a genome wide approach, has been adopted and relatively few genes have been examined. Potential candidate genes have been identified from neurobiological pathways associated with secondary injury cascades as well as non-traumatic epileptogenic mechanisms. Candidate gene studies are preferred within TBI research due to the large sample sizes required to power genome wide association studies (GWAS). No current studies of TBI that collect biological samples are sufficiently large enough to support the use of a GWAS approach.

Apolipoprotein E (apoE) ε4 has been previously associated with poor outcome following TBI and is associated with other neurodegenerative diseases such as Alzheimer's Disease [91-93]. Therefore, apoE ε4 has been investigated as a potential risk factor for late PTS. Initial findings in a small cohort (n=106) of moderate to severe TBI indicate individuals with the ε4 allele are at an increased risk for late PTS (RR: 2.41, 95% CI: 1.15-5.07) compared to individuals without the ε4 allele [94]. Unfortunately, this result was not replicated in a second civilian cohort of moderate to severe TBI or in a military cohort [72; 95].

A single study has identified a significant association between a known functional variant in the methylenetetrahydrofolate reductase (MTHFR) gene, C677T, and late PTS. The MTHFR gene is essential for metabolism of methionine and has previously been found to be associated with neurodegenerative diseases and migraine [96-98]. In a recent case-control study of Service Members, odds of late PTS were significantly greater for individuals with the TT genotype (OR: 1.92, 95% CI: 1.01-3.64) compared to CC individuals [99]. The association was made stronger when the classification of late PTS was revised to include only those with two or more seizures, and remained significant in multivariable analysis (AOR: 2.55, 95% CI: 1.12-5.80) [99].

Interleukin 1-beta (IL-1β) is a pro-inflammatory cytokine produced by activated glia in the CNS. The inflammatory response associated with secondary injury cascades following TBI can increase IL-1β expression, and increased IL-1β levels can be observed chronically post-injury [100]. IL-1β may also contribute to excitotoxicity and epileptogenic mechanisms [101]. To date, one study has examined the effect of genetic variation within the gene encoding IL-1β on risk of PTS. Investigation revealed SNP rs1143634 was associated with differences in IL-1β cerebrospinal fluid (CSF)/serum ratios [102]. Additionally, heterozygous individuals had significantly greater risk of late PTS (hazard ratio: 2.85, 95% CI: 1.37-5.90) after adjusting for

injury severity, SDH, and depressed skull fracture; the relative effect of rs1143634 genotype was greatest of all variables in the model [102].

In addition to single candidate gene studies, previous research has examined multiple genes within pathways related to epileptogenic mechanisms. Various genes related to the adenosine regulatory cycle were examined in a single study of late PTS within a cohort of individuals with moderate to severe TBI. After investigating genes encoding adenosine kinase (ADK), ecto-5'-nucleotidase (NT5E), and equilibrative nucleoside transporter type-1 (ENT-1), Diamond and colleagues found rs11001109 (ADK) minor allele homozygous and rs9444348 (NT5E) heterozygous individuals were at increased risk of late PTS [102]. These findings remained significant after controlling for injury severity and SDH. An additional study in a similar sample examined the potential effect of adenosine A1 receptor (A1AR) genetic variation on PTS. SNP rs3766553 minor allele homozygous individuals were at greatest risk of early PTS [103]. Conversely, rs3766553 major allele homozygous individuals had significantly greater risk of late PTS. SNP rs10920573 heterozygosity was also associated with increased risk of late PTS [103].

Variation in glutamatergic and gamma-amino butyric acid (GABA) related pathways, important for maintenance of the excitatory/inhibitory balance, has also been investigated. Two studies specifically looked at genetic variation within glutamic acid decarboxylase (GAD) genes and possible associations with PTS. In a civilian cohort of moderate to severe TBI, tagging SNP rs3828275 (GAD1) was significantly associated with early PTS; two additional SNPs, tagging SNP rs769391 and functional SNP rs3791878, were associated with risk of PTS from one week to 6 months post-injury [104]. A second study of GAD genetic variation in a military cohort also identified an additional tagging SNP significantly associated with late PTS assessed at 15 years post-injury [72]. In the same cohort, SNP rs11074504 within GRIN, a gene encoding a glutamate

receptor subunit of the N-methyl-D-aspartate (NMDA) receptor, was significantly associated with late PTS [72]. However, no associations identified within the military cohort remained significant after correcting for multiple comparisons.

Although each of these initial candidate gene studies must be further explored to determine if results can be replicated in additional populations, these findings provide preliminary support for the role of genetic variation in the development of PTS. It is likely that, in the future, genetic information may facilitate a more personalized medicine approach to PTS risk assessment, prophylaxis, and treatment.

1.3 SUMMARY

Traumatic brain injury and post-traumatic seizures are a significant public health problem. Chronic complications of TBI incur tremendous costs in healthcare utilization, time from family and caregivers, and increased morbidity and mortality. PTS is a well-recognized complication of TBI that contributes greatly to the cost associated with chronic complications of TBI. As incidence of TBI increases and death rates decrease, more individuals will be living with the chronic complications of TBI, including PTS.

There has been a large effort within previous research to examine the epidemiology of PTS, particularly late PTS, to identify risk factors and propose interventions. Despite these efforts, variability in who will develop PTS remains high. Few studies have examined additional characteristics such as premorbid conditions, acute care complications, or genetic variation that may increase the risk of PTS. Additionally, while prophylaxis is effective for suppressing early

seizures, there remains no effective pharmacotherapy or targeted intervention to prevent late PTS.

Therefore, it is vitally important to continue examining PTS to determine if and how the epidemiology is changing in large, heterogeneous populations, and to identify additional risk factors that may help predict PTS and time of onset. Identifying genetic variation associated with PTS may help provide insight as to why some individuals develop PTS and others with similar injuries do not. Together, these additional data may improve PTS prognostication, expound upon epileptogenic mechanisms following TBI, and identify new targets for intervention.

2.0 SPECIFIC AIMS

2.1 CHARACTERIZATION OF PTS

Multiple studies examining the epidemiology of PTS have been conducted. However, there are limitations regarding generalizability of previous findings. Furthermore, immediate and early PTS are often excluded from large epidemiological studies that focus primarily on late PTS (i.e. PTE). In order to fully understand the potential public health significance of PTS, more detailed information on the epidemiology of all PTS classifications in a large, representative population is required.

2.1.1 Specific Aim 1

Characterize the frequency of post-traumatic seizure at various time points post-injury, within a cohort of individuals with moderate to severe TBI

We expect incidence and prevalence of PTS will be similar to previous studies using similar populations. Stratified analyses may confirm established risk factors for late PTS and provide insight into novel risk factors for immediate and early PTS.

2.2 IDENTIFICATION OF PREDICTORS AND RISK FACTORS FOR PTS

Although many potential risk factors for PTS have been identified, there remains a high degree of variability in who will develop PTS. Previous research has attempted to develop prognostic models to aid clinicians in determining an individual's PTS risk. These attempts were made decades ago, were never adopted for clinical use, and are not representative of current trends in TBI severity, diagnosis, or treatment. To more definitively assess the potential usefulness of prognostic models for PTS in research and clinical care, revised models must be developed.

Similarly, technology to assess potential risk factors related to personal biology is now more accessible in research and clinical practice. Novel genetic risk factors for PTS have recently been identified. Further research regarding the effect of genetic variation on PTS risk is essential to identify potential risk factors and neurobiological mechanisms that may represents points of intervention for PTS prophylaxis and treatment.

2.2.1 Specific Aim 2

Develop prognostic models to predict PTS during acute care hospitalization, at Year 1, and Year 2 following traumatic brain injury.

Hypothesis 2.2.1. A: Personal, medical, and injury characteristics will be identified as significant predictors of PTS

Hypothesis 2.2.1. B: Significant predictors of PTS will vary based on time PTS is assessed post-injury

Hypothesis 2.2.1. C: Prognostic models will be internally validated

2.2.2 Specific Aim 3

Examine the effect of genetic variation within neuronal glutamate transporter genes, SLC1A1 and SLC1A6, on epileptogenesis following severe traumatic brain injury.

Hypothesis 2.2.2. A: Genetic variation in the neuronal glutamate transporter genes, SLC1A1 and SLC1A6, will be significantly associated with epileptogenesis and PTS

Hypothesis 2.2.2. B: Different genetic variants will be associated with epileptogenesis during different subcomponents of the three-year post-injury timeframe

3.0 BACKGROUND

3.1 STUDY POPULATIONS

3.1.1 Traumatic Brain Injury Model Systems

The Traumatic Brain Injury Model Systems (TBIMS) study is an ongoing multi-center, prospective, observational cohort study. Established in 1987, there are 16 currently funded centers including the University of Pittsburgh and four previously funded centers that continue to collect follow-up information [105]. The main objective of the TBIMS program is to study recovery and outcomes after moderate to severe TBI. Individuals participating in the TBIMS study have the potential to be followed from inpatient rehabilitation for TBI throughout the duration of their lifespan. Currently, the longest follow-up time-point is 25 years post-injury. All data collected through the TBIMS program is deposited to the TBIMS National Data and Statistical Center, where it is formatted into the TBIMS National Database (NDB), the central resource for all TBIMS research.

TBIMS Centers are established and funded through center-specific grants, typically awarded for five-year periods [106]. To be eligible, a Center must provide a "multidisciplinary system of rehabilitation care specifically designed to meet the needs of individuals with TBI" [107]. To fulfill this requirement, a participating Center must include emergency medical services

(most commonly at least one Level-1 trauma center), acute care including neurosurgical capabilities, comprehensive inpatient rehabilitation, and long-term interdisciplinary follow-up and rehabilitation services. The number of acute care hospitals within a single TBIMS Center may vary. The TBIMS Center must be able to access emergency and acute care records, however, it is not required that acute medical and rehabilitation facilities be within the same hospital system. TBIMS Centers must include at least one Center specific study, participate in at least one multicenter study, and collect and submit longitudinal data to the TBIMS-NDB.

The TBIMS program specifies inclusion criteria for all individuals enrolled at any participating Center. Throughout its history, the TBIMS program has revised participant inclusion criteria. The current inclusion criteria are defined below. Firstly, an individual must meet the TBIMS case definition of TBI.

> Damage to brain tissue caused by an external mechanical force as evidenced by medically documented loss of consciousness or post-traumatic amnesia (PTA) due to brain trauma or by objective neurological findings that can be reasonably attributed to TBI on physical examination or mental status examination [108]

All participants must have a moderate to severe TBI defined by at least one of the following classifications: post-traumatic amnesia >24 hours, trauma related neuroimaging abnormality, loss of consciousness >30 minutes, or emergency department GCS<13 (not influenced by intubation, sedation, or intoxication). Additionally, all participants must be 16 years or older, present to a TBIMS Center affiliated emergency department within 72 hours of injury (previously 24 hours), receive acute and comprehensive inpatient rehabilitation within the Center's designated facilities, and provide written informed consent.

Data from emergency and acute care, referred to as Form 1, is collected retrospectively. Data from inpatient rehabilitation and at all follow-up interviews, referred to as Form 2, are collected prospectively. Follow-up interviews are currently conducted at 1, 2, 5, and every five years thereafter, post-injury. Over the course of the TBIMS program, follow-up intervals and variables collected have been revised. At the time of analysis, there were 285 variables collected via Form 1 and 243 variables collected at each follow-up using Form 2. Data in the TBIMS-NDB collected through the end of the first fiscal quarter of 2015 (October 2014) were used for the current analyses. This included 13,241 cases with Form 1 data and 41,733 follow-up interviews.

The TBIMS-NDB has previously been extensively studied to determine its generalizability to the United States population [105; 109; 110]. TBIMS investigators have recently compared the TBIMS study population to the Uniform Data System for Medical Rehabilitation (UDS) and eRehabData. The UDS and eRehabData were combined to form a national dataset consisting of individuals 16 years or older with a primary diagnosis of TBI who received inpatient rehabilitation services. Demographic, socioeconomic, and rehabilitation outcomes were then compared between the combined dataset and the TBIMS-NDB. These studies confirm the TBIMS-NDB is largely representative of individuals receiving inpatient rehabilitation services for TBI in the United States [105; 109; 110]. However, the TBIMS-NDB was determined to include a larger proportion of individuals under age 65 and a greater proportion of individuals employed prior to injury [110]. Additionally, individuals in the TBIMS-NDB had a significantly shorter length of stay in inpatient rehabilitation compared to the US TBI rehabilitation population [110]. To address these differences, methods have been developed to weight the TBIMS-NDB to represent the general US population of individuals receiving inpatient rehabilitation

Additional limitations of the TBIMS study include those inherent to other multi-site longitudinal studies such as loss to follow-up. Total loss to follow-up is approximately 24% with varying estimates at each time point. Individuals lost to follow-up may differ across time-points. Similarly, Centers may lose funding during certain cycles resulting in loss to follow-up of the individual Center's cohort during that funding cycle. Attrition due to loss of Center funding is approximately 4% overall and does not surpass 6% for any one follow-up time-point (Table 4).

Table 4. Sample Size and Attrition Rates of the TBIMS-NDB as of March 31, 2015

Time Point	Number Included	% Attrition	% Additional Attrition*
Form 1	13,667	NA	NA
Form 2	45,499	19	4
Year 1	12,973	15	3
Year 2	11,518	16	5
Year 5	8,952	18	5
Year 10	4,684	19	6
Year 15	1,588	14	6
Year 20	449	14	0

**additional attrition due to loss of Center funding*

3.1.2 University of Pittsburgh Local Project

Multiple smaller studies of moderate to severe TBI have been, or are currently being conducted at the University of Pittsburgh. Under the oversight of Dr. Amy Wagner, an overarching study protocol has been developed to collect biological samples in order to analyze various potential biomarkers and their associations with TBI outcomes. The study objectives include investigating the association between genetic variation and development of PTS after moderate to severe TBI.

For the current analyses, individuals 18 years of age or older with a severe TBI (determined by a GCS score ≤8 on admission to the UPMC Level 1 trauma center) were included. Individuals were excluded if they had less than three years of follow-up from the time

of their index TBI. Information on outcomes of interest, including seizure activity, was extracted from the UPMC electronic medical record using a standardized protocol.

3.2 PROGNOSTIC MODELING

Prognostic models are statistical tools that estimate an individual's risk for developing an outcome of interest based on specific characteristics [111]. Prognostication is common in medical research and practice; prognostic models have previously been developed for use in multiple fields such as oncology, cardiology, and neonatology. Within the field of research surrounding TBI, prognostic models have been investigated to predict multiple outcomes such as survival, disability, and global outcome [112-115]. Importantly, especially because the etiology of secondary injury mechanisms in TBI and epileptogenesis are extremely heterogeneous, the aim of prognostic modeling is not to explain causality of the outcome [111]. Prognostic models may include predictors that are not themselves causal, but may be measuring latent variables that have not, or cannot, be measured.

The development of reliable, validated prognostic models is essential for models to be clinically useful. The gold standard for validating a prognostic model is through the use of a second, independent study cohort (i.e. external validation). However, this may be prohibitive in some specialties and recent advances in statistical methods for validation have been made.

Resampling methods are reported to be extremely proficient as a means of internally validating a statistical model [116; 117]. As computing capabilities increase, resampling methods such as bootstrapping, are more readily available and widely used. Bootstrapping is a procedure that involves selecting a sample, with replacement, from an original dataset. Researchers can

indicate the number of samples selected and specify parameters of interest (e.g. sample must always include a designated ratio of males to females). A prognostic model of interest can be identified *a priori* and tested for fit using each bootstrapped sample [118]. Information from bootstrapped samples is aggregated to determine overall fit statistics such as discrimination and calibration of the pre-specified model [116; 117; 119].

Currently, automated programs are available using statistical software, such as R, to streamline these processes [118; 120]. In addition, computer modeling can be used to develop reduced models (i.e. model development using stepwise elimination) while decreasing subjectivity and the potential for investigator bias. Each bootstrap sample may therefore indentify a different set of prognostic variables. These data are simultaneously aggregated to determine the best-fit model allowing for user specification (i.e. AIC or alpha thresholds for variable inclusion/exclusion) [118]. After automated development, the model can be tested for fit using the original sample set and the bootstrapped samples, allowing for computation of fit statistics as above [116; 119].

4.0 MANUSCRIPT ONE

INCIDENCE AND RISK FACTORS OF POST-TRAUMATIC SEIZURES FOLLOWING TRAUMATIC BRAIN INJURY: A TRAUMATIC BRAIN INJURY MODEL SYSTEM STUDY

Anne C Ritter[1, 2], Amy K Wagner[2,3,4,5], William C Walker[6], Anthony Fabio[1], Jerzy P Szaflarski[7], Mary Jo Pugh[8,9], Allen W Brown[10], Ross D Zafonte[11], Flora M Hammond[12,13], Tamara Bushnik[14], Doug Johnson-Greene[15], Timothy Shea[16], Jason W Krellman[17], Joseph A Rosenthal[18], Laura E Dreer[7]

[1]Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA
[2]Physical Medicine & Rehabilitation, University of Pittsburgh, Pittsburgh, PA
[3]Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA
[4]Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA
[5]Center for Neuroscience at University of Pittsburgh, Pittsburgh, PA
[6]Dept of Physical Medicine & Rehabilitation, Virginia Commonwealth University
[7]University of Alabama at Birmingham Epilepsy Center, Department of Neurology, University of Alabama, Birmingham, AL
[8]South Texas Veterans Health Care System Polytrauma Rehabilitation Center, San Antionio, TX
[9]Department of Epidemiology and Biostatistics, University of TX Health Science Center San Antionio, San Antonio, TX
[10]Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN
[11]Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, MA
[12]Carolinas Rehabilitation, Charlotte, NC
[13]Indiana University School of Medicine, Indianapolis, IN
[14]Rusk Rehabilitation, New York University School of Medicine, New York, NY
[15]Miller School of Medicine, University of Miami, Miami, FL
[16]Department of Physical Medicine and Rehabilitation, Ohio State University, Columbus, OH
[17]Department of Rehabilitation Medicine, Icahn School of Medicine at Mount Sinai, New York, NY
[18]Departments of Physical Medicine and Rehabilitation and Ophthalmology

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4.1 ABSTRACT

Objective: Determine the incidence of post-traumatic seizures (PTS) following traumatic brain injury (TBI) among individuals with moderate-to-severe TBI requiring rehabilitation and surviving at least 5 years.

Methods: Using the prospective TBI Model Systems National Database, we calculated the incidence of PTS during acute hospitalization, and at Years-1, 2, and 5 post-injury in a continuously followed cohort enrolled between 1989 and 2000 (n=795). Incidence rates were stratified by risk factors of interest, and relative risk (RR) was calculated. The RR of late PTS following immediate (<24hr), early (24hr-7d), or late seizures (>7d) versus no seizure activity prior to discharge from acute hospitalization was also examined.

Results: PTS incidence during acute hospitalization was highest immediately (<24hrs) after injury (8.9%). New onset PTS incidence was greatest between discharge from inpatient rehabilitation and Year-1 (9.2%). Late PTS prevalence from injury to Year-1 was 11.9% and reached 20.5% by Year-5. The RR of late PTS was significantly greater for individuals self-identifying as a race other than black or white at Year-1 (RR=1.96), and for black individuals at Year-5 (RR=2.86) versus white individuals. Late PTS was greater for individuals with certain intracranial pathologies (i.e. subarachnoid hemorrhage). Penetrating TBI had even higher RR but did not reach significance, likely due to small group size. Individuals with immediate and late seizures during acute hospitalization were at a significantly greater late PTS risk (RR: 2.61 and 3.36, respectively).

Significance: In this prospective, longitudinal, observational study, incidence rates were similar to those in previously published studies. Individuals with immediate and late seizures during acute hospitalization are at an increased late PTS risk after hospitalization. Race and intracranial

pathologies also influenced RR for late PTS. Further studies are needed to examine the impact of seizure prophylaxis in high-risk subgroups and to delineate possible contributors to race associations on long-term seizure outcomes.

4.2 INTRODUCTION

Traumatic brain injury (TBI) is a prevalent public health problem with an annual incidence over 2.5 million in the United States (US), of which approximately 12% result in hospitalization or death [3]. TBI also has many related secondary chronic conditions [121] including shorter life-expectancy after severe TBI versus demographically similar, non-TBI populations [2]. Recent work using the TBI Model Systems (TBIMS) National Database (NDB) found seizure to be an important contributor to premature death among individuals who were hospitalized and received inpatient rehabilitation for TBI, who had a 50-fold risk for subsequent seizure related death compared to an uninjured similarly matched sample [2].

Post-traumatic seizures (PTS) can occur any time post-TBI. Classification is based on the time of seizure post-injury: immediate (<24hrs), early (24hrs-7d), or late (>7d post-TBI) [41]. These cut-offs reflect proposed differences in causal mechanisms and subsequent seizure risk [42; 66]. Seizures occurring within the first week following TBI, also termed acute symptomatic seizures [43], are considered transient, decreasing seizure threshold only temporarily [42]. Late PTS, often used interchangeably with post-traumatic epilepsy (PTE), is characterized by persistent neurobiological changes, attributed to secondary injury biochemical cascades and epileptogenic mechanisms that eventually present as clinical seizures [18; 31]. Individuals with acute symptomatic seizures who have a subsequent late seizure are considered to have late PTS or PTE. The clinical definition of epilepsy, revised in 2014 by the International League Against Epilepsy (ILAE), includes conditions where an individual has a single unprovoked seizure and their risk of a recurrent seizure is similar to, or greater than, the risk of seizure recurrence after two unprovoked

seizures occurring ≥24hrs apart (≥60%) [42]. The recurrent seizure risk following a single, unprovoked seizure >7d post-TBI is high enough to consider late PTS as an epileptic condition [42; 56]. For the current study, late PTS is used, but is equivalent to the current definition of PTE.

Reported PTS incidence varies widely and depends on study design and population characteristics. Few large epidemiological PTS investigations have been conducted in heterogeneous populations. The seminal population-based study in the US examined late PTS in a predominantly white population from 1935 to 1984 [12]. Among individuals with severe TBI, the cumulative probability of late PTS was 10.0% five years after TBI; early PTS occurred in 2.6% of individuals [12]. This study included all ranges of TBI severity, was racially homogenous, and included both adults and children. Inclusion of adults and children may confound risk relationships and complicate accurate risk factor determination since neurological injury/recovery mechanisms post-TBI may vary over the course of neural development or aging. These authors also reported 10.3% of adults with severe TBI developed early PTS [63]. Other, smaller studies report early PTS incidence to range from 2.4 to 8.4% [66; 122]. However, these studies included children and adults with a range of TBI severities. Late PTS cumulative probability rates have been more often reported and findings vary widely [12; 47]; with prevalence ranging from 4%-19% [12; 66; 67; 70; 123]. While these studies provide important information regarding PTS frequency after TBI, many are retrospective, not racially diverse, from single medical centers, and cannot be generalized to large heterogeneous populations. Additionally, they provide little information on immediate and/or early PTS. To address these limitations, the purpose of the current study was to prospectively determine the incidence of PTS following TBI among individuals with moderate-to-severe TBI requiring rehabilitation and surviving at least 5 years using a large-scale, multi-center database.

We used the TBIMS-NDB to calculate multiple PTS frequency measures in a cohort followed out to five years post-injury. Additionally, we stratified PTS incidence at various time-points by demographic and injury characteristics of interest to compute relative risk (RR) for each factor.

4.3 METHODS

4.3.1 Study Design and Population

Data were obtained from the prospective TBIMS-NDB. The TBIMS-NDB is a multi-center, prospective, observational study to investigate recovery and outcomes following acute neurotrauma and inpatient rehabilitation in a heterogeneous population of individuals with moderate-to-severe TBI, across the US. All participating sites have an affiliated trauma center with acute neurosurgical capabilities and associated comprehensive inpatient rehabilitation. Eligibility criteria are: moderate-to-severe TBI (PTA>24hrs, or LOC>30minutes, or emergency department GCS<13, or positive neuroimaging findings), age ≥16yrs, admitted to a participating hospital emergency department within 24hrs of injury, and received both acute care and inpatient rehabilitation within a TBIMS designated hospital system. All enrolled individuals, or legal proxy, provided written informed consent; Institutional Review Board approval exists at all sites.

An additional inclusion criterion for this study was completion of Year-5 post-injury follow-up interview. Further, individuals were then excluded if data regarding seizure activity during acute care hospitalization, or Year-1 *and* Year-2 post-injury, were not available. The acute hospitalization seizure variable was dropped from TBIMS data collection procedures, and

follow-up seizure definitions changed, in 2003 and 2005, respectively. Therefore, all individuals included in analyses were enrolled between 1989 and 2000; follow-up assessments were completed by 2006.

4.3.2 Data Collection

Data were collected at enrollment, Year-1, Year-2, and Year-5 post-injury. Enrollment data included demographic, social, and injury characteristics as well as personal and medical history (pre-injury), and acute outcomes. A proxy interview was completed, for both enrollment and follow-up when participants with TBI were unable to answer questions accurately. Throughout the study duration, data collection protocols changed over time. Therefore, missing data may exist even for individuals who completed assessments at all study time-points.

Outcome Variable

The main outcome variable was PTS status, determined during the course of acute care hospitalization and at each follow-up time-point (Year-1, Year-2, and Year-5).

4.3.3 PTS During Acute Care Hospitalization

The presence/absence of a physician-confirmed clinical seizure during acute hospitalization was identified via medical record review using a standardized form and classified based on time from injury (immediate: <24hrs, early: 1-7days, late: >7days). Only time of first seizure was recorded. Multiple seizures were not captured, therefore, an individual seizing immediately or early after injury may have also seized in a subsequent time category prior to acute discharge.

4.3.4 PTS at Follow-up Interviews

At each follow-up, individuals were asked "*Have you been told by a physician that you have had a seizure since your last follow-up*?". Yes/No answers were recorded; individuals self-reporting seizure activity were documented as having PTS at the specified time-point.

4.3.5 PTS Risk Factors

Risk factors of interest included demographic and injury characteristics. Demographic variables included age, sex, and race. Injury characteristics included admission Glasgow Coma Scale (GCS) score, pathology on computed tomography (CT) scan obtained within 7 days of injury, and penetrating TBI (pTBI; **Supplemental Table 1**). Injury severity was also classified using alternate criteria for moderate-to-severe injury based on duration of post-traumatic amnesia (PTA), loss of consciousness (LOC), and positive neuroimaging findings[124]. CT findings were included as separate variables for specific pathology type [e.g., subdural hematoma (SDH), subarachnoid hemorrhage (SAH)] coded as present/absent, and were not mutually exclusive. pTBI was computed via a coding algorithm previously validated in a subsample of the TBIMS[85]. A contusion load score was calculated by summing the number of regions with contusion on CT reports, then collapsing the sum into 0, 1, 2, 3, and 4 or more regions. Seizures during acute hospitalization were examined as PTS risk factors at follow-up (**Supplemental Table 1**). No data were collected on premorbid seizure activity or history of epilepsy. ICD-9 codes indicating neurosurgical procedures or complications were not collected, nor were medication data.

4.3.6 Statistical Analysis

All statistical analyses were completed using SAS version 9.4 (SAS Institute, Carry NC) and R version 3.0.2 [125]. Seizure incidence was calculated during acute care hospitalization and at follow-up. At Year-1 and Year-2, if data on seizure activity since last follow-up were missing (n=101 and n=99, respectively), seizure status was considered not present at that time-point. If individuals had no prior seizure activity, they were considered "at-risk" for PTS and were included in the denominator of incidence calculations. If individuals had evidence of PTS at a previous time-point, they were not considered "at-risk" for incident PTS. Sensitivity analyses, including Chi-Square and Mann-Whitney, or Fisher's Exact test and Kruskal-Wallis, were conducted to determine whether individuals with missing data at Year-1 or Year-2 differed from those without missing data. Late PTS incidence since last follow-up was calculated at Years-1, 2, and 5. Late PTS prevalence from time-of-injury was also calculated at each follow-up time-point (i.e. >7d to Year-1, >7d to Year-2, etc.). Additionally, the percent of individuals reporting seizure activity at multiple time-points was calculated, stratified by time of onset.

Following primary incidence calculations, immediate/early PTS (injury to 7 days), late PTS from injury to Year-1, and late PTS from Year-1 to Year-5 incidence rates were stratified by risk factors of interest. For demographic variables identified as having significantly different RR of PTS, sensitivity analyses were conducted to determine if there were differences in injury characteristics.

Seizures during acute care (immediate, early, late) were then examined specifically as risk factors. Prior to this analysis, late PTS was recalculated to remove late seizures during acute care (>7days to discharge) from the case definition in order to evaluate RR for late PTS at Year-1 and Year-5 follow-up.

4.4 RESULTS

4.4.1 Population

Data were available for 2,418 individuals injured and enrolled prior to 2001. Of these, 796 were assessed at 5-years post-injury and met inclusion criteria for this analysis (**Figure 1**). Individuals were predominantly male (74.8%), self-identified as white (58.3%), and tended to be in their mid-thirties at time of injury. The predominant cause of injury was motor vehicle collision (51.6%) (**Table 1**). Individuals lost to follow-up at Year-1 or Year-2 who were recaptured at Year-5 tended to have less severe injuries and fewer high-risk pathologies (data not shown).

4.4.2 Frequency Measures

By the Year-5 follow-up, 219 (27.5%) individuals had reported or documented seizure activity at some point post-injury and 163 (20.5%) developed late PTS (**Table 2**). During acute care hospitalization (mean=22.8 days), 98 (12.3%) individuals seized, with highest acute hospitalization PTS incidence immediately (<24hrs) after TBI (**Table 2**). 1.8% of individuals developed incident PTS after 7d post-injury but prior to acute discharge. Incidence of new onset and late PTS since last follow-up peaked at Year-1 (**Table 2**). Of all individuals developing late PTS by Year-5, 58.3% did so by Year-1, and 82.2% did so by Year-2. Further, at Years-1, 2, and 5, more than 50% of individuals reporting seizure activity endorsed multiple seizures since last queried.

Of individuals first seizing prior to Year-5, 38.0% reported seizure activity at one time interval only. Among individuals with incident PTS at Year-1 or Year-2, 64% and 40.1% respectively, reported interval seizure activity at subsequent follow-up(s) (**Supplemental Table 2**).

4.4.3 Stratified Incidence and Relative Risks

Stratified analyses showed PTS incidence was nominally lower among women than men at each time-point, but RR did not reach significance. Individuals self-identifying as white also tended to have lower RR of PTS versus individuals who did not identify as white with significant differences evident at Year-1 and Year-5. Although individuals age 35-44 at injury had greater risk of PTS at Year-5, no consistent pattern was seen (**Table 3a**). Sensitivity analyses showed differences in pTBI frequencies across race [2.4% white, 7.7% black, 6.3% other with pTBI (p=0.01)].

Immediate/early PTS incidence stratification by pathology showed no patterns. When late PTS incidence was stratified by pathology, RR was nominally greater for individuals with each pathology examined versus those without the given pathology, except for intra-ventricular hemorrhage at Year-1. Highest nominal incidence was observed for individuals with pTBI at both Year-1 and Year-5. Despite these patterns, only the RR for SAH pathology (**Table 3b**) and contusion load at Year-1 reached significance (**Table 3c**).

PTS incidence was stratified by two measures of injury severity: GCS, and moderate versus severe injury as determined by duration of LOC, PTA, and neuroimaging findings. Although RR was not significantly greater for individuals with more severe TBI as classified by these measures (**Table 3c**), late PTS incidence at Year-5 was nominally greater for individuals

with more severe. When late PTS incidence at Year-1 and Year-5 were stratified by time of seizure during acute hospitalization, significant RR's were found (**Table 4**). Individuals with immediate/late seizures occurring during acute care were more than twice as likely (RR: 2.61 and 3.36, respectively) to develop late PTS between acute discharge and Year-1. Those with immediate seizures also had higher risk (RR=2.06) at Year-5.

4.5 DISCUSSION

We examined multiple PTS frequency measures and associations with demographic and injury characteristics during and following acute care hospitalization and inpatient rehabilitation for moderate-to-severe TBI from a prospectively followed, large, nation-wide sample. Total PTS incidence during acute hospitalization was slightly higher than previously reported, but incidence of early seizure was consistent with previous reports [12; 48; 50; 65; 80; 122; 126]. However, not all prior PTS studies during acute hospitalization clarify if immediate and early seizures are considered simultaneously. Few previous reports specifically delineate immediate PTS incidence. Annegers and colleagues determined 2.1% of a Rochester county Minnesota study cohort, including children and all injury severities injured from 1935-1974, developed early PTS [63]. Of these, 75.9% first seized during the initial 24hrs post-TBI, similar to our finding that 72.4% of individuals who developed PTS during acute hospitalization first seized within 24hrs of injury. These rates are higher than those reported earlier by Jennett, where only 5% of the cohort exhibited early seizures with 60% occurring within 24hrs [50]. Disparate study findings might be due to differences in participants (i.e., injury severity/age) or methods for capturing

immediate/early PTS. When considering only adults with severe TBI in Annegers' study, 10.3% developed early seizures[63], similar to 10.7% in our study.

Interval new onset seizure (9.2%) and late PTS (10.8%) incidence were highest at Year-1, similar to previously published findings in comparable study samples [66]. Annual late PTS incidence since last follow-up at Year-1 and Year-2 (10.8 and 5.5%, respectively) were higher than annual incidence rates previously reported by Ferguson et al [69]. In our study, late PTS prevalence from injury to Year-1 (11.9%) and Year-5 (20.5%) were ≥2 times higher than for those with severe TBI in Annegers' study [12]. In two studies by Englander and colleagues, using a sample very similar to ours, late PTS incidence at 2 years was 16.4 and 14% [47; 127], similar to the 16.8% in our study. Differences between the percent affected in our analyses may be attributed to the inclusion of individuals with mild TBI or children. Possible differences between our analysis and Annegers' work could also be attributed to differences in study design (e.g. population-based study with medical chart review vs. prospective longitudinal cohort study and self-report). Additionally, due to changes in clinical care over time, differences could also be attributed to increased EEG monitoring of individuals post-TBI, and/or higher survival of the more severely injured in the current study. Future research validating these findings, using a more recent cohort from TBIMS-NDB could be considered with reintroduction of seizure data collection. However, due to changes in TBIMS seizure data collection occurring after 2000, we currently cannot validate the distinct effects of acute symptomatic seizures or late seizures during acute hospitalization in a more recent, independent TBIMS population.

Consistent with other studies, 82.2% of individuals developing late PTS did so by Year-2 post-injury [56]. Yet, it is important to recognize new onset seizures, and subsequently new cases of late PTS, continued to be detected at Year-5. Previous PTE studies show new cases may

develop as many as 30 years post-injury, and epilepsy risk remains significantly elevated versus the general population up to 10 years post-injury among those with severe TBI [12]. Our data provide a more complete picture of the extended risk of late PTS, past the first 1-3 years post-TBI, when other modern observational cohort studies truncate their follow-up [47; 67; 69]. Future work should continue to use the TBIMS-NDB to extend the current follow-up time (earliest participants now at Year-25 post-injury) and determine if current standardized incidence ratios remain similar to or are greater than those found by Annegers.

Late PTS prevalence reached 20.5% by Year-5. This figure is greater than reported rates of 13.7% by 5 years post-injury [66] but remains lower than rates in combat veteran populations [72]. Although both studies' cohorts were recruited from rehabilitation facilities, differences in study design and population (e.g., higher pTBI among military populations) likely contributed to differences.

When PTS incidence was stratified by risk variables, RR patterns were observed for race, but not sex. The lack of significantly greater RR for men could be confounded by survival bias, if men were more severely injured and did not survive to Year-5. However, in a more recently injured TBIMS-NDB cohort, men were at higher risk for seizures during acute care hospitalization, even in multivariable models including markers of injury severity (**Manuscript Two**). Individuals self-identifying as white had a lower late PTS risk at both Year-1 and Year-5 versus non-white individuals. No other known large epidemiological PTS studies have reported significant differences in risk associated with race. One study demonstrated increased RR of late PTS at 2 years for non-white groups versus white individuals, but results were not statistically significant [47]. Differences in PTS frequency by race may be partially explained by differences in injury type (i.e. pTBI). Although, previous work developing prognostic models of PTS using a

more recent cohort from the TBIMS-NDB did not find race to be even nominally associated with PTS and subsequently did not investigate race as a PTS predictor in multivariable modeling (**Manuscript Two**). In addition to more frequent pTBI, differences in late PTS risk by race may reflect differences in environmental exposure (e.g. repeat TBI, substance use) [128] as well as known racial differences in allelic frequencies for genes thought to influence PTS risk [94; 102; 103; 129]. Future work might investigate racial/ethnic differences in epilepsy related outcomes and comorbid burden [130].

Examining incidence stratified by pathology revealed somewhat surprising result. While patterns of nominal rates were as expected, late PTS risk was only significantly elevated for SAH and higher contusional loads at Year-1. These data are inconsistent with most studies, which find that SDH is a significant PTS risk factor. Our findings may be due to variability within SDH and SAH definitions applied in the TBIMS-NDB over time. Due to other limitations in data capture, we cannot examine incidence stratified by other potential risk factors of interest like neurosurgical procedures.

Of special note, at Years-1 and 5, pTBI was associated with the highest nominal late PTS RR of any characteristic studied (18.5 and 18.2%, respectively). Differences in RR were not statistically significant, but the low numbers with pTBI suggests these analyses were underpowered. Additionally, we did not observe consistent increases in PTS incidence with greater injury severity based on GCS or alternative criteria.

When late PTS incidence at Year-1 and Year-5 were recalculated without individuals seizing late during acute hospitalization included in the case definition, there were significant differences in RR based on timing of first acute seizure. Individuals with immediate seizure (<24hrs) had a higher late PTS incidence and greater risk at both time-points versus those not

seizing during acute hospitalization. Those with late seizures (>7d) during acute hospitalization also had a higher late PTS incidence at Year-1 versus individuals not seizing acutely (**Table 4**). Surprisingly, having acute seizure(s) restricted between 1-7 days did not increase late PTS risk. Multiple reports document that early seizure increases late PTS risk [12; 47; 66; 69; 70]. However, these studies do not differentiate between immediate and early seizures. Previous work by Temkin demonstrated increased early seizure risk associated with immediate seizures, but early seizures (1-7 days) were associated with increased late PTS risk [78]. Unlike Temkin's work, we did not observe increased late PTS risk associations with early seizures. It is unclear in Temkin's work whether individuals with early seizures seized immediately or not, and how selecting individuals at high risk of PTS for inclusion impacted late PTS findings. In our analyses, immediate/early seizures were mutually exclusive; therefore, we could not examine the impact of seizing both immediately *and* early on late PTS risk.

The high percentage of individuals in our study seizing immediately, along with the common use of early PTS prophylaxis after phenytoin was shown in clinical trials to reduce early seizures [131], may complicate observed relationships between immediate/early seizures and late PTS reported in our study. Even if seizure prophylaxis was administered immediately upon admission, it is likely that individuals could have already experienced their first immediate seizure [50]. Our data demonstrate lower incidence of early versus immediate seizure and show immediate seizure (but not early seizure) significantly increases late PTS risk out to Year-5. However, seizure prophylaxis during the first week post-injury is shown to only have beneficial effects in reducing early PTS, not late PTS [131]. While not directly measured in the TBIMS-NDB, data from a single TBIMS center from 2000-2007 show 96% received some form of AED prophylaxis during their acute care [102]. Taken together, we hypothesize that early PTS

prophylaxis reduces early seizures but does not reduce the epileptogenic processes initiated by immediate seizures that may (often) occur prior to prophylaxis administration. Therefore, we speculate that individuals in our study who may have seized prior to appropriate prophylaxis use may remain at-risk for late PTS. Future work needs to examine late PTS risk specifically among individuals receiving prophylaxis but who seized prior to or during AED loading, as these data may reshape PTS prophylaxis guidelines. We suggest that antecedent, immediate/early seizure activity can over-activate excitatory amino acid (EAA) receptors [18], perpetuating TBI induced excitotoxicity and contributing to epileptogenesis, regardless of early PTS prophylaxis [6].

While these data provide PTS characterization in a large cohort of individuals from the US, limitations must be considered. Our cohort is restricted to individuals having received inpatient rehabilitation and surviving to Year-5 post-TBI. Additionally, PTS and/or PTE definitions and data collection methods used can bias descriptive analyses. We cannot specify the data source used as evidence of seizure during acute hospitalization other than to cite "documentation from the medical record". It is unclear if evidence was collected using electroencephelogram (EEG). Currently, continuous EEG (cEEG) is common in many trauma centers, and its use identifying subclinical seizures may result in higher incidence of acute symptomatic seizures. How cEEG might have factored into acute symptomatic seizure identification for individuals injured prior to 2001 is less certain. In critically ill patients with TBI, up to 50% of seizures are reported to be subclinical [76]. Additionally, only the first seizure during acute care hospitalization was documented. Therefore, individuals seizing immediately or early, and having later seizures during acute hospitalization, were not accounted for in late PTS calculations if they did not experience a seizure after discharge from acute hospitalization.

Similarly, subsequent seizures during acute care hospitalization if occurring, were not documented.

Another potential limitation is PTS misclassification at follow-up because, while acute symptomatic seizure information was obtained via medical record, all follow-up data were based on participant or proxy self-report of whether a physician told them they had a seizure. Individuals could over-report if they experienced psychogenic non-epileptic seizures or transient neurological symptoms not due to seizure activity. Conversely, individuals experiencing true epileptic seizure activity between follow-ups, who were not aware or did not seek care, may contribute to underestimation of the true number of individuals with late PTS. Nevertheless, in large epidemiological studies of seizure and epilepsy, self-report remains common and necessary [132]. Adding to possible misclassification bias, individuals with missing data at Year-1 or Year-2 were included as at-risk individuals for incidence calculations. By doing so, we may have included individuals as "at-risk" who had seized, underestimating the true incidence. However, we examined individuals with missing data and determined they typically had less severe injury and less intracranial pathology identified via CT than those returning for all follow-ups (data not shown). Within the larger TBIMS-NDB, individuals with less severe TBI who presumably recover better, are more often lost to follow-up than individuals with more complex injuries and presumably worse outcomes [133]. Therefore, excluding individuals with missing data at Year-1 or Year-2, who were subsequently followed at Year-5, would have likely produced a greater degree of bias and overestimated incidence of PTS. Due to limitations in data collection, we were unable to identify, and subsequently exclude, individuals with pre-existing epilepsy disorders. Despite these limitations, the current analyses suggest race as a possible PTS risk factor. The

work also provides insight into temporal PTS risk factors, including immediate/early seizure, and examines the effect of each on late PTS incidence out to 5 years post-injury.

4.6 TABLES

Table 5. Demographic and Injury Characteristics at Baseline Visit N(%)

Sample Size = 796

Age at Injury*		35.4 (15.7)
Sex	Male	595 (74.8)
	Female	201 (25.2)
Race	White	464 (58.3)
	Black	246 (30.9)
	Other	86 (10.8)
Cause of Injury	MVA	411 (51.6)
	Fall	109 (13.7)
	Any Violence	164 (20.6)
	Any Sport	9 (1.1)
	Other	103 (12.9)
Injury Severity	Moderate	79 (9.9)
	Severe	717 (90.1)
PTA (days)*		31.6 (26.3)
LOC (days)*		10.7 (18.9)
Admission DRS*		13.3 (5.4)
Length of Acute Stay (days)*		22.8 (19.4)

**mean(SD); PTA – Post-Traumatic Amnesia; LOC – Loss of Consciousness; DRS – Disability Rating Scale*

Table 6. Frequency Measures of PTS at Follow-up Time Points after TBI

		Incidence of New Onset Seizure	Incidence of Late PTS since last follow-up*	Prevalence of Late PTS since injury
Time Point		N (%)	N (%)	N (%)
Initial Population		796	796	796
Acute Seizure Status	Immediate (<24hrs)	71 (8.9)	---	---
	Early (1 < 7 days)	14 (1.9)	---	---
	Late (>7 days)	13 (1.8)	---	---
Year 1		64 (9.2)	86 (10.8)	95 (11.9)
Year 2		32 (5.0)	39 (5.5)	134 (16.8)
Year 5		25 (4.2)	29 (4.3)	163 (20.5)

**Year-1 represents late PTS incidence since discharge from rehab*

Table 7. Incidence and Relative Risk of PTS Stratified by Variables of Interest

		Immediate and Early PTS			Late PTS Year 1			Late PTS Year 5		
		N(%)		RR (CI)	N(%)		RR (CI)	N(%)		RR (CI)
		No Seizure	Seizure		No Seizure	Seizure		No Seizure	Seizure	
N (%)		711 (89.3)	85 (10.7)		701 (88.1)	95 (11.9)		633 (90.3)	68 (9.7)	
Sex	Male	531 (89.6)	64 (10.8)	1.0	519 (87.2)	76 (12.8)	1.0	466 (89.8)	53 (10.2)	1.0
	Female	180 (89.2)	21 (10.4)	0.97 (0.61-1.55)	182 (90.6)	19 (9.4)	0.74 (0.46-1.19)	167 (91.8)	15 (8.2)	0.81 (0.47-1.40)
Race	White	416 (89.7)	48 (10.3)	1.0	420 (90.5)	44 (9.5)	1.0	395 (94.1)	25 (5.9)	1.0
	Black	218 (88.6)	28 (11.4)	1.10 (0.71-1.71)	211 (85.8)	35 (14.2)	1.50 (0.99-2.27)	175 (82.9)	36 (17.1)	**2.86 (1.77-4.64)**
	Other	77 (89.5)	9 (10.5)	1.0 (0.52-1.98)	70 (81.4)	16 (18.6)	**1.96 (1.16-3.31)**	63 (90.0)	7 (10.0)	1.68 (0.76-3.74)
Age	15-24	224 (88.5)	29 (11.5)	1.0	219 (86.6)	34 (13.4)	1.0	204 (93.2)	15 (6.8)	1.0
	25-34	165 (90.7)	17 (9.3)	0.81 (0.46-1.44)	162 (89.0)	20 (11.0)	0.82 (0.49-1.37)	145 (89.5)	17 (10.5)	1.53 (0.79-2.98)
	35-44	150 (89.3)	18 (10.7)	0.93 (0.54-1.63)	146 (86.9)	22 (13.1)	0.97 (0.59-1.61)	122 (83.6)	24 (16.4)	**2.40 (1.30-4.42)**
	45-54	87 (91.6)	8 (8.4)	0.73 (0.35-1.55)	83 (87.4)	12 (12.6)	0.94 (0.51-1.74)	75 (90.4)	8 (9.6)	1.40 (0.62-3.20)
	55-64	37 (80.4)	9 (19.6)	1.71 (0.87-3.36)	40 (87.0)	6 (13.0)	0.97 (0.43-2.18)	37 (82.5)	3 (7.5)	1.10 (0.33-3.61)
	65+	48 (92.3)	4 (7.7)	0.67 (0.25-1.83)	51 (98.1)	1 (1.9)	0.14 (0.02-1.02)	50 (98.0)	1 (2.0)	0.29 (0.04-2.12)

Table 8. Incidence and Relative Risk of PTS Stratified by Variables of Interest

		Immediate/Early PTS			Late PTS Year 1			Late PTS Year 5		
		N(%)		RR (CI)	N(%)		RR (CI)	N(%)		RR (CI)
		No Seizure	Seizure		No Seizure	Seizure		No Seizure	Seizure	
SDH	No	350 (88.4)	46 (11.6)	1.0	357 (90.2)	39 (9.8)	1.0	332 (93.0)	25 (7.0)	1.0
	Yes	224 (92.2)	19 (7.8)	0.67 (0.40-1.12)	209 (86.0)	34 (14.0)	1.42 (0.92-2.19)	188 (90.0)	21 (10.0)	1.43 (0.82-2.50)
SAH	No	323 (89.0)	40 (11.0)	1.0	333 (91.7)	30 (8.3)	1.0	306 (91.9)	27 (8.1)	1.0
	Yes	251 (90.9)	25 (9.1)	0.82 (0.51-1.32)	233 (84.4)	43 (15.6)	**1.89 (1.22-2.92)**	214 (91.9)	19 (8.2)	1.00 (0.57-1.77)
IVH	No	452 (89.2)	55 (10.8)	1.0	448 (88.4)	59 (11.6)	1.0	413 (92.2)	35 (7.8)	1.0
	Yes	122 (92.4)	10 (7.6)	0.70 (0.37-1.33)	118 (89.4)	14 (10.6)	0.91 (0.53-1.58)	107 (90.7)	11 (9.3)	1.19 (0.63-2.28)
EDH	No	503 (90.5)	53 (9.5)	1.0	497 (89.4)	59 (10.6)	1.0	457 (92.0)	40 (8.1)	1.0
	Yes	71 (85.5)	12 (14.5)	1.52 (0.85-2.72)	69 (83.1)	14 (16.9)	1.59 (0.93-2.71)	63 (91.3)	6 (8.7)	1.08 (0.48-2.45)
pTBI	No	531 (89.7)	61 (10.3)	1.0	525 (88.7)	67 (11.3)	1.0	485 (92.4)	40 (7.6)	1.0
	Yes	25 (92.6)	2 (7.4)	0.72 (0.19-2.79)	22 (81.5)	5 (18.5)	1.64 (0.72-3.73)	18 (81.8)	4 (18.2)	2.39 (0.94-6.08)

Table 9. Incidence and Relative Risk of PTS Stratified by Variables of Interest

		Immediate/Early PTS			Late PTS Year 1			Late PTS Year 5		
		N(%)		RR (CI)	N(%)		RR (CI)	No Seizure		RR (CI)
		No Seizure	Seizure		No Seizure	Seizure		No Seizure	Seizure	
Contusion Load	0	230 (88.5)	30 (11.5)	1.0	238 (91.5)	22 (8.5)	1.0	222 (93.3)	16 (6.7)	1.0
	1	99 (90.0)	11 (10.0)	0.87 (0.45-1.67)	101 (91.8)	9 (8.2)	0.97 (0.46-2.03)	96 (95.1)	5 (4.9)	0.74 (0.28-1.96)
	2	126 (92.0)	11 (8.0)	0.70 (0.36-1.35)	117 (85.4)	20 (14.6)	1.73 (0.98-3.05)	104 (88.9)	13 (11.1)	1.65 (0.82-3.32)
	3	57 (86.4)	9 (13.6)	1.18 (0.59-2.37)	56 (84.9)	10 (15.1)	1.79 (0.89-3.60)	50 (89.3)	6 (10.7)	1.59 (0.65-3.89)
	>=4	62 (93.9)	4 (6.1)	0.53 (0.19-1.44)	54 (81.8)	12 (18.2)	2.15 (1.12-4.11)	48 (88.9)	6 (11.1)	1.65 (0.68-4.03)
Injury Severity	Mod	69 (87.3)	10 (12.7)	1.0	69 (87.3)	10 (12.7)	1.0	63 (91.3)	6 (8.7)	1.0
	Severe	642 (89.5)	75 (10.5)	0.83 (0.45-1.53)	632 (88.2)	85 (11.8)	0.94 (0.51-1.73)	570 (90.2)	62 (9.8)	1.13 (0.51-2.51)
GCS	>13	169 (88.5)	22 (11.5)	1.0	172 (90.1)	19 (9.9)	1.0	158 (91.9)	14 (8.1)	1.0
	9-12	113 (86.9)	17 (13.1)	1.14 (0.63-2.05)	113 (86.9)	17 (13.1)	1.31 (0.71-2.43)	105 (92.9)	8 (7.1)	0.87 (0.38-2.01)
	8	347 (90.4)	37 (9.6)	0.84 (0.51-1.38)	334 (87.0)	50 (13.0)	1.31 (0.79-2.16)	296 (88.6)	38 (11.4)	1.40 (0.78-2.51)

**Injury severity as determined by GCS, duration of loss of consciousness and post-traumatic amnesia, and neuroimaging findings*

Table 10. Incidence and Relative Risk of Late PTS* Stratified by Variables of Interest

Acute Seizure Status	Late PTS Year 1			Late PTS Year 5		
	N(%)		RR (CI)	N(%)		RR (CI)
	No Seizure	Seizure		No Seizure	Seizure	
	710 (89.2)	86 (10.8)		641 (90.3)	69 (9.7)	
None	634 (90.8)	64 (9.2)	1.0	577 (91.0)	57 (9.0)	1.0
Immediate	54 (76.1)	17 (23.9)	2.61 (1.62-4.20)	44 (81.5)	10 (18.5)	2.06 (1.12-3.78)
Early	13 (92.9)	1 (7.1)	0.78 (0.12-5.22)	12 (92.3)	1 (7.7)	0.86 (0.13-5.72)
Late	9 (69.2)	4 (30.8)	3.36 (1.44-7.84)	8 (88.9)	1 (11.1)	1.24 (0.19-7.97)

*No seizures during acute hospitalization (including late) contribute to definition of late PTS

Table 11. Supplemental Table: Definitions of Risk Factors for Stratified Incidence

	Variable	Definition	Method
Demographics	Sex	Biological sex	
	Race	Self-identified race	Self-report
	Age	Age categorized into 10 year increments, beginning at age 15 and ending with those 65 and older	Medical Record Review
Injury Characteristics	Injury Severity	**Moderate**: normal or abnormal imaging with 30min <LOC< 24 hours, or 1day<PTA<7days, or GCS 9-12 **Severe**: normal or abnormal imaging with LOC >24 hours, or PTA>7days, or GCS 3-8	Medical Record Review
	Subdural Hematoma (SDH)	Presence of extra-axial collection within sub-dural space including hematoma and hygroma	Medical Record Review
	Subarachnoid Hemorrhage (SAH)	Blood in ambient, basal, interpenduncular cisterns or cisterna magna, or along falx or tentorium	Medical Record Review
	Intra-ventricular Hemorrhage (IVH)	Blood documented within intra-ventricular space	Medical Record Review
	Epidural Hematoma (EDH)	Presence of extra-axial collection within epidural space	Medical Record Review
	Contusion Load	Based on medical record review. Calculated by summing the number of regions with parenchymal contusions documented in medical record. Regions were specified by cortical area or non-cortical focal contusion. A maximum of 6 regions were documented (frontal, temporal, parietal, occipital, focal non-cortical, not specified)	Calculated Variable
	Penetrating TBI	Calculated using validated algorithm using imaging reports of retained fragment and mechanism of injury from medical record review.	Calculated Variable
	Seizure during Acute Care Hospitalization	Documents time of first seizure during acute care hospitalization (no seizure, immediate, early, late). Seizure must be confirmed by a physician.	Medical Record Review

Table 12. Supplemental Table: Individuals Reporting Seizures at Multiple Time Points

Time of First Seizure (N)		Seizure at 1 Time Point	Seizure at 2 Time Points	Seizure at 3 Time Points	Seizure at 4 Time Points
Acute Seizure Status	Immediate (71)	44 (62.0)	15 (21.1)	9 (12.7)	3 (4.2)
	Early (14)	12 (85.7)	2 (14.3)	0	0
	Late (13)	8 (61.5)	1 (7.7)	1 (7.7)	2 (15.4)
Year 1 (64)		23 (35.9)	26 (40.6)	15 (23.4)	NA
Year 2 (32)		19 (59.9)	13 (40.1)	NA	NA
Year 5 (25)		25 (100)	NA	NA	NA

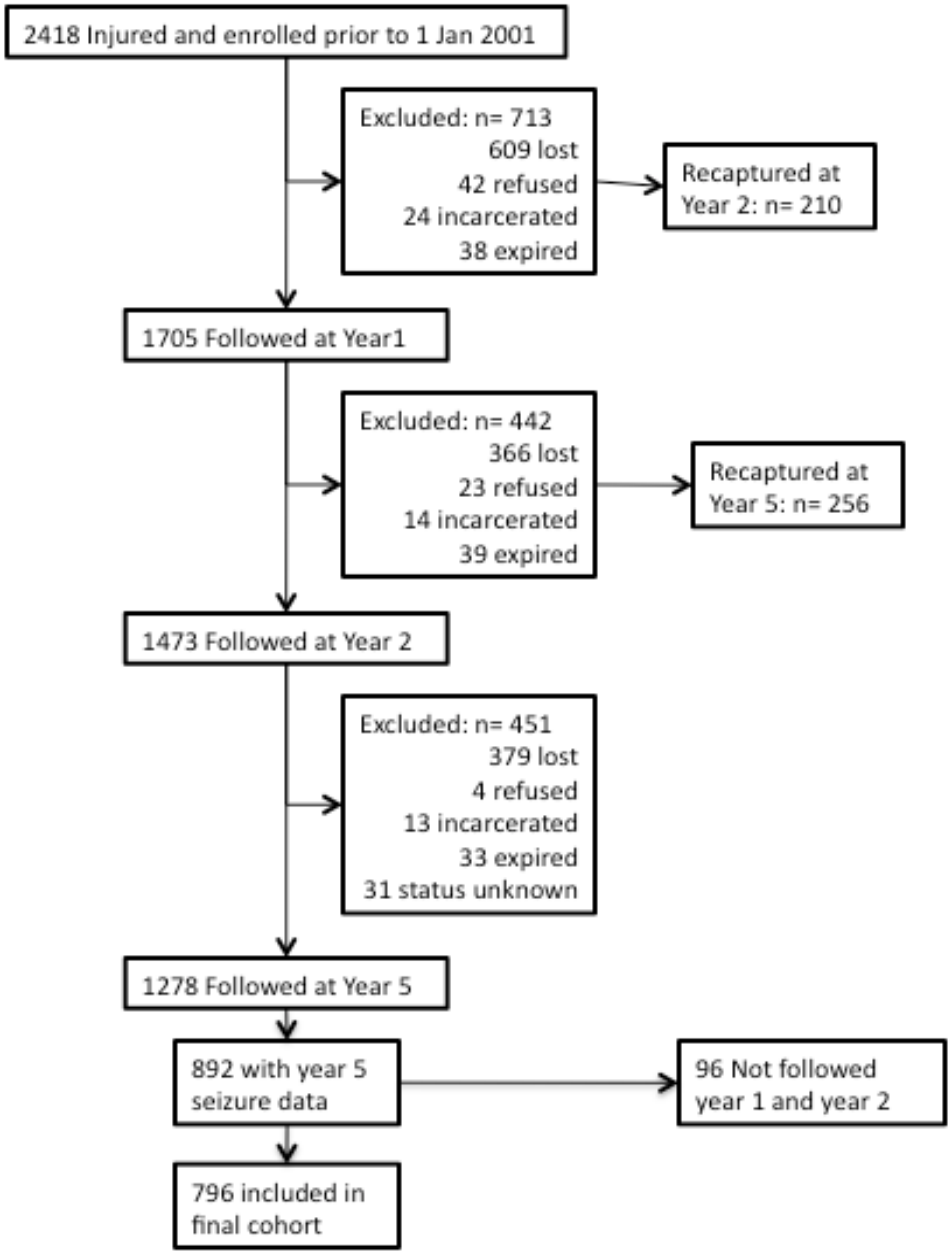

Figure 2. Traumatic Brain Injury Model System Five Year Follow-up CONSORT Figure

5.0 MANUSCRIPT TWO

PROGNOSTIC MODELS FOR PREDICTING POST-TRAUMATIC SEIZURES DURING ACUTE HOSPITALIZATION, AND AT 1 AND 2 YEARS FOLLOWING TRAUMATIC BRAIN INJURY

Anne C Ritter[1, 2], Amy K Wagner[2,3,4,5], Maria M Brooks[1], Ross D Zafonte[6], Mary Jo Pugh[7,8], Anthony Fabio[1], Flora M Hammond[9,10], Jerzy P Szaflarski[11], Laura E Dreer[12], Tamara Bushnik[13], William C Walker[14], Allen W Brown[15], Doug Johnson-Greene[16], Timothy Shea[17], Jason W Krellman[18], Joseph A Rosenthal[17]

[1]Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA
[2]Physical Medicine & Rehabilitation, University of Pittsburgh, Pittsburgh, PA
[3]Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA
[4]Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA
[5]Center for Neuroscience at University of Pittsburgh, Pittsburgh, PA
[6]Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, MA
[7]South Texas Veterans Health Care System Polytrauma Rehabilitation Center, San Antionio, TX
[8]Department of Epidemiology and Biostatistics, University of TX Health Science Center San Antionio, San Antonio, TX
[9]Carolinas Rehabilitation, Charlotte, NC
[10]Indiana University School of Medicine, Indianapolis, IN
[11]University of Alabama at Birmingham Epilepsy Center, Department of Neurology, University of Alabama, Birmingham, AL
[12]Departments of Physical Medicine and Rehabilitation and Ophthalmology
[13]Rusk Rehabilitation, New York University School of Medicine, New York, NY
[14]Dept of Physical Medicine & Rehabilitation, Virginia Commonwealth University
[15]Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN
[16]Miller School of Medicine, University of Miami, Miami, FL
[17]Department of Physical Medicine and Rehabilitation, Ohio State University, Columbus, OH
[18]Department of Rehabilitation Medicine, Icahn School of Medicine at Mount Sinai, New York, NY

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5.1 ABSTRACT

Objective: Post-traumatic seizures (PTS) are well-recognized acute and chronic complications of traumatic brain injury (TBI). Risk factors have been identified, but considerable variability in who develops PTS remains. Existing PTS prognostic models are not widely adopted for clinical use and do not reflect current trends in injury, diagnosis, or care. We aimed to develop and internally validate preliminary prognostic regression models to predict PTS during acute care hospitalization, and at Year-1 and Year-2 post-injury.

Methods: Prognostic models predicting PTS during acute care hospitalization and Year-1 and Year-2 post-injury were developed using a recent (TBI 2011-2014) cohort from the TBI Model Systems National Database. Potential PTS predictors were selected based on previous literature and biological plausibility. Bivariate logistic regression identified variables with a p-value<0.20 that were used to fit initial prognostic models. Backward-stepwise elimination was used to determine reduced prognostic models and to internally validate using 1000 bootstrap samples. Fit statistics were calculated, correcting for over-fitting (optimism).

Results: The prognostic models identified sex, craniotomy, pre-injury mental health treatment/psychiatric hospitalization, and pre-injury limitation in learning/remembering/concentrating as significant PTS predictors during acute hospitalization. Year-1 significant PTS predictors were injury severity, subdural hematoma (SDH), contusion load, craniotomy, craniectomy, pre-injury condition-limiting physical activity, mental health treatment/psychiatric hospitalization, and incarceration were significant PTS predictors. Year-2 significant predictors included seizure during acute hospitalization, SDH, intracranial fragment, craniectomy, and pre-injury condition-limiting physical activity. Corrected concordance (C)

statistics were 0.549, 0.756, and 0.724 for acute hospitalization, Year-1, and Year-2 models, respectively.

Significance: The prognostic model for PTS during acute hospitalization did not discriminate well. Year-1 and Year-2 models showed fair to good predictive validity for PTS. Cranial surgery, while medically necessary, requires ongoing research regarding potential benefits of increased monitoring for signs of epileptogenesis, PTS prophylaxis, and/or rehabilitation/social support. Future studies should externally validate models and determine clinical utility.

5.2 INTRODUCTION

Traumatic brain injury (TBI) is a well-recognized public health problem. Over 2.5 million TBIs occur annually in the United States [3]; approximately 11% require hospitalization, primarily for moderate/severe injury. TBI is increasingly recognized as a chronic disease, significantly impacting morbidity and mortality [2; 134]. As medicine advances, more individuals are expected to survive moderate/severe TBI, increasing the number affected by injury-associated complications.

Post-traumatic seizures (PTS) and epilepsy (PTE) are well-recognized TBI complications. PTS can develop at any point after TBI and is classified by time of first seizure (immediate: <24hrs, early: 24hrs to 7d, and late: >7d post-TBI). Immediate and early PTS are considered directly related to the primary injury. Late seizures are attributed to secondary injury cascades and persistent epileptogenic mechanisms and eventually present as clinical seizures [18; 31]. PTS incidence and prevalence vary widely throughout the literature and depend on study design (e.g. length of follow-up), population characteristics (e.g. injury severity), and PTS definition. Previous reports after primarily closed-head injury indicate a broad range of percent affected (early: 1.4-12%; late: 4.4-18.9%) [12; 47; 50; 66; 69; 123]. Work using the NIDILRR Traumatic Brain Injury Model System (TBIMS) National Database, including individuals with predominantly closed-head moderate/severe TBI, demonstrated incidence rates of 8.9% and 1.9% for immediate and early PTS, respectively (**Manuscript One**). By 1yr post-injury, 20.4% of the cohort reported seizures, and approximately 12% met criteria for late PTS (i.e. PTE). Late PTS incidence from injury to 2yrs and injury to 5yrs post-TBI increased to 16.8% and 20.5%

(**Manuscript One**). Seizure risk after severe TBI, beyond 10yrs post-injury, remains significantly elevated compared to the general population [12]. These data suggest epileptogenesis can follow a prolonged course, and TBI related pathology exerts long-term epileptogenic effects.

Prognostic models can estimate an individual's risk for developing an outcome of interest based on specific characteristics [111]. While many studies examined injury characteristics and associations with PTS, few have developed prognostic PTS models. Of these, none have been integrated into routine clinical practice. Such models were developed decades ago, using small samples, and examining probability based on a single risk factor [46; 135]. A multivariable mathematical model was developed in the 1970's and validated using datasets from TBI studies available at the time [136]. However, these prognostic models do not reflect current trends in injury severity, TBI detection and treatment, or seizure prophylaxis. Since then, improved neuroimaging allows greater specificity when characterizing intracranial pathology. Neurosurgical procedures, including craniectomy, are now more common for treatment of intracranial pathology. Therefore, new prognostic models reflecting current injury patterns, diagnosis, and treatment trends are required if models are to be clinically useful. Accurate PTS risk prediction could help define high-risk populations in support of clinical intervention trials. Predictive models could also inform clinical algorithms to identify individuals likely to benefit from tailored seizure prophylaxis or treatment.

The TBIMS National Database (TBIMS-ND) is an ongoing, multi-center, longitudinal observational study. Currently, there are 16 funded centers collecting demographic, premorbid personal and medical history, and injury-specific data upon study enrollment, as well as chronic medical conditions, psychosocial, and rehabilitation outcomes. The TBIMS-ND is an excellent source of data for prognostic model development involving a variety of TBI-related outcomes for

individuals surviving acute injury and receiving inpatient rehabilitation. The purpose of this study was to develop and internally validate prognostic models predicting PTS during acute care hospitalization, at Year-1, and Year-2 post-injury for a recent cohort in the TBIMS-ND.

5.3 METHODS

5.3.1 Study Design and Population

Data were obtained from the TBIMS-ND. All participating centers have a Level-I or Level-II Trauma Center, acute neurosurgical capabilities, and associated comprehensive inpatient TBI rehabilitation. Individuals with moderate/severe TBI (PTA>24 hrs, or LOC>30minutes, or emergency department GCS<13, or positive neuroimaging findings), admitted to a participating hospital emergency department within 72hrs of injury, age ≥16yrs, receiving acute care and inpatient rehabilitation within a TBIMS designated hospital system were eligible for study inclusion. All subjects, or legal proxy, provided written informed consent.

Two variables related to follow-up PTS were collected at different times within the TBIMS-ND, with the most recent variable added in 2012. To ensure data reflected current population trends and standards of care, current analyses included participants injured between January 1, 2012 and August 31, 2014.

5.3.2 Data Collection

Data were limited to those collected at enrollment, Year-1, or Year-2 post-injury. All data were collected using standardized protocols. Enrollment data, collected though chart review and interview included demographic, social, and injury characteristics, pre-injury personal and medical history, and acute outcomes. CT scan data were classified by trained raters based on a composite of the worst findings on CT scan over the first 7d post-injury. Follow-up data collection was completed via telephone or mailed self-administered battery. Proxy interviews were completed if an individual with TBI was unable to provide reliable responses.

5.3.3 Outcome Variable

PTS status, dichotomized as present or absent, was the main outcome, determined during the course of acute hospitalization, at Year-1, and Year-2. Following discharge from acute hospitalization, TBIMS Center data collectors record up to 20 ICD-9 codes in the participant's medical chart related to their TBI admission. To determine PTS status during acute hospitalization, all recorded acute care ICD-9 codes were reviewed. ICD-9 codes relating to convulsion (780.39), PTS (780.33), and epilepsy (345.0x → 345.9x) were included as evidence of seizure activity following TBI.

PTS status at Year-1 and Year-2 were determined solely via participant (or proxy) self-report. Study participants were asked "*Have you had a seizure since your TBI?*" at follow-up interviews. If participants answered yes, they were asked, "*Since your discharge from rehabilitation, have you had a seizure?*" at the Year-1 follow-up interview and, "*In the past year,*

have you had a seizure?" at the Year-2 follow-up interview. If patients answered yes to the second question, they were counted as having PTS at Year-1 and Year-2, respectively.

5.3.4 Predictors of Interest

Predictors of interest included baseline demographics, personal and medical history information, and injury characteristics. All predictors were selected *a priori* based on biological plausibility and possible risk factors identified in previous literature [12; 47; 48; 66; 69; 70; 78] (**Table 1**). Demographic variables included age, sex, and race. Personal and medical history variables included *pre-injury*: prior moderate/severe TBI, condition significantly limiting physical activity, limitation in learning, remembering, or concentrating, substance abuse, mental health treatment, psychiatric hospitalization, suicide attempt, military service, and incarceration.

Injury characteristics included injury severity (**Table 1**), duration of post-traumatic amnesia (PTA) in Year-1 and Year-2 models, confirmed pathology on CT scan, intraparenchymal fragment, penetrating TBI (pTBI), craniotomy, craniectomy, and associated spinal cord injury. CT findings were included as separate variables for specific pathology type [e.g. subdural hematoma (SDH), epidural hematoma (EDH)], coded as present or absent and were not mutually exclusive. pTBI was computed via a coding algorithm previously validated in a subsample of the TBIMS [85]. Also, a contusion load score was calculated by summing the number of regions with reported contusion (**Table 1**). This score was collapsed into 0, 1, 2, 3, 4, and 5 or more regions. At Year-1 and Year-2, seizure during acute hospitalization was included as a risk factor. No data were collected on premorbid seizure activity or history of epilepsy.

Prognostic Modeling

PTS prognostic models during acute hospitalization, Year-1, and Year-2 were developed and internally validated with resampling. For each time-point [PTS during acute hospitalization, PTS status since discharge from rehabilitation (Year-1) and PTS status in the past year (Year-2)], all potential risk factors described above were examined using bivariate logistic regression. All variables with p-value<0.20 were retained for inclusion in model building.

A saturated regression model, including all variables identified in the above step, was fit for each of the three PTS outcomes. After fitting a saturated model, variables were preliminarily examined for multicollinearity using Spearman correlation matrices. For each model, retained fragment and pTBI were highly collinear (r>0.9); pTBI occurred much less frequently versus retained fragment, and therefore, was not included in further prognostic modeling. Premorbid history of mental health disorder and premorbid psychiatric hospitalization were also highly collinear and were combined to form a four-level categorical variable (no mental health disorder or hospitalization; mental health disorder no hospitalization; hospitalization without mental health disorder; both mental health disorder and hospitalization). The saturated model was refit, and variance inflation factors (VIF) and condition indices were calculated.

Next, backward (step-down) variable selection was performed with an exit criterion of alpha=0.05. The reduced model was internally validated via resampling in an automated process using the *rms: Regression Modeling Strategies* package for R [120]. Specifically, 1,000 bootstrap samples were drawn with replacement from the original data such that each bootstrap sample had an equal number of observations as the original dataset. In each bootstrap sample, stepwise backward elimination with an exit criterion of alpha=0.05, was used to validate the reduced model. The C-statistic, a measure of concordance equal to the area under the receiver operating characteristic (ROC) curve, was calculated using Somers' D_{xy} for the saturated model [118]. The C-

statistic was calculated for the final, reduced model selected from the original data, with and without adjustment for optimism.

All statistical analyses were completed using SAS version 9.4 (SAS Institute, Cary NC) and R version 3.0.3 [125].

5.4 RESULTS

5.4.1 Population

2,160 participants injured January 1, 2012 through August 21, 2014 had PTS related ICD-9 codes during acute hospitalization recorded (**Figure 1**). Of the 2,160 participants, 1,941 had data available on all predictors identified in simple logistic regression for seizure during acute hospitalization. For Year-1 analyses, 1,164 participants had PTS data, and 1,039 had data available for all predictors included in the saturated regression model. For Year-2 analyses, 410 participants had PTS data, and 375 had data for predictors included in the saturated model. At each time point, demographics were similar to previous TBI studies (**Table 2**).

5.4.2 Prognostic Models

Following bivariate examination of predictors, 12 variables met inclusion criteria ($p<0.20$) for the initial, saturated prognostic model for PTS during acute hospitalization (**Table 3**). After backward elimination and bootstrapping, the final model included sex, craniotomy, pre-injury limitation in learning/concentrating/remembering, and preinjury mental health treatment

and/or psychiatric hospitalization. Craniotomy was the most statistically significant predictor in the final prognostic model and was selected in 70.9% of bootstrapped models (**Table 3**). In the saturated model, the calculated C-statistic was 0.595. However, after correction for optimism, the C-statistic in the final model was 0.549 (**Table 3**).

The Year-1 saturated prognostic model of PTS included 16 predictor variables (**Table 4**). After validation, the final model included injury severity, SDH, contusion load, craniotomy, craniectomy, pre-injury condition limiting physical activity, mental health treatment/psychiatric hospitalization, and incarceration. The model failed to converge in 10 bootstrap samples. Craniectomy was the most statistically significant predictor ($p<0.001$) and was selected in 99.9% of bootstrap samples (**Table 4, Figure 1**). Intraparenchymal fragment and seizure during acute care hospitalization were the last variables removed from the prognostic model during backward elimination, prior to determination of the final model (data not shown). The calculated C-statistic for the Year-1 saturated model was 0.797, which was reduced to 0.756 after adjustment for optimism (**Table 4**)

The Year-2 saturated model included 14 predictor variables (**Table 5)**. For Year-2, contusion load was collapsed into 0, 1, 2, 3, or 4 or more regions due to low sample size when 4 and 5 or more regions were separate. Similarly, mental health disorder and/or psychiatric hospitalization were collapsed into three levels (hospitalization no mental health disorder [n=2], combined with mental health disorder *and* hospitalization). Following bootstrapping with backward elimination, SDH, intraparenchymal fragment, craniectomy, seizure during acute hospitalization, and pre-injury condition limiting physical activity were significant predictors. With the exception of seizure during acute hospitalization, each predictor was indicative of at ≥3X greater odds of PTS at Year-2 versus those without the predictor (**Table 5**). Similar to Year-

1, craniectomy was the most statistically significant predictor of PTS at Year-2 and was selected in 90.4% of bootstrap samples. Craniotomy was not included in model generation based on bivariate results ($p>0.20$). The C-statistic for the saturated model was 0.785, which decreased to 0.724 after correction for optimism (**Table 5**).

5.5 DISCUSSION

We developed prognostic models of PTS during acute hospitalization, Year-1, and Year-2 following TBI for individuals requiring hospitalization and inpatient rehabilitation at designated TBIMS centers. We internally validated these models using resampling techniques and generated discrimination statistics. Within each model, multiple risk factors were significant predictors of PTS at each time-point. C-statistics demonstrated that models had fair to good ability to discriminate between individuals with and without PTS at Year-1 and Year-2. However, the prognostic model for acute hospitalization did not perform much better than chance for predicting those who had PTS. Nonetheless, variables identified as PTS predictors over time may shed light on vulnerable risk groups and the temporal nature of specific clinical and demographic PTS risk factors.

Sex was the only significant demographic PTS predictor (men at increased risk) and only in the acute care model. This finding must be interpreted with caution because of the model's poor discrimination ability and data showing that sex was included in less than half (45.5%) of the bootstrap models, indicating sex is not a reliable predictor of acute PTS. Two large late PTS studies reported increased seizure risk in men, but differences were non-significant [47; 69]. Previous early PTS studies have not examined extensively demographic characteristics as risk

factors other than finding young children at increased risk for early seizure versus adults [48; 66]. We identified pre-injury limitation in learning, remembering, or concentrating as a significant PTS predictor during acute hospitalization. This variable was included in less than half (48.9%) of bootstrap samples. However, pre-injury limitation in learning may capture latent premorbid neurobiological differences that increase seizure susceptibility, as evidenced by increased epilepsy rates among individuals with developmental disabilities [137].

Pre-injury mental health treatment and/or psychiatric hospitalization was a significant PTS predictor during acute hospitalization and Year-1. Previous work suggests depression history, prevalent in 21% of the study population, is associated with increased late PTS risk [69]. Notably in our analyses, 22% of individuals reported history of mental health treatment and/or hospitalization for psychiatric disorder. Existing research indicates bidirectional relationships between psychiatric conditions (especially major depression) and epilepsy [138]. These associations may be attributable to common neuropathological mechanisms, like regional monoaminergic dependent derangements in glutamate management and neurotransmission [138]. Additionally, medications for mental health disorders like antipsychotics (e.g. chlorprothixene, clozapine) and specific antidepressants (e.g. maprotiline, venlafaxine), can decrease seizure threshold, further increasing seizure risk after TBI [139]. Importantly, post-injury depression occurs commonly after TBI [140]. Many individuals take anti-depressants to address clinical symptoms. Therefore, clinicians may need to weigh seizure risk into their selection of antidepressants in this population. The findings also suggest further studies to evaluate the combined seizure risk among individuals receiving psychotropic medications after TBI.

Pre-injury condition limiting physical activity was a significant PTS predictor at Year-1 and Year-2. This variable may include individuals with conditions attributable to central nervous

system (CNS) pathology that limit activity and could increase seizure susceptibility independent of TBI [e.g. cerebral palsy [141]]. This variable may also include those with pre-morbid epilepsy if the condition impacts (or the individual perceives it impacts) physical activity (e.g. if seizures are not well controlled). Generally, PTS studies exclude individuals with pre-morbid seizure disorders due to inability to distinguish PTS from seizures due to non-traumatic etiology. The TBIMS does not collect information on premorbid seizure/epilepsy. However, individuals with premorbid seizure/epilepsy may be at risk for increased seizure frequency and/or severity post-TBI. Future work should evaluate the impact of TBI on changes in seizure frequency/severity among those with pre-existing epilepsy.

Pre-injury incarceration was a significant PTS predictor at Year-1. One study reports higher percentages of prior arrest and incarceration among individuals with late PTS versus those without late PTS [142]. Incarceration is associated with increased impulsivity [143] and associated behaviors (e.g. aggression, risk taking, substance use) [144]. These individuals may have underlying neuropathologies involving limbic structures and neurotransmitter disruption in the nucleus accumbens that impact cortical cognitive control [144], predisposing them to risky behaviors that may result in TBI and PTS. Incarceration may also be associated with developmental disability and history of violence, including previous TBI. However, these variables were already accounted for in prognostic modeling. Thus, incarceration may represent latent neurobiological traits not otherwise accounted for by data collected.

Contrary to expectation, previous moderate/severe TBI did not predict PTS. To our knowledge, no previous study has examined the risk of incident PTS after multiple moderate/severe TBI. We hypothesized pathology from prior injury increases PTS risk after subsequent injury. However, the lack of significant findings may be related to low event rates,

with less than 3.5% of the population reporting prior moderate/severe TBI. Future work should investigate how multiple TBI affects biosusceptibility to complications like PTS. Intraparenchymal fragment was a significant PTS predictor at Year-2, consistent with previous research demonstrating higher PTS rates among those with depressed skull fracture [12; 47; 66] and pTBI [72]. In our analyses, pTBI was very rare, but also partially defined by the intraparenchymal fragment variable, and was therefore not examined in prognostic models. Injury severity was a significant Year-1 predictor, where PTS odds were greater in those with severe versus moderate injury. At Year-2, PTS rates were 2 times higher for those with severe versus moderate TBI (data not shown), but injury severity did not reach statistical significance in multivariable modeling. The lack of predictive ability may be attributed to low sample size or inclusion of other variables associated with injury severity (i.e. intracranial pathologies, craniectomy).

SDH was a significant predictor at Year-1 and Year-2, consistent with previous literature [12; 47; 78]. SDH was not a significant PTS predictor acutely, but the propensity for temporal glial scarring in SDH regions, and the fundamental role of glial scarring in epileptogenesis [40; 62], may explain the temporality of this finding. Contusion load is a marker of multifocal injury throughout the brain, and contusion has been identified previously as a risk factor for PTS [12; 47; 78]. As contusion load increases, neuronal injury and apoptosis likely increase, disrupting neuronal circuits and predisposing focal areas to ictal discharges. Vascular damage after TBI leads to regional blood extravasation and subsequent generation of blood breakdown products within CNS tissues, perpetuating oxidative stress, another mechanism of epileptogenesis [18; 31].

Seizure during acute hospitalization was the last variable removed from the Year-1 PTS model and was a significant PTS predictor at Year-2. Although there is debate regarding the "seizure begets seizure" construct [145], research consistently demonstrates early seizure is

associated with increased risk of late PTS [47; 66; 69; 70]. Immediate/early seizures are considered provoked and non-epileptogenic. However, provoked seizures may exacerbate secondary injury cascades affecting neurochemical and synaptic regulation [146]. Seizures cause reactive astrocytosis and altered glutamate management, further promoting TBI-induced excitoxicity [62]. Reactive astrocytosis also perpetuates the injury-induced inflammatory response, propagating an inflammation/excitation cycle that may result in subsequent seizures [62]. Thus, early seizures, and associated disruptions in critical neuroregulatory mechanisms after injury, may alter neuronal homeostasis, further causing maladaptive neuronal circuit reorganization (plasticity) in what are already seizure-prone systems [147]. While the acute hospitalization period for the TBIMS population often extends beyond the first week post-TBI, the finding that these seizures contribute to longer term PTE risk underscores the critical need for effective PTS prophylaxis and revisiting whether or not current guidelines for medications and treatment duration are preventing immediate/early PTS effectively and reducing PTE risk [8].

Craniotomy and craniectomy are common procedures following severe TBI. Recently, decompressive craniectomy (DC) has become a widely used procedure for management of intractable intracranial pressure. Cranial surgeries were among the strongest and most statistically significant PTS predictors in our models, confirming previously published findings [47; 78]. However, cranial surgery type reaching statistical significance within models varied across time. We hypothesize this association may stem from both anatomic and physiologic changes from the craniectomy and associated cranioplasty as well as late surgical complications.

Craniotomy and craniectomy are implicated as risk factors for seizure, even when used to address non-traumatic CNS pathologies [89]. Craniectomy carries increased risk for additional brain tissue damage during surgery and secondary to post-operative hematoma and edema [148].

Chronic complications (>1month post-surgery) can occur post-craniectomy, including poor wound healing, infection, and hydrocephalus [148]. Complications and increased morbidity can also occur secondary to subsequent duraplasty/cranioplasty [149]. Thus, delayed pathological mechanisms associated with chronic complications and subsequent cranioplasty may explain the temporality of craniectomy as a significant PTS predictor. Observational and retrospective studies note more severe injury among individuals undergoing craniectomy versus craniotomy or standard care [150]. Our prognostic models include multiple injury severity and pathology measures, yet craniectomy remained among the strongest predictors, supporting the idea that craniectomy is associated with increased PTS risk, independent of injury severity. PTS prophylaxis guidelines [8], do not reflect new pharmacological agents or trends in neurosurgical intervention for treatment of TBI, yet may benefit from additional research that considers these issues.

Although these models elucidate potentially important PTS predictors, there are limitations to consider. Relative to prognostic studies in general, sample sizes in current analyses were small. Ability to discriminate PTS was poor during acute hospitalization. Low acute model performance may be due to the fact that seizure status during acute hospitalization does not differentiate between immediate, early, and late seizures. Differentiating between these time points as outcomes could improve individual model performance as PTS risk factors temporally evolve. Alternatively, factors predicting acute seizures may be so diverse that prognostic models would not be effective. Acute seizures may include those detected via electroencephalogram (EEG). However, we do not know if EEG was used to capture seizure activity, if specific individuals only were monitored using continuous EEG, or if EEG monitoring/screening practices differed across TBIMS centers. Misclassification of PTS status from ICD-9 codes, and

inability to determine premorbid seizure/epilepsy disorder, also limit model performance.

Importantly, PTS status misclassification at Year-1 and Year-2 may also have occurred because PTS determination was based exclusively on self-report. Individuals who experienced psychogenic may have reported seizure activity. However, for large population-based epidemiological studies, it is not feasible to determine PTS status through in-depth neurological examine or medical history. Therefore, self-report remains the gold standard for seizure/epilepsy research. Lack of information on medication use prohibited investigating how psychotropics affect PTS risk. Therefore, we cannot determine if inclusion of mental health disorder/psychiatric hospitalization is predictive or if this variable represents increased PTS risk secondary to psychotropic medication use. We were also unable to control for AED effects on acute hospitalization or Year-1/Year-2 seizure risk, including differential effects of specific medication type. However, in a single TBIMS center, 96% of individuals with severe TBI received seizure prophylaxis during acute care [129]. It is possible, but cannot be confirmed, that other TBIMS centers would have similar prophylaxis rates. Additionally, the TBIMS-ND includes only individuals surviving their acute injuries and receiving acute inpatient rehabilitation after moderate-severe TBI. Results here may not extrapolate to all individuals with moderate-severe TBI. Lastly, the observational design does not provide causal evidence among relationships with PTS outcome.

Despite limitations, these prognostic models may have added benefit compared to prior models, which were not used clinically even though they were reliable in different study populations [46; 136]. Previous models focused on calculating PTS probability or seizure recurrence over time [46; 135; 136], while our prognostic models reflect current trends in TBI diagnosis, treatment, and population characteristics, and investigate multiple risk factors identified in

previous PTS studies. Regardless, these models should be examined in independent study populations to determine discriminability and validity outside the TBIMS population. Individuals with characteristics identified in prognostic models as predictive of PTS represent subpopulations that may benefit from tailored seizure prophylaxis guidelines addressing unique premorbid characteristics, pathologies, and procedures.

Further study is required to determine whether new evidence of biological risk factors for PTS improves the clinical utility of prognostic models. Year-1 and Year-2 models had optimism-corrected C-statistics greater than 0.70 (0.756, 0.724, respectively). While these values indicate good discriminatory ability, there remains room for improvement. Of particular interest are genetic factors previously shown to be associated with accelerated epileptogenesis and seizure risk after TBI [104; 129]. These studies suggest genetic variation remains a significant PTS risk factor after controlling for other factors including injury severity and SDH. Data regarding genetic variation in epileptogenic pathways could improve prognostic ability for PTS, much the way genetic information improved breast cancer prognostication [151]. As modern medical and prevention efforts for PTS move toward personalized medicine approaches, personal biology metrics like genetic variation and inflammation may contribute meaningfully to prognostication and treatment development.

Acknowledgements

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. No authors have any conflict of interest. Grant support: NIDILRR: 90DP0041-02-01.

Figure Legends

Figure 1. Histogram depicting predictors of interest included in prognostic models of PTS during acute care hospitalization (blue), at Year-1 (red), and Year-2 (green) post-injury. Y-axis represents the percent of bootstrap models the predictor of interest was retained in after backward stepwise elimination. Variables without a column for a specific time-point were not considered as a predictor of interest for the time-point. PTA=post-traumatic amnesia; SDH=subdural hematoma; SAH=subarachnoid hemorrhage; EDH=epidural hematoma; SCI=associated spinal cord injury; MH=mental health; Psych Hosp=psychiatric hospitalization.

Supplemental Figure 1. Consort like diagram depicting the flow of individuals used for development of prognostic models at progressive time-points within the TBIMS-ND.

5.6 TABLES

Table 13. Risk Factors Selected for Consideration in Prognostic Models

	Variable	Definition	Method
Demo-graphics	Sex	Biological sex	
	Age	Age at Injury	
	Race	Self-identified race	SR
Personal and Medical History	Condition Significantly Limiting Physical Activity	A condition that substantially limits one or more basic physical activities such as walking, climbing stairs, reaching, lifting, or carrying prior to injury	SR
	Limitation in Learning, Remembering, Concentrating	Difficulty in learning, remembering, or concentrating due to a physical, mental, or emotional condition that has been present for at least 6 months prior to injury	SR
	Treatment for Mental Health Condition	Received previous treatment for any mental health problems prior to injury (e.g. depression, anxiety, schizophrenia, and alcohol & drug abuse)	SR
	Psychiatric Hospitalization	Any psychiatric hospitalizations prior to injury	SR
	Suicide Attempt	Suicide attempt prior to injury	SR
	Substance Use Problem	Based on self-reported alcohol (drinks per week, binge drinker, alcohol use) and drug use prior to injury	CV
	Incarceration	Any penal incarcerations with conviction for felony prior to injury	SR
	Military Service	Any military service prior to injury	SR
Injury Characteristics	Injury Severity	**Moderate**: normal or abnormal imaging with 30min < LOC < 24 hours, or 1day<PTA<7days, or GCS 9-12 **Severe**: normal or abnormal imaging with -->LOC >24 hours, or PTA>7days, or GCS 3-8	MRR
	Post Traumatic Amnesia (PTA)	Days of post-traumatic amnesia	MRR
	Subdural Hematoma (SDH)	Presence of extra-axial collection within subdural space including hematoma and hygroma	MRR
	Subarachnoid Hemorrhage (SAH)	Blood in ambient, basal, interpeduncular cisterns or cisterna magna, or along falx or tentorium	MRR
	Intra-ventricular Hemorrhage (IVH)	Blood documented within intra-ventricular space	MRR
	Epidural Hematoma (EDH)	Presence of extra-axial collection within epidural space	MRR

Table 13 Continued.

	Contusion Load	Calculated by summing the number of regions with parenchymal contusions documented in medical record. Regions were specified by cortical area or non-cortical focal contusion. A maximum of 6 regions were documented (frontal, temporal, parietal, occipital, focal non-cortical, not specified)	CV
	Retained Fragment	Intraparenchymal fragment including fractures displaced >2mm, excluding existing surgical clips or coils	MRR
	Penetrating TBI	Calculated via validated algorithm using imaging reports of retained fragment and mechanism of injury from medical record review.	CV
	Associated Spinal Cord Injury	Injury to neural elements of spinal cord present or absent	MRR
	Seizure during Acute Care Hospitalization	Inclusion of ICD-9 codes 780.39, 780.33, and 345.0x → 345.9x within first 20 ICD-9 codes reported during acute care hospitalization	MRR
Surgical Procedures	Craniotomy	Surgical procedure, defined as "cranium opened, something removed, cranium closed"	MRR
	Craniectomy	Surgical procedure, define as "cranium opened and left open"	MRR
Method abbreviations: MRR: medical record review; CV: calculated value; SR: self-report			

Table 14. Demographic and Injury Characteristics at Baseline Visit

		Acute	**Year 1**	**Year 2**
Sample Size		2160	1164	410
Age at Injury*		45.5 (20.3)	44.3 (20.2)	41.8 (19.9)
Sex	Male	1574 (72.9)	868 (74.6)	310 (75.6)
	Female	586 (27.1)	296 (25.4)	100 (24.4)
Race	White	1455 (67.6)	791 (68.0)	295 (72.0)
	Black	329 (15.3)	177 (15.2)	59 (14.4)
	Other	369 (17.1)	196 (16.8)	56 (13.6)
Admission Glasgow Coma Scale	Mild	773 (37.4)	401 (35.5)	126 (32.0)
	Moderate	227 (11.0)	123 (10.9)	48 (12.2)
	Severe	687 (33.2)	407 (36.1)	143 (36.3)
	Intubated	381 (18.4)	197 (17.5)	77 (19.5)
	Unknown	92 (4.3)	36 (3.0)	16 (3.9)
Post Traumatic Amnesia*		22.3 (22.8)	1208 (24.6)	24.2 (27.2)
Length of Acute Stay (Days)*		20.6 (19.5)	21.7 (21.2)	22.2 (24.6)
**mean(SD)*				

Table 15. Prognostic Model for Prediction of PTS during Acute Care Hospitalization

Variables in Saturated Model	**Retained in Reduced Model**	**Adjusted Odds Ratio**	**P-value**
Sex (ref=female)	Yes	1.51	0.039
Age	No	---	---
Injury Severity (ref=moderate)	No	---	---
Subdural Hematoma	No	---	---
Contusion Load	No	---	---
Craniotomy	Yes	1.72	0.005
Concurrent Spinal Cord Injury	No	---	---
Pre-Injury Condition Limiting Physical Activity	No	---	---
Pre-Injury Limitation in Learning/Remembering/ Concentrating	Yes	1.68	0.033
Pre-Injury Treatment for MH Condition/Psych Hosp (ref=Neither)	Yes	---	---
Treatment for MH Condition		1.34	0.187
Both		1.66	0.095
Psychiatric Hospitalization		5.01	0.004
Pre-Injury Substance Use Problem	No	---	---
Fit Statistics	**Sample Size**	**Seizure Prevalence**	**C Statistic**
Saturated Model	1941	171 (8.8%)	0.595
Reduced Model			0.593
Optimism Corrected Reduced Model			0.549
Final Prognostic Model for PTS during Acute Care Hospitalization			
Seizure During Acute Care Hospitalization = 0.02 + 0.41*Sex + 0.54*Craniotomy + 0.29*TreatMentalHealth + 0.51*TreatMentalHealth&PsychHosp + 1.61*PsychHosp + 0.52*PreInjuryLimitationLearning			
**Unless noted, reference group for adjust odds ratio is variable not present* *MH: Mental Health; Psych Hosp: Psychiatric Hospitalization*			

Table 16. Prognostic Model for Prediction of PTS at Year 1

Variables in Saturated Model	Retained in Reduced Model	Adjusted Odds Ratio	P-value
Post-Traumatic Amnesia	No	---	---
Injury Severity (ref=moderate)	Yes	2.23	0.030
Seizure during Acute Care Hospitalization	No	---	---
Subdural Hematoma	Yes	1.77	0.26
Subarachnoid Hemorrhage	No	---	---
Epidural Hematoma	No	---	---
Retained Fragment	No	---	---
Contusion Load (ref=0)	Yes		
1		2.55	0.008
2		3.35	0.001
3		3.03	0.004
4		1.44	0.447
>=5		3.08	0.036
Craniotomy	Yes	2.58	<0.001
Craniectomy	Yes	4.49	<0.001
Previous Moderate/Severe TBI	No	---	---
Pre-Injury Condition Limiting Physical Activity	Yes	3.09	<0.001
Pre-Injury Treatment for MH Condition/Psych Hosp (ref=Neither)	Yes		
Treatment for MH Condition		1.70	0.060
Both		2.87	0.009
Psychiatric Hospitalization		4.87	0.061
Pre-Injury Suicide Attempt	No	---	---
Pre-Injury Substance Use Problem	No	---	---
Pre-Injury Incarceration	Yes	2.27	0.012
Fit Statistics	**Sample Size**	**Seizure Prevalence**	**C Statistic**
Saturated Model	1039	107 (10.3%)	0.797
Reduced Model			0.770
Optimism Corrected Reduced Model			0.756
Final Prognostic Model for PTS at Year 1			
PTS at Year 1 = -0.35 + 0.80*InjurySeverity + 0.57*SDH + 0.94*$ContusionLoad_1$ + 1.21*$ContusionLoad_2$ + 1.11*$ContusionLoad_3$ + 0.37*$ContusionLoad_4$ + 1.13*$ContusionLoad_5$ + 0.95*Craniotomy + 1.50*Craniectomy + 1.13*PhysicalLimitation + 0.53*TreatMentalHealth + 1.05*TreatMentalHealth&PsychHosp + 1.58*PsychHosp + 0.82*Incarceration			
**Unless noted, reference group for adjust odds ratio is variable not present* *MH: Mental Health; Psych Hosp: Psychiatric Hospitalization*			

Table 17. Prognostic Model for Prediction of PTS at Year 2

Variables in Saturated Model	Retained in Reduced Model	Adjusted Odds Ratio	P-value
Injury Severity	No	---	---
Duration Post-Traumatic Amnesia	No	---	---
Seizure during Acute Care Hospitalization	Yes	2.71	0.038
Subdural Hematoma	Yes	3.73	0.004
Subarachnoid Hemorrhage	No	---	---
Retained Fragment	Yes	3.03	0.049
Contusion Load	No	---	---
Craniectomy	Yes	3.34	0.002
Previous Moderate/Severe TBI	No	---	---
Pre-Injury Condition Limiting Physical Activity	Yes	3.67	0.022
Pre-Injury Treatment for MH Condition/Psych Hosp	No	---	---
Pre-Injury Suicide Attempt	No	---	---
Pre-Injury Substance Use Problem	No	---	---
Pre-Injury Incarceration	No	---	---
Fit Statistics	**Sample Size**	**Seizure Prevalence**	**C Statistic**
Saturated Model	375	45 (12.0%)	0.785
Reduced Model			0.763
Optimism Corrected Reduced Model			0.724
Final Prognostic Model for PTS at Year 2			
PTS at Year 2 = -0.44 + 1.00*SeizureDuringAcuteCare + 1.32*SDH + 1.11*Fragment + 1.21*Craniectomy + 1.30*PhysicalLimitation			
**Unless noted, reference group for adjust odds ratio is variable not present* *MH: Mental Health; Psych Hosp: Psychiatric Hospitalization*			

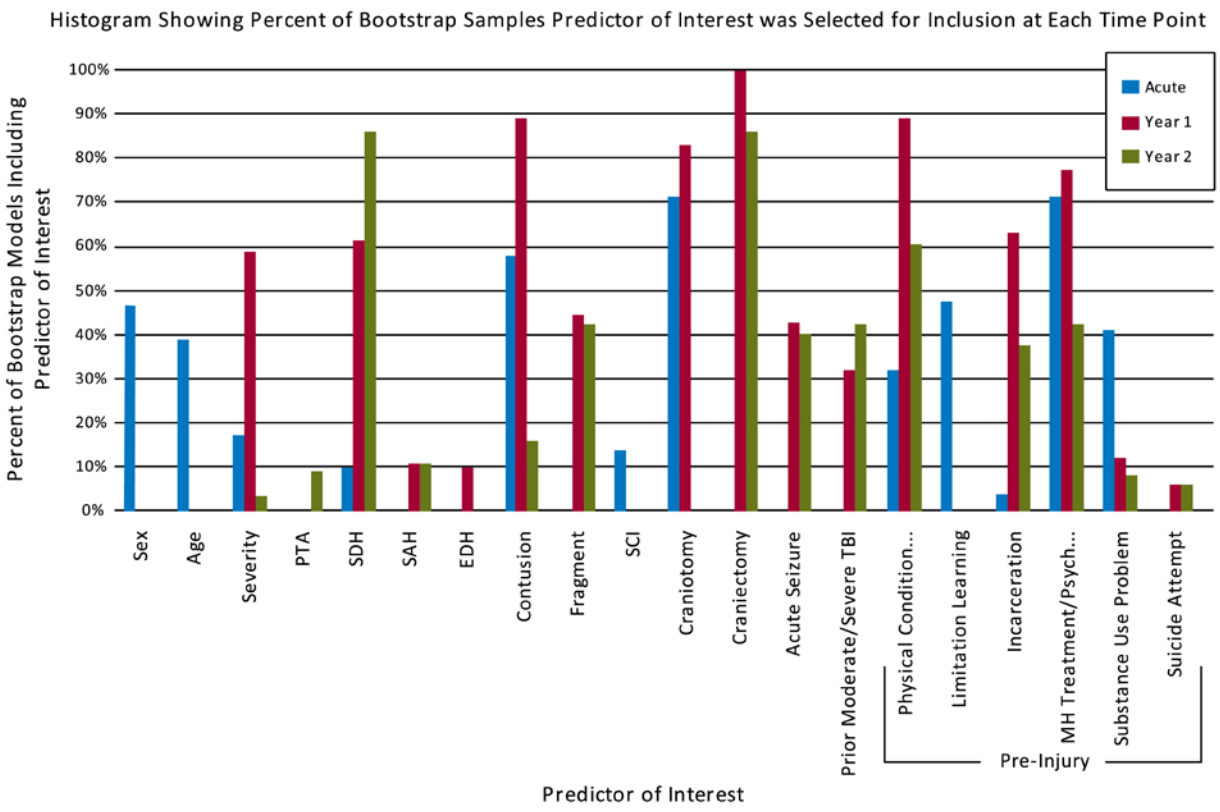

Figure 3. Histogram Depicting Variables Included in Bootstrap Samples for Prognostic Models

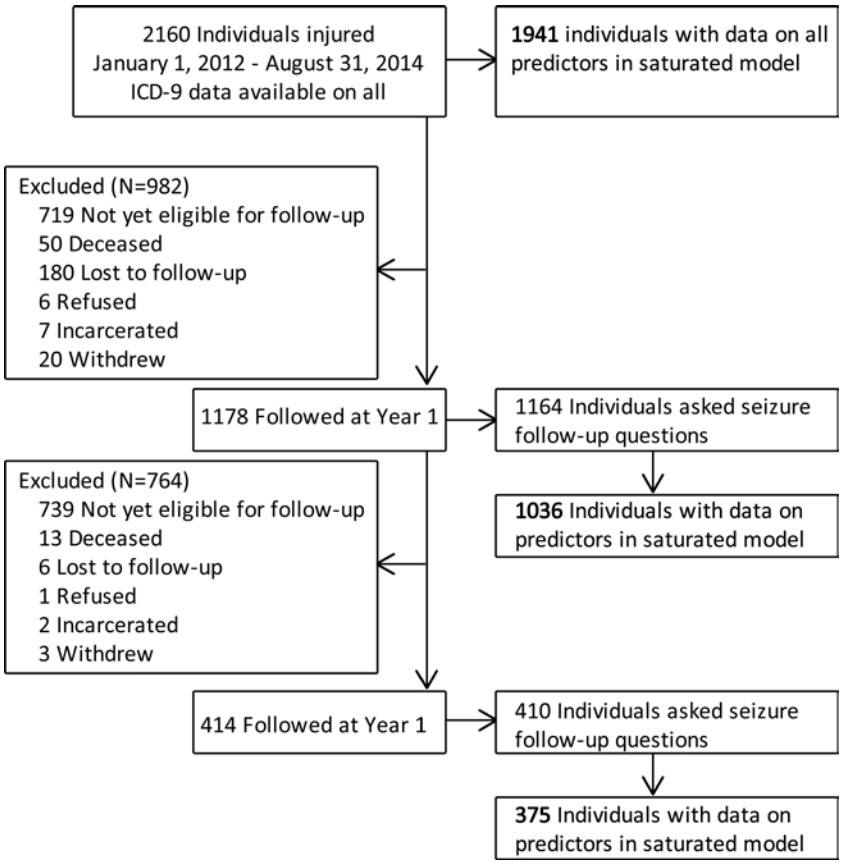

Figure 4. Traumatic Brain Injury Model System Prognostic Model CONSORT Figure

6.0 MANUSCRIPT THREE

GENETIC VARIATION IN NEURONAL GLUTAMATE TRANSPORT GENES AND ASSOCIATIONS WITH POST-TRAUMATIC SEIZURE

Anne C Ritter[1,2], Candace M Kammerer[3], Maria M Brooks[1], Yvette P Conley[4,5], Amy K Wagner[2,5,6,7]

[1]Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA
[2]Physical Medicine & Rehabilitation, University of Pittsburgh, Pittsburgh, PA
[3]Human Genetics, University of Pittsburgh, Pittsburgh, PA
[4]Department of Health Promotion and Human Genetics, University of Pittsburgh, Pittsburgh, PA
[5]Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA
[6]Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA
[7]Center for Neuroscience at University of Pittsburgh, Pittsburgh, PA

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6.1 ABSTRACT

Objective: Post-traumatic seizures (PTS) commonly occur following severe traumatic brain injury (sTBI). Risk factors for PTS have been identified, but variability in who develops PTS remains. Excitotoxicity may influence epileptogenesis following sTBI. Glutamate transporters manage glutamate levels and excitatory neurotransmission and have been associated with both epilepsy and TBI. Therefore, we aimed to determine if genetic variation in neuronal glutamate transporter genes is associated with accelerated epileptogenesis and increased PTS risk after sTBI.

Methods: Individuals (N=253), 18-75yrs with sTBI, were assessed for genetic relationships with PTS. SNPs within *SLC1A1* and *SLC1A*6 were assayed. Kaplan-Meier estimates and log-rank statistics were used to compare seizure rates from injury to 3yrs post-injury for SNPs by genotype. Hazard ratios were estimated using Cox proportional hazards regression for SNPs significant in Kaplan-Meier analyses adjusting for known PTS risk factors.

Results: 32 tagging SNPs were examined (*SLC1A1*: n=28, *SLC1A6*: n=4). 49 (19.37%) subjects had PTS. Of these, 18 (36.7%) seized within 7days, and 31 (63.3%) seized between 8d-3yrs post-TBI. Correcting for multiple comparisons, genotypes at SNP rs10974620 (*SLC1A1*) were significantly associated with time-to-first seizure across the full 3yr follow-up (seizure rates: 77.1% minor allele homozygotes, 24.8% heterozygotes, 16.6% major allele homozygotes; p=0.001). When follow-up started on day 2, genotypes at SNP rs7858819 (*SLC1A1)* were significantly associated with PTS risk (seizure rates: 52.7% minor allele homozygotes, 11.8% heterozygotes, 21.1% major allele homozygotes; p=0.002). Adjusting for covariates, rs10974620

remained significant (p=0.017, minor allele versus major allele homozygotes HR: 3.4, 95%CI: 1.3-9.3). rs7858819 also remained significant in adjusted models (p=0.023, minor allele versus major allele homozygotes HR: 3.4, 95%CI: 1.1-10.5).

Significance: Variations within SLC1A1 are associated with risk of epileptogenesis following sTBI. Future studies need to confirm findings, but variation within neuronal glutamate transporter genes may represent a possible pharmaceutical target for PTS prevention and treatment.

6.2 INTRODUCTION

Traumatic brain injury (TBI) represents an ever-growing public health problem. In the United States, over 2.5 million TBIs occur annually; of these, approximately 300,000 result in hospitalization or death [3]. TBI is a major cause of morbidity and mortality and is increasingly recognized as a disease process with many associated chronic health outcomes. Those with severe TBI (sTBI) have significantly shorter life-spans versus demographically similar, non-TBI, populations [2]. Recent data also show individuals with sTBI are 50 times more likely to die of seizure than age, sex, and racially similar populations [2].

Post-traumatic seizures (PTS), defined as any seizure occurring after TBI, are classified based on time-to-first seizure relative to injury: immediate (<24hours), early (1-7days), and late (>7days post-injury) [41]. Temporal classification cut-offs are attributed to differences in causal pathology and risk of seizure recurrence [18; 31]. PTS incidence varies widely across adult TBI studies, likely due to differences in study design, population, and PTS definitions. In predominantly closed-head injury populations, incidence of immediate/early PTS and late PTS range from 1-12% and 4-19%, respectively [12; 47; 66; 69; 123]. PTS risk factors like injury severity, specific intracranial pathologies, and patient characteristics have been identified [12; 47; 66; 69; 123]. Yet, a high degree of variability regarding who develops PTS remains. Evidence about factors affecting time-to-first seizure and the process of epileptogenesis after TBI is limited. Factors affecting time-to-first seizure can provide information on potential mechanisms associated with epileptogenesis. Previously, using time-to-event analysis, we reported variation in adenosine regulatory and IL-1b genes as associated with time to first PTS [102; 129]. Pathological mechanisms

involving other secondary injury cascades, such as excitotoxicity, are likely contributors to epileptogenesis and may contribute to increased PTS risk chronically after injury [18].

Glutamate is the most prominent excitatory neurotransmitter in the human brain. In response to the primary TBI, there is an immediate release of glutamate into the extracellular space and ion channel activation [58]. These phenomena can lead to neuronal depolarization, disrupted cellular metabolism, and excitotoxic glutamate levels [31]. Excitotoxicity may lead to neuronal and astrocytic swelling, mitochondrial damage, cell death, and immediate/early PTS. Seizures can cause over-activation of excitatory amino acid receptors, inducing calcium dependent production of nitric oxide and reactive oxygen species and free radical damage to DNA and cellular membranes [18]. Moreover, after experimental TBI, studies show regional decreases in glutamate transporter expression that is maintained chronically post-injury and is manipulable with levetiracetam [57]. These observations suggest decreased glutamate clearance, and low-level excitoxicity, is an ongoing mechanism of TBI pathology and contributor to epileptogenesis. Antecedent immediate/early seizure activity may, along with altered glutamate transporter expression, perpetuate excitoxicity and cell death and contribute to epileptogenesis [6].

Genetic variation within glutamate transporter genes may predispose individuals to excitotoxicity after TBI. There are five glutamate transporters in the human central nervous system (CNS), encoded by separate genes. Of these, the *SLC1A1* and *SLC1A6* genes encode the neuronal glutamate transporters, excitatory amino acid transporters (EAAT) 3 and 4. EAAT3/4 contribute to, but are not the main transporters responsible for extracellular glutamate uptake in most brain regions. However, in brain regions where astrocyte expression is limited, neuronal glutamate transporters may play a more dominant role in glutamate clearance. EAAT4 expression is limited to Purkinje cells, while EAAT3 is expressed on and within multiple neuron

types in various regions [152]. In addition to its role in glutamate signal termination, studies suggest EAAT3 is vital to glutathione and GABA synthesis [35; 153; 154]. Evidence of intracellular EAAT3 localization suggests yet other functions in addition to glutamate reuptake and GABA synthesis. However, disruption of these functions could potentially increase seizure susceptibility through excitoxicity, decreased antioxidant reserves, or decreased inhibitory neurotransmission. Previous research demonstrates variation within *SLC1A1* and *SLC1A6* and augmented EAAT3/4 expression, is associated with multiple neurological conditions including multiple sclerosis, schizophrenia, and epilepsy [154]. Neuronal glutamate transporters associations with multiple neurological disorders reflects the importance of the excitatory/inhibitory balance and suggests multiple variants may alter function and/or expression and contribute to individual phenotypes and pathologies.

Therefore, we hypothesized genetic variation in neuronal glutamate transporter genes *SLC1A1* and *SLC1A6* would be significantly associated with epileptogenesis, measured as differences in time-to-first seizure, following sTBI. Additionally, we hypothesized different variants would be associated with PTS in sub-components of a 3-year time frame.

6.3 METHODS

6.3.1 Study Design and Population

Individuals were recruited to participate in a larger study assessing genetic relationships with TBI outcomes. Patients ages 18-75 presenting consecutively to a Level 1 trauma center with sTBI (Glasgow Coma Scale≤8), with positive head CT findings and requiring extra-ventricular

drainage catheter placement for intracranial pressure management, were screened. Patients were excluded if they had penetrating head injury, prolonged cardiac or respiratory arrest prior to admission, or legal proxy consent could not be obtained. To remove genetic effects of population stratification, analyses were limited to individuals listed as white by self/proxy-report (n=25 participants excluded). Individuals with a premorbid seizure history (n=5) were also excluded due to inability to attribute seizure to injury or pre-existing pathology, leaving 253 individuals analyzed. The University of Pittsburgh Institutional Review Board approved all informed consent and study procedures.

6.3.2 Critical Care Management of Severe TBI

All patients were admitted to the neurotrauma intensive care unit and received treatment consistent with The Guidelines for the Management of Severe Head Injury [155]. Generally, patients with sTBI received PTS prophylaxis for 1 week.

6.3.3 Demographic and Injury Related Data

Demographic and injury related data were documented at study enrollment. Intracranial pathology type was separated into seven categories using ICD-9 classification derived from radiological findings. These categories were dichotomized by injury types (present or absent) and were not mutually exclusive. Admission GCS was used to establish study eligibility, but the best GCS score during the first 24 hours after admission was used as a covariate in analyses. Injury severity score (ISS) is an overall body injury measure extracted from medical records,

with a maximum score of 75 based on survivability of injuries within and across body regions [156]. Medical records were reviewed for antiepileptic drug use during acute care.

6.3.4 Single Nucleotide Polymorphism Selection

Tagging single nucleotide polymorphisms (SNPs) for *SLC1A1* and *SLC1A6* were evaluated based on data available from the National Center for Biotechnology Information, HapMap Build 36. SNPs with minor allele frequency (MAF) ≥20% and pairwise r^2 ≥80% with respect to other known SNPs in the genes selected to optimize the heterozygosity of the SNPs and to facilitate analysis of common variants among unrelated individuals. Identified SNPs captured variability in the genes including 1000 bases 5' upstream into the promoter region.

6.3.5 DNA Extraction and Genotyping

DNA was extracted from cerebrospinal fluid (CSF), collected via passive drainage, using Qiamp DNA extraction protocol (Qiagen) or from whole blood using a published salting out procedure [157]. DNA samples were genotyped using iPLEX Gold SNP Assay (Sequenom). Double-masked genotype assignments were made for each SNP, and discrepancies were addressed using raw data or re-genotyping. Assays included blind duplicates for quality assessment. Genotypes for SNPs representing variability within *SLC1A1* and *SLC1A6* were evaluated. All SNPs were evaluated for Hardy-Weinberg Equilibrium (HWE), MAF, and LD (**Figure 1**) specific to the study population using Haploview [158].

6.3.6 Outcome Measure: Post-Traumatic Seizure

Time-to-first seizure following TBI was the primary outcome of interest. PTS status was obtained by retrospective review of all electronic inpatient and outpatient medical records available from our medical center. Date of first seizure was determined by ambulance and/or emergency room report, inpatient progress or nursing note, EEG report, patient history, and discharge or transfer summaries. Medical record notation referring to convulsions, seizures, status epilepticus, or seizure disorder was considered evidence of seizure occurrence. Date of death was also extracted from medical records or from social security death data (http://www.ssa.gov/sitemap.htm). All participants were followed until date of first seizure or date of death. Follow-up was censored at 3 years post-injury.

6.3.7 Statistical Analysis

Analyses were completed using SAS-9.4 (Carry NC) and R-3.0.3. All genotyped participants meeting eligibility criteria were included in analyses. Demographic and injury characteristics were compared between individuals who did not seize and those who seized at different time-points post-injury, using chi-square and Kruskal Wallis tests as appropriate. Individuals who seized were separated into groups based on time of first seizure (i.e. immediate, early, late). Due to small sample sizes, immediate and early groups were collapsed for comparison of demographic and injury characteristics.

Among individuals who seized, Chi-square analyses were conducted, using Fisher's exact test when appropriate, to determine if genotype frequencies differed by time of first seizure (i.e.

immediate, early, late). Immediate and early seizures were again combined due to small sample size and compared to late seizure.

Time-to-event analyses were used to address the primary hypothesis regarding genetic variation and epileptogenesis after TBI over a 3-year time period. Due to linkage disequilibrium (LD), i.e. correlation among selected SNPs, the effective number of tests conducted was smaller than the number of SNPs screened. The minimum number of effective tests (M_{eff}) was calculated using methods based on eigenvalues[159; 160]. The M_{eff} was calculated for *SLC1A1* and *SLC1A6* independently, and results were summed. A Bonferroni correction was then applied to the original α=0.05 using the total M_{eff} as the number of independent tests for subsequent time-to-event analyses. SNPs that were statistically significant after multiple comparison correction were further evaluated using Cox regression.

Kaplan-Meier curves were used to estimate seizure rates at three years post-injury, considering the full follow-up period (i.e. time of injury through 3 years post-TBI), for individual SNPs by genotype, and rates were compared using the log-rank statistic. Cox proportional hazards regression was used to estimate hazard ratios (HR) for SNP genotypes that demonstrated significantly different Kaplan-Meier estimated rates of PTS based on Bonferroni corrected p-values derived from log-rank statistics. Cox regression models were then adjusted for demographic and injury characteristics that differed significantly across seizure groups (no seizure, immediate/early, late seizure). Proportionality assumptions were examined for all variables. All time-to-event analyses were repeated 1) where immediate seizures were removed by beginning the follow-up period day 2 post-injury (individuals seizing or expiring before day 2 excluded) and 2) where both early and immediate seizures were removed by beginning the

follow-up period on day 8 post-injury (individuals seizing or expiring before day 8 excluded), to specifically examine late PTS.

For each follow-up timeframe, a gene risk score (GRS) was created using all SNPs that were nominally significant ($p<0.05$) in Kaplan-Meier analyses in order to explore possible additive effects of having multiple risk genotypes. The number of SNPs for which an individual was homozygous for the minor allele (risk genotype for all SNPs based on Kaplan-Meier results) was summed. The subsequent GRSs were then analyzed for associations with time-to-first seizure in their respective follow-up period.

6.4 RESULTS

We identified and genotyped 32 SNPs from *SLC1A1* and 4 SNPs from *SLC1A6*. Four SNPs on *SLC1A1* failed to genotype for >20% of the total population and were excluded. All other SNPs were in HWE. Therefore, we examined a total of 28 SNPs from *SLC1A1* and 4 from *SLC1A6* (**Supplemental Table 1**). M_{eff} calculations indicated a total of 16 independent tests (14 and 2 for *SLC1A1* and *SCL1A6*, respectively), resulting in a Bonferroni adjusted significance level of 0.003.

Two hundred fifty-three individuals met all inclusion criteria and were genotyped. Similar to other studies of severe TBI, our study population was predominantly male (79.5%), average 35.3 years old. The majority of individuals had severe TBI as determined by best in 24hr GCS score (91.7%); the average best in 24hr GCS score for the total cohort was 6 (**Table 1**).

Overall, 49 individuals (19.4%) developed PTS. Of these, 12 (24.5%) seized within 24 hours, 6 (12.2%) seized within the first 7 days, and 31 (63.3%) seized between 8d-3yrs post-

injury. Depressed skull fracture and subdural hematoma (SDH) occurred more frequently in individuals who seized compared to those who did not seize (**Table 1**). Among individuals who seized, there were no differences in genotype frequencies between individuals seizing immediately and early versus those seizing late (data not shown).

SDH and depressed skull fracture frequencies differed significantly by seizure status. Therefore, we adjusted for these factors in all Cox regression models. SDH was included as a covariate. Since depressed skull fracture did not meet proportionality assumptions, models were stratified by presence/absence of depressed skull fracture. All SNPs met the assumption of proportionality.

6.4.1 Time-to-Event Across Full Follow-up Period

We found significant differences in seizure rates by genotype for rs10974620 (p=0.001), located on *SLC1A1*, when assessing the full follow-up period (**Table 2**). 24.8% of major allele homozygous (CC), 16.6% of heterozygous, and 77.1% of minor allele homozygous (GG) individuals seized during follow-up. Among those who seized, the average time-to-first seizure was twice that for heterozygous and major allele homozygous individuals versus minor allele homozygotes (786, 794, 384 days, respectively). Three additional SNPs in *SLC1A1* (rs10815020, rs7858819, and rs301430) were nominally associated with differences in seizure rates. For each SNP, minor allele homozygotes had the highest risk of seizures 3yrs post-injury and the shortest time-to-first seizure (**Figure 2**).

In both unadjusted and multivariable adjusted Cox regression (adjusting for covariates noted above) we found significant differences in seizure risk by SNP rs10974620 genotypes (p=0.004 and p=0.017, respectively). Individuals homozygous for the minor allele had a

significantly higher hazard of seizure (unadjusted HR=4.08; adjusted HR=3.43) versus major allele homozygotes (**Table 3**). There was no significant difference in hazards between heterozygotes and individuals homozygous for the major allele. In adjusted models, SDH was significantly associated with increased seizure risk (HR=2.36, p=0.016).

No SNPs on *SLC1A6* had significantly different time-to-first seizure based on Kaplan Meier analyses, and were not further included in Cox models.

6.4.2 Time-to-Event Removing Immediate Seizures

When follow-up began on post-injury day 2 (i.e. only early and late seizures included), there were significant differences in the 3-year seizure rates by genotype for *SLC1A1* SNP rs7858819 (p=0.002). Minor allele homozygous (TT) individuals had the highest seizure rates (52.7%) versus heterozygotes (11.8%) and major allele homozygotes (CC; 21.1%) (**Figure 3**). Among those who seized, minor allele homozygous individuals had the shortest time-to-first seizure (270 days) versus major allele and heterozygotes (520 and 937 days, respectively). There were nominal differences by genotype for *SLC1A1* SNP rs10974620 (p=0.004) (**Table 2**). In Cox regression, seizure risk for SNP rs7858819 minor allele homozygous individuals was significantly greater versus major allele homozygotes (HR=3.9, p=0.005). After adjusting for SDH and stratifying by depressed skull fracture status, the rs7858819 genotype effects were attenuated, but remained significantly associated with seizure risk (HR=3.39, p=0.023; **Table 3**). In the adjusted model, SDH was also significantly associated with seizure (HR=3.11, p=0.013).

6.4.3 Time-to-Event Examining Late Seizures Only

To determine if there were significant associations with genotypes in epileptogenesis of late PTS, analysis was restricted to follow-up beginning on post-injury day 8. Associations with genotypes at SNPs rs10974620 (p=0.044) and rs7858819 (p=0.009) were nominally significant (**Table 2**).

6.4.4 Gene Risk Scores

Across the full follow-up, Kaplan-Meier estimates differed significantly for individuals with no risk genotypes, one, or more than one risk genotypes (3yr seizure rates: 16.7, 45.5, 42.9%, respectively; log-rank p-value <0.001). Results were similar when a gene risk score was calculated for follow-up beginning on day 2. 3yr seizure rates were 12.8, 33,3, 33.3% for individuals with 0, 1, or more than 1 risk genotype (log-rank p-value=0.005). However, there were no significant differences between individuals having one risk genotype versus those with more than one risk genotype (data not shown).

6.5 DISCUSSION

Variation in genotype, and changes in neuronal glutamate transporter expression, has been associated with seizure and epilepsy. TBI results in decreased glutamate transporter expression [57], potentially perpetuating ongoing excitotoxic injury and damage after TBI, as well as facilitating a pro-epileptogenic environment. However, it remains unclear whether genetic

variation in neuronal glutamate transporters affects epileptogenesis or seizure development following TBI. Therefore, we examined associations between *SCL1A1* and *SLC1A6* genetic variation and epileptogenesis, measured by time-to-first seizure, among individuals with severe TBI.

We found genetic variation in *SLC1A1*, but not *SLC1A6*, was associated with reduced time-to-first seizure and increased seizure risk during a 3-year post-injury follow-up. Individuals homozygous (GG) for the minor allele at SNP rs10974620 had significantly higher seizure risk over the 3yr follow-up period, even after adjusting for relevant covariates. Individuals homozygous (TT) for the *SLC1A1* SNP rs7858819 minor allele also had greater risk of PTS in multivariable models when follow-up began on day 2 post-injury (after the immediate seizure period). Both SNPs were nominally associated with the other time periods characterized. We found no significant differences in seizure risk when comparing individuals with one risk genotype to those with more than one risk genotype in each timeframe, suggesting no additive genetic effects. The high degree of LD (**Figure 1**) among genotypes for SNPs significantly associated with seizure risk within our study population, approximately 2,600bp from one another within the same intron, is one possible explanation for this finding. Although the sample size is small, possible differences in SNP associations with PTS over time may indicate that genetic variants within *SLC1A1* influence temporally dynamic PTS risk post-injury. Future follow-up studies should examine this hypothesis.

The *SLC1A1* locus encodes EAAT3, is located on chromosome 9p24, and is approximately 97kb in length. SNPs rs10974620 and rs7858819 are both located within the second intron. We used the most recent Genome Reference Consortium data, GRCh38, from Utah residents with northern/western European ancestry (CEPH;

http://hapmap.ncbi.nlm.nih.gov/citinghapmap.html.en) to explore gene regions around rs10974620 and rs7858819. In the CEPH population, little information regarding LD for rs10974620 is available. However, rs7858819 may be tagging a region that contains multiple functional variants as it is in LD with a 13.5kbp region extending from intron two into intron five that includes multiple missense polymorphisms.

EAAT3 terminates post-synaptic action and maintains physiological levels of glutamate. However, EAAT3 is not as critical for terminating glutamate signaling when compared to glial glutamate transporters (EAAT1/EAAT2) [161]. EAAT3's binding affinity and synaptic location suggests it may have a more prominent role in glutamate signal termination in pathological conditions involving elevated extracellular glutamate levels [59]. EAAT3 also facilitates cysteine transport [162], and thus, cysteine dependent glutathione (antioxidant) production [35]. EAAT3 is highly expressed on glutamatergic and GABAergic neurons [152] in the cortex, hippocampus, cerebellum, and basal ganglia [163]. Regional expression suggests EAAT3 mediated glutamate transport supplies GABAergic neurons with intracellular glutamate required for GABA production. Using a similar cohort, our laboratory previously reported variation in the gene encoding glutamic acid decarboxylase (GAD1), responsible for synthesizing GABA from glutamate, is also associated with increased late PTS risk [104]. Therefore, disruption in EAAT3 function and/or expression may reduce antioxidant reserves and impair GABA production, subsequently increasing excitatory tone and contributing to epileptogenesis.

Animal models examining EAAC1 (EAAT3 rodent analog) in epilepsy and seizure induction show EAAC1 antisense treatment reduces EAAC1 availability and increases epilepsy development in a dose dependent manner [164]. In a similar antisense treatment model, functional EAAC1 loss was associated with proportional increases in epileptiform activity and EEG

abnormalities [153]. The same study showed greater excitability in EAAC1 anti-sense treated animals, and decreased GABA levels in EAAC1 antisense treated animals versus controls [153]. Other experimental models involving chemically induced epilepsy demonstrate significantly increased EAAC1 levels compared to controls [165], suggesting increased EAAC1 expression is needed to manage elevated glutamate levels associated with seizure. Kainic acid induced epilepsy models also show EAAC1 can translocate from the membrane to the intracellular space during early epileptic activity [166]. Another study using pentylenetetrazol kindling showed increased EAAC1 expression 24 hours after seizure, but animals with lower EAAC1 levels were more easily induced into an epileptic state [167]. Taken together, low EAAC1 expression increases seizure susceptibility, and changes in EAAC1 expression or location within the epileptic brain may compensate for or contribute to glutamatergic mechanisms of epileptogenesis.

EAAT3 expression studies in humans are few, but they report individuals with temporal lobe epilepsy (TLE) having altered neuronal EAAT3 mRNA compared to controls [165; 168]. Studies report differences in EAAT3 immunoreactivity linked to the presence of hippocampal sclerosis, with increased EAAT3 immunoreactivity occurring on granule cells from sclerotic regions [168]. Conversely, among individuals with pharmacoresistent neocortical epilepsy, EAAT3 expression was decreased in epileptic regions compared to non-epileptic tissues from the same individuals [169].

Multiple candidate gene studies have reported *SLC1A1* genetic variation with psychiatric conditions including post-traumatic stress disorder [170], autism spectrum disorder [171], and schizophrenia [154]. The most data regarding human *SLC1A1* genetic variation and psychiatric disorders is reported with obsessive-compulsive disorder (OCD). Both family based linkage studies and case-control association studies of unrelated individuals have reported *SLC1A1*

variation is associated with OCD diagnosis and age of onset [172; 173]. *SLC1A1* haplotypes associated with OCD have been reported and include SNPs examined within our current analysis [172; 173], but SNPs included and risk allele designations differ across studies. One study reported that a three SNP haplotype, including rs301430 and rs7858819 C-alleles, was significantly associated with OCD [173]. These investigators also examined mRNA levels of *SLC1A1* from brain tissue of individuals with bipolar and schizophrenic disorders and healthy individuals. They reported that an increasing the number of minor alleles for rs301430 and rs7858819 was associated with increased mRNA levels [173]. Overall, these studies, while not drawing consistent conclusions regarding risk alleles, provide evidence that genetic variation within specific regions of *SLC1A1* is associated with pathological phenotypes. Further investigation is required to identify potentially functional variants and establish how they may be associated with phenotypes under investigation. Similarly, our study suggests further work is needed to evaluate potentially functional SNPs in regions tagged by rs7858819 and rs10974620 and their potential association with PTS.

In addition to activity-related expression changes, EAAT3/EAAC1 expression and trafficking can be modified via post-translational mechanisms and interaction with multiple kinases [154], potentially affecting glycosylation and phosphorylation sites important for transporter function and post-translation regulation [152]. EAAT3 also interacts with intracellular proteins for proper anchoring on cell membrane and EAAT3 trafficking [154]. Specific alleles may result in changes to amino acid residues or protein misfolding, disrupting these interactions and affecting membrane protein expression.

Additional studies are needed to examine differences in *SLC1A1* expression among individuals with TBI who do and do not develop PTS. Further research regarding how specific

genetic variation may effect translation, expression, and/or trafficking of EAAT3 is essential to assess whether pharmacological modulation may mitigate or prevent PTS. Specifically, animal TBI models that lead to post-traumatic seizure may also provide specific opportunities to investigate, not only glutamate concentration and reduced EAAC1 expression after TBI, but also if levetiracetam associated increases in post-TBI glutamate transporter expression [57] translate at all to reduced PTE. Additionally, EAAT3's function differs across neuro-developmental phases. Thus, effects of *SLC1A1* genetic variation on PTE may vary across the age spectrum of those sustaining TBI. Also, TBI-induced EAAT3 disruption may interact with genetic variation to impact neuroplasticity during the post-injury period. In addition to examining the role of neuronal glutamate transporters, future work should assess whether glial transporters are independently associated with epileptogenesis post-TBI and how these transporters may work collectively with other candidate gene variants to affect excitatory and inhibitory pathways influencing PTS.

Our results represent novel insights regarding the relationship between genetic *SLC1A1* variation and PTS risk. However, our results are limited by small sample size and low event rates. Although we did not find statistically significant relationships between SNPs and late PTS risk, it is possible there was not sufficient power to detect differences. Time-to-first seizure was classified based on an intensive medical record review of individuals cared for through the largest health care provider in the geographic region. However, seizure status and time-to-first seizure may have been misclassified due to missing data on healthcare provided outside of this system. To minimize differences in allelic frequency by race and ancestry, we limited our analyses to individuals self-reporting race as white, however, residual population stratification may still remain. Furthermore, our results cannot be generalized to other non-European ancestry

populations. We also included only individuals with severe TBI, and our results may not generalize to less severe TBI populations. Critically, additional studies are needed to replicate our findings in similar populations and also assess associations with functional SNPs within the LD block implicated in our study. Increasing our knowledge of genetic variants affecting PTS development may improve prognostic seizure models (In Review), possibly enabling researchers and clinicians to assess more accurately the probability of individual PTS development. If validated, these results may represent an innate factor by which to identify individuals with increased PTS risk; also EAAT3 may be a potential therapeutic target for PTS prevention and treatment.

Disclosure: We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. No author has any conflict of interest.

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Figure Legends

Figure 1. Haploview generated gene map displaying linkage disequilibrium (D') for SNPs located on *SLC1A1* (panel A) and *SLC1A6* (panel C). Deeper red colors are indicative of greater D' values. Panel B shows a magnified view of SNPs on *SLC1A1* shown to be associated with

time to first seizure in the current analyses (19=rs10974620, 20=rs10815020, 21=rs7858819, 24=rs301430).

Figure 2. Kaplan Meier estimates for time to first seizure by *SLC1A1* SNP rs10974620 genotypes for full follow-up (Time of Injury to Three Years).

Figure 3. Kaplan Meier estimates for time to first Seizure by *SLC1A1* SNP rs7858819 genotypes for follow-up beginning day 2 post-injury to three years (individuals seizing or expiring before day 2 excluded).

6.6 TABLES

Table 18. Population and Injury Characteristics by Seizure Status

	No Seizure	Immediate/Early Seizure	Late Seizure	P value*
Sample Size	204 (80.6)	18 (7.1)	31 (12.3)	---
Age at Injury, mean (SD)	35.40 (15.71)	37.17 (16.57)	33.65 (13.47)	0.825
Sex, males	163 (79.9)	14 (77.8)	24 (77.4)	0.935
Admission GCS				0.543
Severe (3-8)	187 (91.7)	17 (94.4)	28 (90.3)	
Moderate (9-12)	16 (7.8)	1 (5.6)	2 (6.5)	
Mild (13-15)	1 (0.5)	0 (0)	1 (3.2)	
ISS, mean (SD)	35.87 (9.69)	34.94 (8.21)	31.7 (7.8)	0.057
Received Acute Seizure Prophylaxis	192 (94.1)	18 (100)	31 (100)	0.422
Depressed Skull Fracture	28 (13.7)	3 (16.7)	10 (32.3)	**0.039**
Subdural Hematoma	119 (58.3)	13 (72.2)	26 (83.9)	**0.013**
Subarachnoid Hemorrhage	140 (68.6)	12 (66.7)	20 (64.5)	0.874
Diffuse Axonal Injury	65 (31.9)	5 (27.8)	8 (25.8)	0.814
Epidural Hemorrhage	27 (13.2)	4 (22.2)	7 (22.6)	0.226
Contusion	101 (49.5)	8 (44.4)	14 (45.2)	0.863
Intraventricular Hemorrhage	66 (32.4)	5 (27.8)	8 (25.8)	0.788
Intracerebral Hemorrhage	73 (35.8)	7 (38.9)	10 (32.3)	0.880
* p-value for chi-square and Kruskal-Wallis tests comparing 3 groups				

Table 19. SNPs in SLC1A1 with Significantly Different Seizure Rates Determined by Comparison of Kaplan Meier Curves using Log Rank Statistic

	Full Follow-Up		**No Immediate Events**		**No Immediate or Early Events**	
	3Yr Seizure Rate (%)	**P-value**	**3Yr Seizure Rate %**	**P-value**	**3Yr Seizure Rate %**	**P-value**
rs10974620		**0.001**		0.004		0.044
CC	24.8		20.3		19.7	
GC	16.6		12.0		10.7	
GG	77.1		71.4		66.7	
rs10815020		0.007		0.050		0.134
AA	25.8		22.6		21.9	
AG	21.1		14.9		13.8	
GG	58.4		48.0		42.9	
rs7858819		0.035		**0.002**		0.009
CC	23.7		21.1		20.5	
CT	21.2		11.8		10.6	
TT	56.4		52.7		47.5	
rs301430		0.033		0.018		0.072
TT	25.8		22.1		20.7	
CT	18.9		12.3		12.3	
CC	49.3		42.5		38.1	
All genotypes in order of major allele homozygous, heterozygous, minor allele homozygous						

Table 20. Results from Unadjusted and Adjusted Cox Proportional Hazards Regression Models for Two SNPs in SLC1A1

Model	Hazard Ratio	95% Confidence Interval	P-Value
Unadjusted Models			
rs10974620 Ref = CC			**0.004**
CG	0.67	0.34 – 1.34	
GG	4.08	1.58 – 10.55	
rs7858819 Ref = CC			**0.005**
CT	0.51	0.22 – 1.18	
TT	3.90	1.35 – 11.31	
Adjusted Models*			
rs10974620 Ref = CC			**0.017**
CG	0.68	0.34 – 1.35	
GG	3.43	1.26 – 9.34	
rs7858819 Ref = CC			**0.023**
CT	0.56	0.24 – 1.32	
TT	3.39	1.10 – 10.46	
rs10974620 from full follow-up model; rs7858819 from model beginning day 2 post-injury *Adjusted for subdural hematoma, stratified by depressed skull fracture			

Table 21. Supplemental Table: Allele Frequency and Location Information for SNPs Examined in Time to First Seizure Analyses

Gene	SNP	Base Pair Position	Minor Allele Frequency	Alleles
SCL1A1				
	RS7045401	4493526	0.34	T:G
	RS10814991	4495254	0.43	C:T
	RS10814993	4497428	0.33	A:C
	RS10739062	4502848	0.45	C:G
	RS10491732	4506655	0.35	C:T
	RS7030825	4509735	0.32	C:T
	RS7041093	4512200	0.25	T:C
	RS12342908	4516255	0.29	G:A
	RS17755777	4516768	0.29	T:C
	RS10815002	4524549	0.30	T:C
	RS7021569	4527113	0.31	C:G
	RS7025968	4528150	0.47	C:G
	RS7848533	4539377	0.47	A:C
	RS10758631	4546319	0.46	C:A
	RS6476876	4548122	0.34	C:G
	RS10739065	4550752	0.32	A:C
	RS10758632	4552509	0.21	G:C
	RS10739066	4555923	0.41	A:T
	RS10974620	4557296	0.20	C:G
	RS10815020	4557770	0.26	A:G
	RS7858819	4559892	0.22	C:T
	RS7022772	4566210	0.26	C:A
	RS2072657	4576451	0.29	T:G
	RS301430	4576680	0.27	T:C
	RS301979	4576851	0.29	C:G
	RS6476879	4577346	0.41	C:A
	RS301434	4582082	0.46	G:A
	RS3087879	4586808	0.35	G:C
SCL1A6				
	RS10414225	15062223	0.36	G:T
	RS873599	15068860	0.30	T:A
	RS10403281	15080117	0.28	G:C
	RS3746295	15083693	0.43	A:C

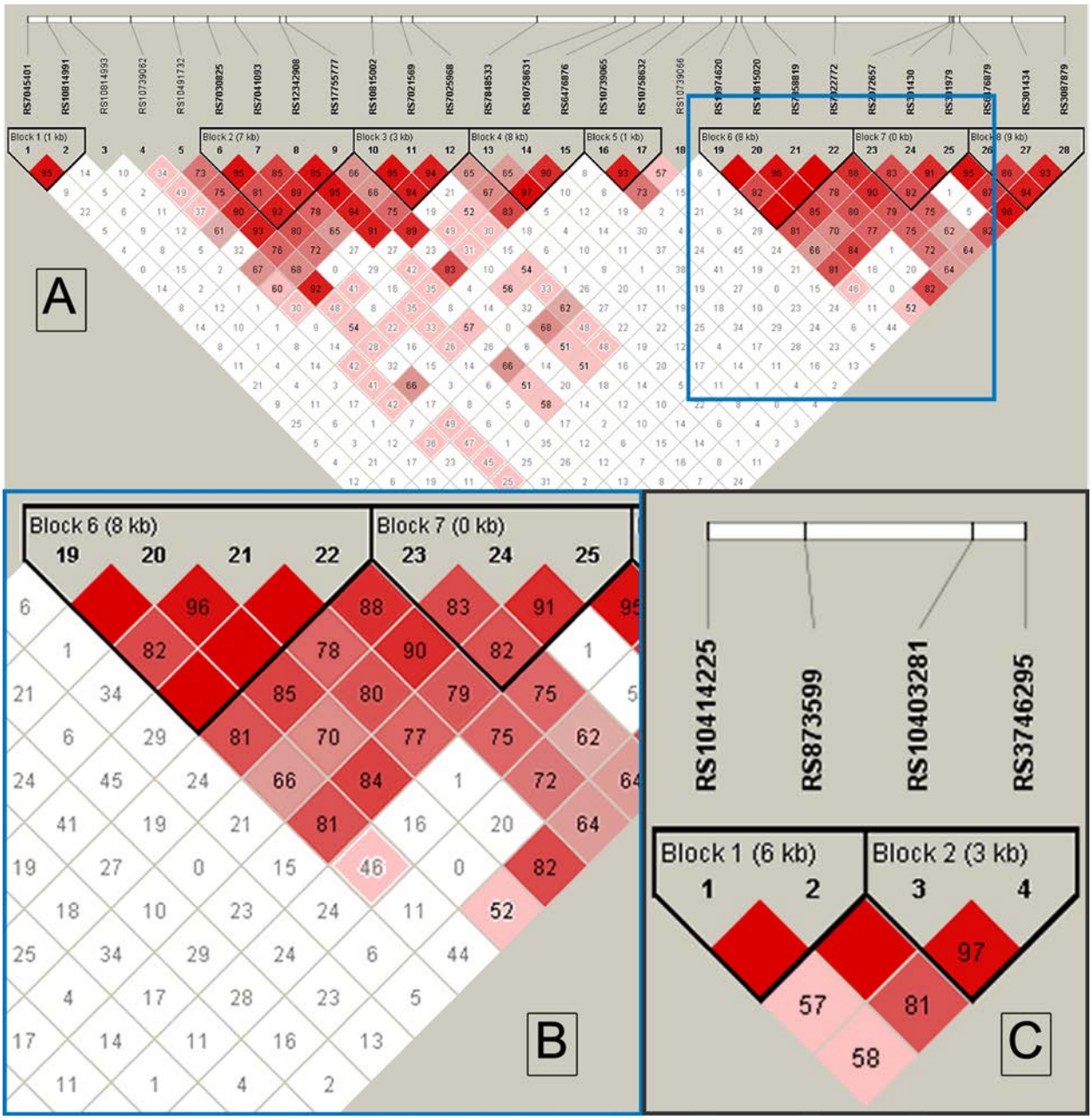

Figure 5. Linkage Disequilibrium Maps for SLC1A1 and SLC1A6

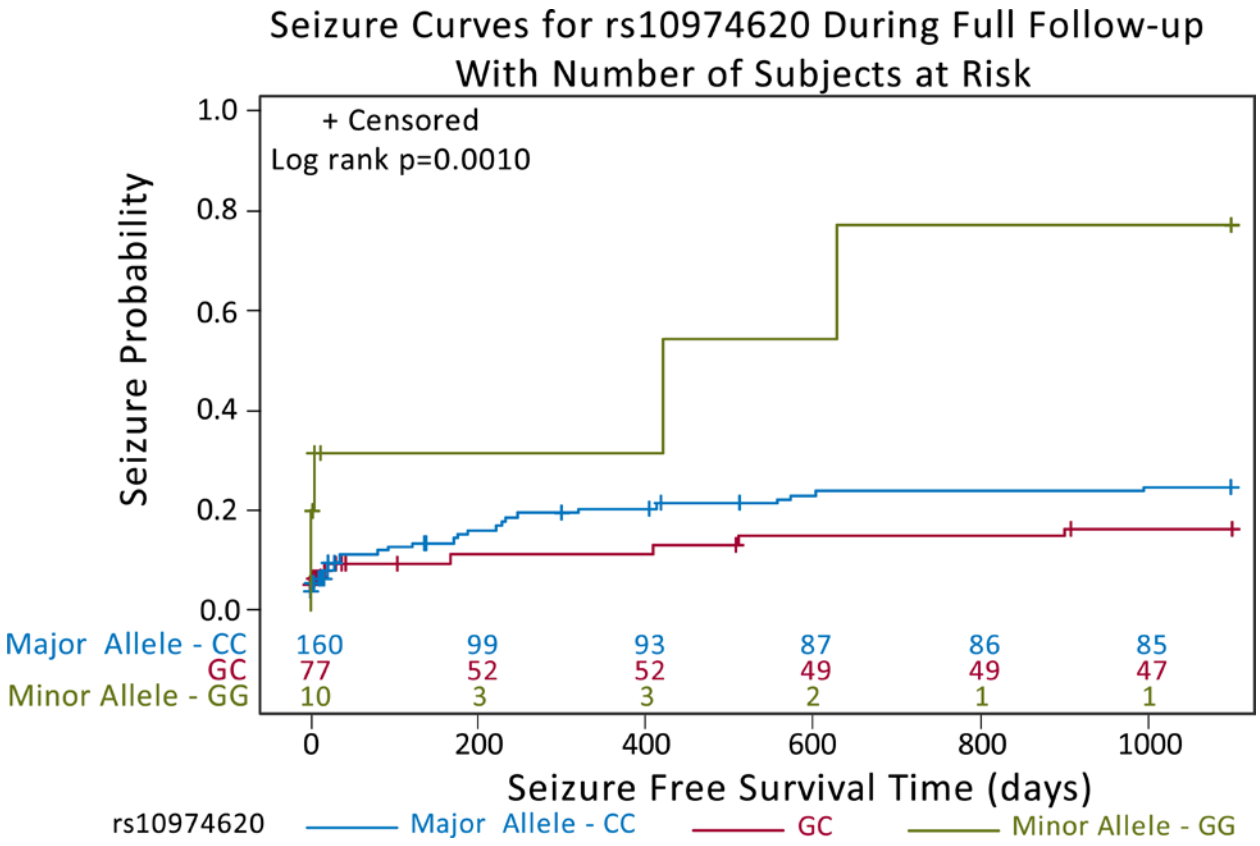

Figure 6. Seizure Curves for rs10974620 During Full Follow-up

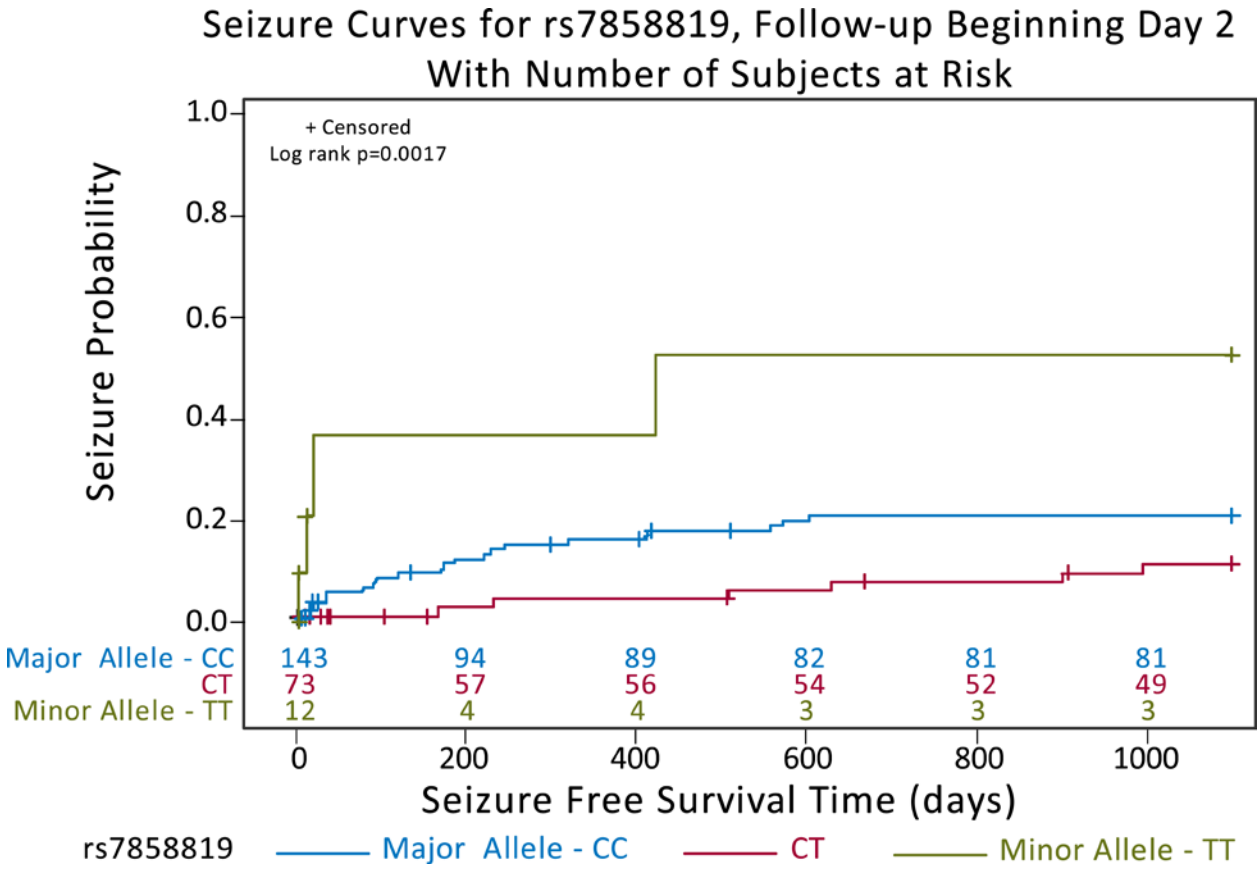

Figure 7. Seizure Curves for rs7858819, Follow-up Beginning Day 2

7.0 GENERAL DISCUSSION

7.1 SUMMARY

In the current body of work, we present relatively recent epidemiological trends and risk factors of PTS and developed prognostic models for PTS at different time-points post-TBI.

Immediate seizures are usually considered a direct response to the primary head injury, non-epileptic in nature, and do not influence treatment decisions in an acute setting, with the exception of standard seizure prophylaxis[44]. Yet, **Manuscript One** demonstrates individuals who developed immediate seizures have a significantly greater risk of late PTS at 1 and 5 years post-TBI compared to individuals with no seizure activity during their acute hospitalization. Conversely, those with early seizure were not at greater risk. Any seizure during acute hospitalization was also identified as a predictor of late PTS. Although an earlier seizure is consistently cited as a risk factor for late seizure [12; 47; 69; 77; 78], the current analysis is one of the first reports to specifically delineate the impact of immediate and early seizure separately on late PTS. These findings support the concept that immediate seizures may themselves initiate pathological mechanisms that facilitate/accelerate epileptogenesis.

Prognostic models developed in **Manuscript Two** identified significant predictors, such as personal history and neurosurgical procedures, which could impact screening of patients with TBI for potential risk of late PTS and could justify research re-evaluating seizure prophylaxis

guidelines, particularly for those deemed at high risk. We also identified relationships between seizure during acute hospitalization and late PTS risk, with additional implications for the potential efficacy of current seizure prophylaxis guidelines.

Manuscript Three identified SNPs within neuronal glutamate transporter genes that are significantly associated with seizure risk and epileptogenesis following moderate to severe TBI. These findings add to existing research regarding personal biology in PTS risk and have multiple implications regarding future directions for PTS genetics research, stratified clinical trials, and novel drug discovery or repurposing for PTS that may impact practice and policy.

7.2 IMPLICATIONS AND RECOMMENDATIONS

Findings form the current body of work highlight existing and introduce new concepts that may affect future research and programmatic decisions impacting those at risk for PTS. The finding that immediate seizure increases late PTS risk must be further examined to determine if this result can be replicated. If validated in external populations, individuals with immediate seizures following TBI may represent a subpopulation that would benefit from increased and/or prolonged seizure monitoring. Individuals seizing immediately post-injury who receive prophylaxis and develop late PTS may also represent a high-risk population with genetic variation within pathways relevant to excitotoxic injury that could provide insight into epileptogenesis and biological pathways outside of the effects of AEDs. Future work should examine the effect of immediate seizures on late PTS risk, controlling for other known risk factors. Additionally, the effects of prophylactic and anti-epileptic medications on late PTS risk

and pathology must be examined in more detail, including temporal relationships regarding AED administration and attaining effective serum levels.

Additionally, novel prognostic markers of acute symptomatic and late PTS were identified. Future research to validate the models in external populations is necessary. If validated, the prognostic models could be used to develop clinical decision algorithms and for providing a much-needed tool to assess the probability of developing PTS at an individual level, such as readily accessible risk assessment calculators similar to the Breast Cancer Risk Assessment Tool based on validated prognostic models[174]. These tools could enable physicians to more accurately discuss the risk of PTS with individual patients, encourage more frequent monitoring, inform and extend PTS prophylaxis periods when needed, and provide greater education regarding late PTS and signs and symptoms of seizure activity.

Future research should also continue to examine personal biology, including genetic variation, as risk factors for PTS. Incorporating genetic risk into our current prognostic models may account for (at least some) risk variation attributable to personal biology and may help improve the model's reliability and provide even more accurate probability estimates. Ultimately, accurately screening individuals with moderate to severe TBI, and stratifying these individuals by PTS risk, could facilitate future research and improve care. Identifying individuals with immediate seizures who received prophylaxis yet developed late PTS, and genetic risk factors could provide information regarding potential mechanisms of epileptogenesis. This information may then lead to reverse-translational (i.e. from bedside to bench) research for the development of novel pharmaceutical treatments. Future clinical trials to investigate the effectiveness of tiered prophylaxis regimens could also benefit from the ability to identify subpopulations at high risk of PTS. Based on the body of work, and the potential impact on

future research, the recommendation to re-investigate the current PTS prophylaxis guidelines should be made. Updated guidelines may be particularly beneficial for individuals with immediate seizures and those undergoing neurosurgical procedures, particularly craniectomy.

7.2.1 Traumatic Brain Injury Model Systems (TBIMS)

The findings from **Manuscripts One and Two**, examining incidence and prognostic models for PTS, directly address future implications for the TBIMS and its potential impact on TBI survivors with PTE. As one of the longest running, federally funded prospective observational studies of recovery and outcomes following moderate to severe TBI, TBIMS research efforts are vital to our understanding of the natural history of TBI, and its complications and recovery course, including PTS. However, many lessons, including the importance of assessing the impact of changes to data collection, can be learned and recommendations made from the current work.

Primarily, there are major limitations for longitudinal research when variables are dropped, added, or changed throughout the course of the study. For example, due to changes in variables collecting seizure information at baseline and during follow-up interviews, we were unable to leverage for our analysis the full number (over 13,000) of individuals enrolled and followed in the TBIMS-NDB. Instead, changes to seizure variables resulted in two mutually exclusive cohorts, one with data differentiating time of seizure during acute hospitalization and one without. Therefore, we could not examine immediate, early, and late seizure during acute care as separate risk factors for the majority of individuals in the dataset, limiting statistical power. It is understood that as research outside the TBIMS progresses, novel risk factors or outcomes may be identified and variables added. Dropping or changing variable definitions is detrimental to ongoing research efforts. However, adding a substantial number of variables to a

longitudinal study could strain available resources and add to participant burden. Therefore, researchers must assess planned changes and dropping or revising variables to determine the future effect of research efforts involving the variables in question. Prospective longitudinal studies should clearly define variables of interest prior to study initiation and continue to collect variables important to the study's specific aims and objectives throughout study duration. For studies such as the TBIMS, which has been continuously funded for 27 years and may continue to be funded well into the future, there are likely additional stakeholders to consider when revising data collection. Such stakeholders may potentially include the funding agency and associated Knowledge Translation Centers, as well as the larger TBI research community.

The TBIMS research efforts focus primarily on psychosocial and behavioral outcomes. However, the TBIMS provides an excellent infrastructure for collecting and analyzing data regarding clinical complications and long-term sequelae of TBI, including PTS. Additional variables regarding clinical endpoints, especially those pertinent to PTS (e.g. AED use), should be considered for inclusion in data collection procedures. Similarly, the TBIMS research efforts would likely benefit from the inclusion of additional clinical variables pertaining to acute care as well as premorbid condition variables, particularly premorbid history of epilepsy. Including previous history of epilepsy would allow investigation of the effects of TBI on seizure frequency and severity.

In the future, Model Systems investigators, as well as outside investigators, should capitalize on the existing infrastructure, as well as researcher experience and expertise, for collecting medication and biological data (e.g. blood draw for biomarker and genetics). Medication data during acute hospitalization and throughout study participation would allow researchers to examine the effect of medication treatments on outcomes. Information pertaining

to AED use (acute and chronically) would allow comparison of late PTS incidence, seizure frequency, comorbidities, and adverse effects between different AEDs. Including collection of biological specimens would allow researchers to examine individual variability in genetic pathways relevant to epileptogenic mechanisms to identify novel risk factors, prognostic factors, and possible points of intervention to prevent PTS.

7.2.2 Federally Funded Research Efforts

Despite multiple federal institutes and agencies funding and attempting to streamline TBI and epilepsy research, the capability to examine PTS and examine many of the questions raised by the current body of work remains low. The NINDS Common Data Elements (CDE) includes two case report forms (CRFs) to collect information regarding seizure activity. However, none of the suggested variables can delineate immediate from early seizures, nor do they capture information on seizure frequency. One of the CRFs focused on post-traumatic epilepsy (i.e. late PTS) screening does differentiate seizures occurring before and after 7 days post-injury and documents if AEDs are prescribed. Yet, no information regarding seizure prophylaxis, specific AED medications, or late seizure frequency is captured.

Even if future PTS research collected data specified by existing CRFs, researchers would *still* not be able to examine important questions related to PTS risk, prophylaxis, or treatment. Therefore, more detailed information including specific time of first seizure, frequency of seizures, and prophylaxis and AED use must be collected. Including immediate and early classification categories and details on prophylaxis within the NINDS CDEs, and providing a standardized CRF, may encourage researchers to collect this information. PTS CDEs could then be incorporated into the Federal Interagency Traumatic Brain Injury Research (FITBIR;

https://fitbir.nih.gov/) database and pooled across multiple studies. This infrastructure would enable researchers to examine immediate and early seizures, as well as the effect of prophylaxis, in a larger more heterogeneous sample, providing greater statistical power to examine risk factors for immediate verse early seizure, and immediate and early PTS as risk factors for late PTS.

Future efforts from the NIH and other federal funding sources should explore the possibility of establishing a biorepository in partnership with the TBIMS network. Biorepositories are a critical tool in translational, clinical, and epidemiological research[175]. Prior to establishment of a biorepository, it would be necessary to examine the associated costs and potential added value associated with a large repository. In many highly prevalent diseases, heavily influenced by environmental factors and less so by inherited traits, genetic information does not substantially add to the prediction of disease on an individual basis [176; 177]. PTS, particularly late PTS (i.e. PTE), is likely a condition not heavily influenced by external environmental factors. As such, genetic predisposition may play a large part in risk estimation. Few studies have examined genetic risk factors for late PTS controlling for known environmental risk factors like repetitive head injury or post-injury alcohol and substance sue. Yet, studies do control for injury associated risk factors (e.g. subdural hematoma, depressed skull fracture, and/or injury severity) and demonstrate significant associations, between genetic markers and late PTS [102; 103; 129]. Analyses should be completed to investigate potential improvement to a C-statistic, or area under the receiver operating characteristic curve, when genetic risk factors are added to a predictive model. The reasonably large relative effect sizes for genetic risk factors (hazard ratios estimated to range from 2.9 to 4.5 for various SNPs) [102; 129] in

prior studies increases optimism that genetic risk factors would provide sufficient added benefit to justify the cost of a future repository.

Due to the large number of sites across different regions involved in the TBIMS, and with different laboratory capabilities and experience, establishment of a centralized biorepository would be essential. Many organizations, including NINDS, have established "best practices" for the successful development and execution of biorepositories[178]. One, central biorepository for the TBIMS would help to eliminate multiple challenges when trying to combine information from biological specimens processed at multiple sites and overcome issues inherent to small, fragmented studies of biomarkers, particularly genetics. A centralized biorepository for the TBIMS, and collection of biospecimens for genetics research, would allow investigation of research questions critical to our understanding of PTS.

In addition to a TBIMS biorepository, a TBI specific biorepository at an NIH institute would also greatly enhance the capability of researchers to investigate genetic questions related to PTS. The NIH has already established biorepositories within the National Cancer Institute and the National Heart Lung and Blood Institute. Additionally, the National Institute on Neurological Disorders and Stroke funds multiple biorepositories, primarily for banking of post-mortem tissue in neurodegenerative research. The establishment of a central TBI biorepository overseen by a specific NIH institute would enable the organization to sponsor, oversee, and report on biospecimen collection protocols[179]. A central body could also oversee the use of banked biospecimens, which is critical to prevent misuse of samples and unnecessarily redundant research, and for scientific review of proposals to use banked samples to preserve quantities.

In addition to a central biorepository and governing body to facilitate sharing of banked biospecimens, a review of current data sharing policies and data repositories could facilitate

future PTS research. The FITBIR was recently established by the NIH and Department of Defense (DoD) to share TBI related data and aid collaboration between investigators. While in theory, a centralized data repository could enhance data sharing, there are many reservations from federally funded investigators regarding FITBIR's feasibility and effective stewardship with managing the equitable and ethical use of the large datasets mandated for inclusion under its governance. FITBIR includes a committee to approve requests to use deposited data. However, it is not clear if requests must include research proposals previously reviewed for scientific merit, potentially by a federal funding agency, or by an IRB. Additionally, there is no notification system to inform investigators that the data they contributed is being requested for release, utilized or published. Therefore, as it stands, FITBIR does not foster a sense of collaboration among investigator or an effective roadmap for increasing the impact of TBI research datasets on clinical research and practice.

7.3 PUBLIC HEALTH SIGNIFICANCE

PTS, including PTE, pose a significant public health burden. Using TBI data from the CDC, and the incidence of late PTS in our cohort, of the 275,000 TBIs in the US that result in hospitalization annually, approximately 32,000 individuals will develop late PTS within the first year post-injury. An additional 24,000 individuals hospitalized for TBI will develop late PTS by 5 years post-injury. Similarly, using this estimate in conjunction with data regarding the annual incidence of epilepsy[180], we can calculate that incident cases of late PTS within the first year post-injury account for approximately 20% of the annual incidence of all epilepsy cases.

The Institute of Medicine's Committee on the Public Health Dimensions of the Epilepsies recognizes the role of continued prevention efforts for established epilepsy risk factors, including TBI, to decrease the public health burden of epilepsy[180]. Yet, CDC statistics indicate no decrease in the incidence of moderate and severe TBI, but it does document decreases in TBI resulting in death over the past decade. Similarly, data presented in **Manuscript One** demonstrate incidence of acute symptomatic and late PTS is slightly higher in the TBIMS cohort examined compared to previous PTS studies. Reasons for these modest differences are multifactorial and likely include more individuals surviving moderate/severe TBI and being at risk of PTS, more common use of EEG to monitor for seizure activity resulting in increased detection, as well as differences in study design. As research and modern medicine continue to advance, it is likely that survival after moderate to severe TBI will continue to increase. Subsequently, the number of individuals at risk of developing chronic complications, including PTS, will also increase.

Although antiepileptic drugs (AEDs) are recommended during the first seven days following TBI to suppress seizure activity during this time period, there is no effective prophylactic treatment for late PTS (i.e. PTE). Compounding this problem, individuals who develop seizures immediately after injury, likely prior to administration of early seizure prophylaxis, are at an increased risk for late PTS.

Prophylactic antiepileptic drug (AED) use to prevent late PTS is not recommended due to common multifaceted adverse effects, potential for interaction with other medications, and the need for recurrent healthcare visits to monitor for therapeutic levels. Many common adverse effects are related to AED mechanism of action and manifest as CNS signs and symptoms including drowsiness, dizziness, cognitive impairment, and psychiatric effects, among others[181]. Individual variability, such as premorbid and family history and genetic variance, may contribute

to who will develop adverse symptoms following AED administration. The impact of individual variability on adverse effects is likely magnified following TBI due to highly heterogeneous secondary injury cascades, which may affect multiple neuro-chemical processes. Future research is needed to examine differences in adverse drug effects among individuals with late PTS compared to individuals with epilepsy of non-traumatic etiology.

The heterogeneous nature of TBI may also be a complicating factor in treating late PTS once a diagnosis is made. There are no specific medications for the treatment of late PTS compared to epilepsies of different etiology. Late PTS is often refractory to clinical management[182], possibly resulting in numerous dose and/or medication changes as well as polytherapy. Even if effective medication and dosing levels are identified, individuals with cognitive or behavioral deficits secondary to TBI may have difficulty adhering to complicated medication regimens.

In addition to adverse effects of AED treatment, psychiatric comorbidities, particularly anxiety and depression, are highly prevalent among individuals with epilepsy (including those with late PTS). Despite their already high prevalence in individuals with epilepsy, research shows these psychiatric disorders are underdiagnosed[183]. Furthermore, presence of multiple psychiatric comorbidities that may go untreated in individuals with epilepsy can significantly decrease health-related quality of life[184].

Multiple psychiatric comorbidities are also prevalent following TBI and are associated with decreased quality of life measures[140; 185; 186]. Therefore, individuals with late PTS are likely at an even greater risk of psychiatric and quality of life comorbidities compared to those with TBI or epilepsy alone. Further research is required to more closely examine the risk of psychiatric comorbidities and poor quality of life outcomes for individuals with late PTS, paying

particular attention to the effect of AEDs and psychiatric medications. However, allocating additional resources now toward services for those at high-risk and already diagnosed with late PTS could greatly benefit affected individuals by improving social support and potentially reducing negative psychosocial effects associated with epilepsy.

Late PTS, its associated comorbidities, and need for frequent and specialized healthcare utilization pose a significant quality of life, socioeconomic, and health care burden on individuals with this condition. The work presented adds to the growing body of PTS literature by characterizing incidence, developing prognostic models, and identifying novel genetic variation associated with PTS. Classifying high-risk populations could facilitate future research on the effectiveness of tiered prophylaxis and novel pharmacological interventions. Ultimately, recognition of an individual's risk for PTS may help patients and caregivers by providing education regarding their risk, signs of seizure activity, and connecting them to social support as well as proper medical resources for monitoring, prophylaxis and treatment. These actions may help to improve long-term outcomes in individuals at high risk for PTS.

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