EXPLORATION OF MTDNA VARIANTS IN RELATION TO POST-TRAUMATIC SEIZURE AFTER SEVERE TRAUMATIC BRAIN INJURY

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Post-traumatic seizures (PTS) are heterogeneous and their development has been proposed to depend on variation in the type and severity of injuries and differing genetic backgrounds of the patients. Identification of patients at risk for developing PTS has significant public health implications as those who suffer from PTS have limitations of daily living such as driving and may have increased caretaker burden and difficulty with reemployment.

Genetic studies have revealed that several nuclear genetic variants may act as risk factors in the development of PTS. Many mitochondrial diseases due to variations in mitochondrial DNA (mtDNA) have seizure as part of their phenotype. However, no studies have yet focused on the role that mtDNA may play in the development of PTS.

The goals of this study are to explore roles that mtDNA variants may play as independent risk factors for the development of both early (EPTS) and late (LPTS) post-traumatic seizures. To explore whether mtDNA variants act as effect modifiers of other injury related factors in relation to the development of EPTS/LPTS, and to explore if mtDNA variants effect the relationship between LPTS and GOS measures at 6 months post-injury.

Nineteen mtDNA variants were genotyped in a population of 136 severe TBI patients. Additional genotypes were collected for 6 of the variants in the second stage of the analysis (n=332). No variant was significantly associated (at $p<0.05$) in the univariate analysis with EPTS or LPTS in either the smaller or larger subsets of the population. The A8701G variant

showed the strongest association with EPTS in the univariate analysis (p=0.084) and was entered into a multivariate model. This model showed a significant effect for the variant (p=0.041). T16519C had the strongest univariate association with LPTS (p=0.053) and retained borderline significance when entered into a multivariate model (p=0.095). T16519C appeared to have the strongest trend towards a potential modifying effect with SDH and cranial surgery on the outcome of LPTS. T16519C and T195C may also have possible trends towards lower functional outcomes in those with LPTS.

While no significant independent associations were found between the 19 mitochondrial variants and the outcome of PTS, the findings from this study may help guide future research in this area. This study was able to provide limited evidence that mitochondrial polymorphisms may in fact play a role in unraveling the complex phenotype of PTS.

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PREFACE

I would like to express my sincerest thanks and gratitude to the members of my dissertation committee for guiding me throughout the completion of this project. I'd like to thank my dissertation advisor Dr. Thomas Songer for the weekly meetings to keep me on track in the final push to completion. I'd also like to thank Dr. Anthony Fabio for his assistance in helping me to organize and set up my statistical models and his ability to calm the panic when things didn't always go as planned with my data analysis. I am indebted to Dr. Yvette Conley for helping me with the original development of this project, supplying the mitochondrial genotyping data and offering her lab for the additional genotyping during the project. I'd also like to thank Dr. Amy Wagner and her staff who were instrumental in the collection of the PTS data. I'd like to give special thanks to Dr. Janice Dorman for being my editor and biggest cheerleader throughout this whole process. Finally, I want to thank Melissa and Isaac for their love, support and most of all their patience throughout the final stages of this project.

1.0 INTRODUCTION

Traumatic brain injury (TBI) is one of the leading causes of death and disability in the United States, and its effects are felt across all demographic boundaries.[1] Approximately 1.7 million people in the United States sustain a traumatic brain injury (TBI) each year accounting for 1.4 million emergency department visits and 275,000 hospital admissions. [2] Current estimates suggest that between 3.2-5.3 million Americans live with the sequelae associated with TBI that include; reduced quality of life, and loss of one or more physical and mental functions[3]. Furthermore, more than $60 billion is spent yearly to cover health expenses, disability and lost wages due to TBI [4]**.** These personal, public health and financial impacts warrant further investigation of the potential physiologic and genetic risk factors related to the identification, prevention, and treatment of TBI and its related sequelae.

Post-traumatic seizures (PTS) are a serious complication after TBI. They can occur soon after injury as a consequence of acute pathophysiological changes due to the neurological insult, or they may develop months to years after the initial injury [5]. TBI accounts for as much as 20% of all symptomatic cases of epilepsy in the general population [6]**.** Those with TBI are twelve times more likely than a person in the general population to suffer seizures [7]. Up to 80% of PTS occur within the first 24 months of injury [8]. Recurrence is also a major concern as up to 86% of those who have one seizure after TBI will have another within two years [5]. The likelihood of developing PTS increases with injury severity as more than 15% of those with

severe TBI develop seizures, a risk factor that remains elevated for many years post injury [9]. PTS also has significant implications for future treatment and quality of life of the TBI patient, including loss of independence, mobility, and employment opportunities [5]. Anticonvulsant medications are often given prophylactically to PTS patients to prevent further secondary brain injury and possibly aid in improving their functional outcomes [5]. However, anticonvulsant medications used to treat PTS can produce unwanted side effects, including further neurobehavioral impairment, and sedation [10]. While many of the drugs studied to prevent PTS have shown some effectiveness at decreasing early seizures, they have not shown any real success in preventing late seizures or the development of post-traumatic epilepsy as they primarily suppress seizure activity [11]. Early seizures may be more of a direct reaction to brain damage, while late seizures may be due to damage of the cortex by free radicals generated following iron deposition in brain from some form of intracranial hematoma[12]. Temkin suggests that further work needs to be done to understand the underlying mechanisms of PTS in order to develop therapies that could identify targets for slowing, stopping and more effectively treating seizures.

The search to uncover these underlying mechanisms in PTS has led to studies that have explored genetic links to certain seizure disorders [13-15], and to studies that have explored how nuclear genetic variability affects the development of PTS [16-20]. In addition to potential nuclear genetic variants having an impact on seizure development, it is also known that many inherited mitochondrial diseases include seizure as part of their pathogenesis [21, 22]. It has been estimated that as many as 40-50% of patients with mitochondrial encephalopathy suffer from seizures [23], and specific mitochondrial mutations are associated with some forms of myoclonic epilepsy [23]. For example, Myoclonic Epilepsy with Ragged Red Fibers (MERRF)

involves several polymorphisms in mtDNA, with clinical hallmarks that include progressive epilepsy and a distinct appearance of mitochondria in muscle fibers [24]. Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis and Stroke (MELAS) syndrome can be caused by multiple mtDNA mutations and has clinical features that include seizures, hemiparesis, hemianopia, and blindness among others [25]. From the publication of the complete mitochondrial genome in 1981 [26], over 200 pathogenic point mutations and larger rearrangements (i.e. substitutions, deletions) have been associated with disease [27].

To date there has been scant mention in the literature investigating the relationship between TBI and mtDNA variations (polymorphisms). The few articles pertaining to the topic have focused primarily on deletions of the mitochondrial genome as a consequence of acute brain injury [28, 29]. McDonald reported that a deletion at mtDNA base pair 7346 was less frequent in long term survivors of TBI and hypothesized that since the survivors lacked the deletion, they were able to better cope with the injury compared to those who died of similar injury. Lifshitz investigated whether specific mtDNA deletions would be seen as a consequence of TBI due to altered metabolic states and oxidative stresses incurred during the injury. He did not find any association between TBI and mtDNA deletions, but did find that mitochondrial protein yields from the injured brains were lower than those of uninjured controls.

Despite the paucity of published papers looking at mtDNA and TBI and the complete lack of literature regarding mtDNA and PTS, at least one other study has explored specific mtDNA variants as predictors of mortality [30]. Canter and colleagues sought to determine whether specific genetic variations in mtDNA known to impact energy production were associated with the risk of in-hospital mortality after severe trauma as assessed by Injury Severity Scores (ISS). They selected three mtDNA polymorphisms at positions 4216, 10398, and

4917 because variants at these positions are known to alter the amino acid sequence of key subunits of the Complex I enzyme of the respiratory chain. They demonstrated that one mtDNA variant (the 4216 T allele) was an independent predictor of increased mortality in the multivariate model [30]. Furthermore, the findings that seizure activity is an important sign in the early presentation of several mitochondrial encephalopathies implies that preexisting variants in mtDNA might predispose one to developing seizures after traumatic brain injury.

1.1 RATIONALE

Traumatic brain injury (TBI) represents a major public health issue with some 1.7 million people sustaining TBI, 275,000 requiring hospitalization and more than 50,000 deaths attributed to it annually in the United States [1]. Up to $60 billion is spent yearly to cover health expenses and lost wages due to TBI [31], and 3.2-5.3 million people suffer from the long term sequelae of TBI that include reduced quality of life, and the loss of one or more physical or mental functions [3]. One common and potentially debilitating consequence of TBI are post-traumatic seizures (PTS), which are seizures that occur after head injury and thought to be causally related to the trauma itself [32]. Post-traumatic seizures are typically defined as early (provoked) when they occur within the first seven days of injury or as late (unprovoked) if they occur after 7 days [33]. Post-traumatic epilepsy (PTE) is defined by two or more late seizures after TBI [34]. The probability of developing PTS or PTE is significantly correlated with type (closed versus penetrating) and severity of TBI (mild, moderate and severe). The prevalence of PTS ranges from as high as 53% in military populations with penetrating TBI [35], to approximately 10-15% among civilians with severe TBI [9].

PTS is a heterogeneous condition and it has been proposed to depend on variation in the type, location, severity, number of injuries, secondary brain insults, as well as on the different genetic backgrounds, ages and genders of the patients [36, 37]. Injury severity is by far the best predictor of who will go on to develop PTS [32, 38]. However, animal models of TBI that use a fluid percussion technique to simulate moderate to severe closed head injury have also demonstrated that certain regions of the cerebrum (e.g. the frontal neocortex and temporal lobes) are more likely display seizure activity [39-42]. Lesion type and lesion location have also been associated with the development of PTS in several human studies [38, 43-45].

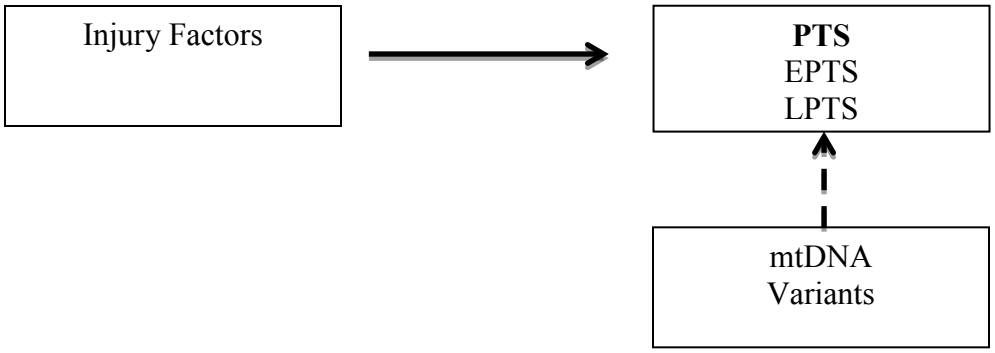
PTS may also play a role in moderating functional outcomes after TBI. PTS has been associated with poorer GOS scores and higher incidence of behavioral abnormality in patients with severe TBI in India [46]. Late PTS has also been associated with a higher incidence of depression [47]. A 5 year follow-up study of rehabilitation and reemployment in Finland showed that late PTS was associated with poorer functional and social outcomes and was a considerable factor in hospital readmission [48]. Overall, it appears that PTS can result in greater psychiatric problems as well as reduced general health and poorer functional and social outcomes [12]. Studies exploring genetic susceptibility to seizure disorders have expanded significantly the number of potential candidate genes involved with seizures [14, 15, 49], while others have begun to expand the concept of genetic susceptibility to TBI outcomes [50, 51]. Only recently have genetic studies revealed that several nuclear genetic variants (APOE, A1AR, GAD) may act as risk factors in the development of PTS [16-20]. While no studies have yet to focus on the role of mitochondrial DNA in the development of PTS, strong evidence has emerged that variations in the mitochondrial genome are linked to mitochondrial encephalopathies that have disrupted cellular metabolism and seizure as part of their phenotype [52-54].

1.2 SPECIFIC AIMS

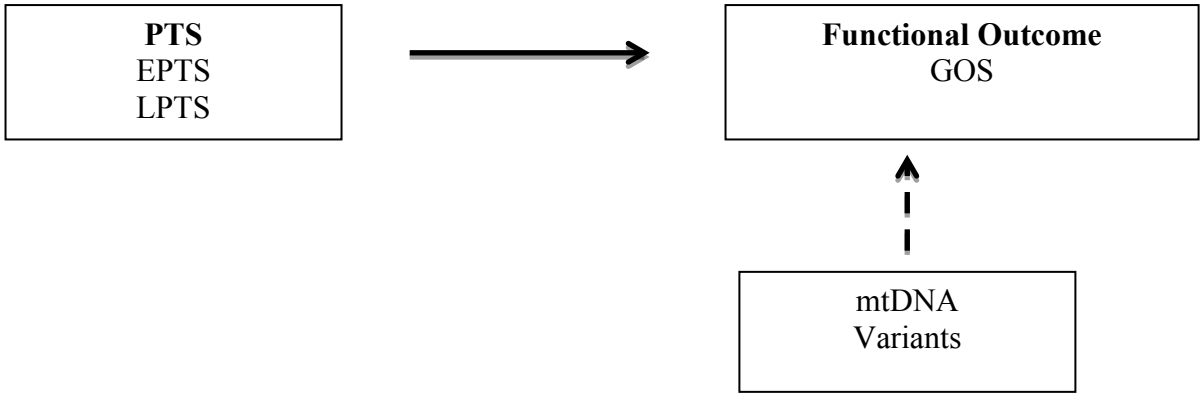
The goals of this proposed project are to use data from our current cohort of severe TBI patients to explore roles that mtDNA variants may play as independent risk factors for the development of both early PTS (EPTS) and late PTS (LPTS) (**Aim 1**). To explore whether mtDNA variants act as effect modifiers of other injury related factors (mechanism, lesion type) in relation to the development of EPTS or LPTS (**Aim 2**), and to explore if mtDNA variants affect the relationship between LPTS and functional outcome measures (GOS) at 6 months post-injury (**Aim 3**).

Specific Aim 1: Examine the contribution of mtDNA variation to the development of both EPTS and LPTS in this cohort of severe TBI patients.

Specific Aim 2: Examine if selected injury factors associated with increased risk of both EPTS and LPTS, are affected by the presence of specific mtDNA variants in this cohort of severe TBI patients.



Specific Aim 3: To examine if EPTS and LPTS are associated with functional outcomes and if this relationship is affected by mtDNA variants in this population of severe TBI patients.



These data present a unique opportunity to test hypotheses that mtDNA variants can help explain the variability of PTS in severe TBI patients either as independently acting risk factors or as interacting variables in regards to other injury related factors such as location or lesion type that are thought to affect the development of PTS. Furthermore, the combined effects of mtDNA variants and other injury characteristics may influence functional and social outcomes after

severe TBI. Knowledge of the contribution of mtDNA variants may lead to changes in how anti-convulsive drugs are administered in the acute and long-term phases of TBI recovery. A better understanding of the injury factors may aid in the design of future injury prevention studies. The effects of mtDNA variation coupled with injury characteristics may serve as predictors of long term outcome after injury.

2.0 LITERATURE REVIEW

2.1 TRAUMATIC BRAIN INJURY

2.1.1 Definition

The impact of traumatic brain injury (TBI) on the population has been recognized as a national public health issue that requires a response from the public health community to prevent and understand their long-term consequences [55]. In response to a Federal Interagency Head Injury Task Force Report, the National Center for Injury Prevention and Control (NCIPC) of the CDC began developing surveillance systems to study TBI at the population based level [55]. In 1995, the CDC published the standard clinical case definition of TBI as an occurrence of injury to the head (arising from blunt or penetrating trauma or from acceleration-deceleration forces) that is associated with symptoms or signs attributable to the injury including: decreased level of consciousness, amnesia, other neurological or neuropsychological abnormalities, skull fracture, diagnosed intracranial lesions or death as well as the case definition for data systems using ICD 9 codes [56]. The NCIPC also defined a four-step public health model for studying TBI in which each step occurs simultaneously and informs the others. The first step is to create surveillance systems to collect basic information regarding TBI events. The second is to identify factors that increase the risk of TBI or are protective. The third step is to design and test interventions. And

the final step is the implementation of successful strategies and the continued use of surveillance to evaluate their impact at the national level [57]. This ongoing system has led to a more accurate understanding of the impact that TBI has on the population in both physical and economic terms.

2.1.2 Impact and Burden

TBI's can result in loss of the ability to work or perform daily activities, and are often associated with the need for increased hospitalization or long-term care[58]. TBI is also a known comorbidity for other health adverse conditions including binge drinking [59], depression [60], Alzheimer's and dementia development [61], and overall increased mortality [62]. Failure to recognize the signs and symptoms of TBI can result in under diagnosis and become yet another barrier to obtaining appropriate services that adds to the overall personal and economic burden [58]. Injuries in general exact an enormous burden on the health care system of the United States as over 50 million medically treated injuries per year have resulted in a total lifetime cost of over 400 billion dollars in medical treatment and lost productivity [31]. It is estimated that of the total expenditures, between $50-60 billion is spent yearly to cover health expenses, disability and lost wages due to TBI [4, 55]. TBI has a tremendous impact on both the civilian and military populations as one of the leading causes of death and disability.

2.1.3 TBI Epidemiology in Civilians

TBI is one of the leading causes of death and disability in the United States, and its impacts are felt across all demographic strata. Approximately 1.7 million civilians in the United States sustain a TBI each year accounting for 1.4 million emergency department visits, 275,000 hospital admissions and approximately 53,000 deaths, which accounts for over 30% of all injury related deaths in the civilian populations of the US[2]. The majority of TBI related deaths are caused by falls, motor vehicle accidents and firearms. Children 0-14, adolescents 15-19 and adults older than 65 are the most likely to sustain a TBI, and in the youngest and oldest strata falls accounted for a third of all TBI related injuries [1]. Overall, males are 1.4 times more likely to suffer a TBI than females [2]. There is also evidence that African Americans have a higher overall risk of TBI compared to whites or other minorities, and recent analyses by Faul and colleagues found that African Americans have an overall incidence rate of Emergency Department visits of 568.7 compared to 456.6 for whites and 345.2 for American Indians, Alaskan Natives, or Pacific Islanders [2]. Other work has shown that African Americans were 2.76 times more likely to be nonproductive than whites one year after TBI [63].

Current prevalence estimates place the number of American civilians living with the long-term effects and disabilities of TBI at between 3.17-3.32 million. This number may actually be an underestimate as it only includes hospitalizations for TBI and doesn't account for those who were treated and released by emergency departments or those who never sought treatment [3]. Other work has shown that approximately 200,000 TBIs are treated yearly in outpatient settings or doctor's offices [4].

2.1.4 TBI in Military Populations

As substantial as the TBI numbers are in the civilian populace, they do not include injuries sustained by military personnel during the wars in Afghanistan and Iraq. TBI has been called the "signature wound" of U.S. troops because it appears to account for a larger proportion of casualties than it has in other recent wars [64]. Advances in protective body armor and helmets have reduced the incidence of penetrating head injuries and death, but closed head TBIs continue to occur. Since the beginning of these two wars, over 1.6 million personnel have served in the theater and estimates of TBI among wounded soldiers has been estimated to be as high as 22% [64]. In a sample of 433 individuals with TBI who were treated at Walter Reed Medical Center, 56% had moderate to severe head trauma, and 88% of all TBI patients sustained closed head injuries confirming that closed head injuries have become more common in these conflicts than in the past. [65]. Blast injury is a significant factor in many of these injuries due to the widespread use of improvised explosive devices [66]. Military sources report that nearly two-thirds of army war zone evacuations are due to blast injury [65]. One recent analysis of TBI hospitalizations of US Army soldiers deployed in Afghanistan and Iraq between 2001 and 2007 identified 2898 inpatient episodes of TBI. 46% of injuries were classified as Type I (most severe), 54% were Type II (mild to moderate), and <1% were Type III (most mild). The overall admission rates for TBI were 24.6 for Afghanistan and 41.8 for Iraq per 10,000 soldier years. Over time, hospitalization rates for TBI rose for both campaigns as well [67].

2.1.5 TBI Classification and Severity

There are different systems of classifying TBI: clinical indices of severity; pathoanatomic indices for describing injury type; and physical mechanisms for describing causative forces of injury. Pathophysiological, and injury cascade markers have also been incorporated as additional measures of TBI severity [68].

The most widely used tool for classifying TBI patients is the Glasgow Coma Score (GCS) that was developed by Teasdale and Jennett in 1974. This scale provides an effective way for clinicians to determine the depth and duration of impaired consciousness after TBI by measuring three independent components of behavior: eye opening, motor responsiveness, and verbal performance (see Figure 1) [69]. The scale runs from 3 (comatose and nonresponsive) to 15 (no deficits in any of the three domains). Once the patient is stabilized, the test is administered and the scores for each domain are summed to create the composite score. The GCS scale is the most commonly used neurological injury scale for adults due to its high intra-observer reliability and good predicative capabilities [70]. Many studies have classified Severe TBI from 3-8, Moderate 9-12 and Mild from 13-15 [71, 72]. There have been other variations used as well within and outside the U.S. [73]. While the extreme ends of the scale are relatively easy to interpret, scores in the middle are more subjective and may lead to potential misclassification.

The Defense and Veterans Brain Injury Center uses a modification of the GCS scale to determine the severity of TBI in those serving in the military (see Figure 2). Mild TBI is often referred to as concussion and may or may not involve a brief loss of consciousness (LOC) of <1 hour or an episode of post-traumatic amnesia (PTA) of <24 hours and a Glasgow Coma Score (GCS) between 13-15. Moderate TBI typically has LOC of >1 hour, but <24 hours, PTA lasting

from 24hours to 7days and a GCS between 9-12. Severe TBI is typically defined as LOC for >24 hours, PTA lasting more than 7 days, and a GCS <8. (from http://www.traumaticbraininjuryatoz.org/Home.aspx)

Glasgow Coma Score		
Eye Opening (E)	Verbal Response (V)	Motor Response (M)
4 – Spontaneous Eye opening 3 – Eyes Open to Speech 2 – Eyes Open to Pain 1 – No Eye Opening	5 – Alert and Oriented 4 – Confused, yet coherent 3 – Inappropriate words and jumbled phrases of words 2 – Incomprehensible sounds 1 – No Sounds	6 – Obeys commands fully 5 – Localizes to noxious stimuli 4 – Withdraws from noxious stimuli 3 – Abnormal flexion 2 – Abnormal extension 1 – No response

Figure 1: Glasgow Coma Score
Adapted from Teasdale and Jennet, 1974 [69]

GCS as used by DVBIC			
Severity	Glasgow Coma Scale (total)	Loss of Consciousness (duration)	Post-Traumatic Amnesia (duration)
Mild	13-15	< 1 hr.	< 24 hrs.
Moderate	9-12	1-24 hrs.	24 hrs. to < 7 days
Severe	3-8	> 24 hrs.	7 days or more

Figure 2 TBI Severity as used by Defense and Veterans Brain Injury Center
Adapted from Helmick et al. **[74]**

2.1.6 Complications Related to TBI

The complications of TBI vary with severity of injury, with moderate and severe patients experiencing much greater dysfunction than those diagnosed with mild TBI. Approximately 80% of patients presenting to Emergency Departments are classified as mild (GCS 13-15), 10% are moderate (GCS 9-12) and 10% severe (GCS <8) [75].

Presenting clinical symptoms of mild TBI include headache, nausea and vomiting, but may also include disorientation, confusion and/or amnesia. Patients with moderate TBI have a widely varied immediate clinical presentation that can include LOC, post-traumatic seizures (PTS), confusion and some amnesia, but they can typically still follow commands. Severe patients present with a GCS <8, and are often unconscious and nonresponsive [76].

Mortality is an important consideration in TBI studies, of the 1.7 million TBI's that occur each year in the United States, 3.0% (53,000) result in death [2]. The risk of mortality increases with the severity of injury and can act as confounder when trying to investigate other outcomes related to TBI.

TBI is a heterogeneous condition that can have both immediate and longer-term complications associated with it. The risk of immediate complications increases with severity of injury and can include: immediate seizures, hydrocephalus (post-traumatic ventricular enlargement), CSF leaks, infections, cranial nerve injuries, organ system failure and multi-organ trauma [77]**.** Long-term complications or outcomes are typically those that persist for more than 6 months after injury and can include: changes in neurocognition, neurologic deficits, psychiatric disorders and social impacts [73]. Although the morbidity associated with TBI is highly variable, this review will focus on one common and serious complication, Post-Traumatic Seizures (PTS).

PTS is a major neurologic outcome after TBI. The review committee of Gulf War and Health: Volume 7: Long-term Consequences of Traumatic Brain Injury evaluated a total of 29 studies regarding the association between TBI and the development of seizures. Across those studies the risk of seizures after severe TBI was 17-95 times higher than the uninjured population, and 2.9-6.6 times higher in those with moderate TBI. In the studies of mild TBI, the risk of seizure was 1.5 times higher than in the uninjured population. The committee concluded that there was sufficient evidence of an association between both severe and moderate TBI and the development of seizures after injury, but concluded that there was limited evidence of an association between mild TBI and development of seizures ([73] see Chapter 7, pp. 197-209).

2.2 POST-TRAUMATIC SEIZURES

2.2.1 Definitions

The association between head injuries and the development of seizures has been recognized for thousands of years. References of seizures developing in patients surviving severe head injury date back as far as 3000 B.C. [78]. According to Caveness**,** "the relation of craniocerebral trauma to convulsive seizures was recognized by Hippocrates (460-357BC) in his treatise "*Injuries of the Head*" [79]. The terms post-traumatic seizure (PTS) and post-traumatic epilepsy (PTE) are often used to describe the same thing: seizures occurring after head trauma that are thought to be causally related to the trauma itself [32]. In some settings, the diagnosis of epilepsy has been reserved for patients who have had two or more unprovoked seizures [6]. PTE is typically defined as recurrent, late seizures that occur more than one week after injury [80]. As Frey points out there are disadvantages of waiting for a second seizure to occur before applying the term PTE. She notes that some patients will be excluded because of the long period between the initial seizure and the second seizure. Others may not have their first seizure until long after the injury has occurred, and the practice of using antiepileptic drugs after a first seizure may alter the course of seizure development [32]. While there is still debate as to what differentiates late occurring PTS and PTE, the term late post-traumatic seizure (LPTS) will be used throughout this literature review when referring to seizures that occur later than 1 week after injury

2.2.2 Early and Late PTS

Jennett was one of the first authors to divide seizures occurring after TBI into early PTS (EPTS) and late PTS categories (LPTS). He defined early seizures as those occurring between 1-7 days after injury and late as those occurring beyond 7 days [33]. It is also common in the literature for early seizures to be referred to as provoked, whereas late seizures are termed unprovoked [34]. It has also been suggested that seizures occurring within the first few weeks of injury share more in common with early seizures than late seizures, and more data on early seizure timing is needed to allow for identification of the point after which the occurrence of an early seizure would be predicative of future seizures [81]. To date, Jennett's classification it is still one of the most widely used methods of categorizing PTS. More recently, PTS have been typically divided into three categories: immediate, early and late seizures [32, 78]. Immediate seizures are those that occur less than 24 hours after injury. Early seizures occur between 1-7 days after the injury. Late seizures are those that appear greater than 7 days after the injury. In some studies of PTS, immediate seizures are not included as an early seizure in analysis, while other research has shown that both immediate and early onset seizures are risk factors for developing LPTS [82].

2.2.3 Frequency of PTS

The frequency of PTS has been well documented in both civilian and military populations. Civilian studies have included both clinical studies and population level studies that have assessed the frequency of both early and late PTS. Some of the largest population based studies to assess risk of seizure were conducted by Annegers et al., as part of the Rochester

Epidemiology project in Minnesota. In their first retrospective cohort they reported on the risk of seizures in a cohort of 2747 patients (1132 children and 1615 adults) in Olmstead County, Minnesota who had sustained TBI (defined by LOC, PTA, or skull fracture) between 1935-1974 and were compared with age and sex specific rates of seizure in the general population. TBI data were obtained from physician diagnoses in the medical records [83]. An additional 4541 children and adults with TBI (characterized by LOC, PTA, or skull fracture) who sustained TBI's between 1975-1984 were added to the second series as a continuation of the project [9]. In total they studied post-traumatic seizures for a period of 50 years, with some patients followed for over 30 years from the date of their injury. The overall frequency of PTS was 4.4%, with 2.6% developing EPTS and 2.1% developing late PTS. Their focus, however, was primarily on the risk of developing LPTS. The overall risk of LPTS was 3.6 times higher than in the uninjured population. The risk of late seizure was strongly tied to injury severity with the highest risk after severe TBI (SIR = 17.0, 12.3-23.6), followed by moderate TBI (SIR = 2.9, 1.9-4.1), and those with mild TBI (SIR= 1.5, 2.5-3.8). They also found that late seizure risk was the highest in the first year after injury (SIR = 12.7) and fell to 1.4 by 5 or more years after injury. In the univariate analysis, EPTS was a strong predictor of the development of LPTS (Rate Ratio = 5.5), but when adjusted for other factors it was no longer a significant independent risk factor for the development of late PTS [9]. Thus, seizure risk was highest initially in the most severely injured and that risk remained elevated over the course of many years relative to those with more mild injuries.

In a more recent population based study of the development of PTE, Ferguson and colleagues conducted a retrospective study of 4519 South Carolina Residents 15 and older who had sustained a TBI resulting in hospital admission who were discharged alive between 1999-

2002. The cohort was randomly selected based upon hospital size and level of TBI severity. All of the hospitals involved were part of the South Carolina TBI Follow-Up Registry. They determined seizure activity from discharge billing and abstraction of TBI related data from medical charts. Pre and Post injury seizure activity was assessed via follow-up interviews of either survivors or proxies. Of the 4519 patients identified, they excluded 773 due to death, deteriorating medical status, incarceration status or other reasons. There were 3746 patients who were eligible to be screened for seizures at one-year post-hospitalization and 1628 declined to participate, were unable to be located or were contacted, but did not respond.

One year after discharge, they were able to interview 2118 patients, at two years they interviewed 1536, and at the end of the third year they interviewed 1173. A total of 945 patients were lost to follow-up over the course of the three years. Initial reports of seizure activity were abstracted from discharge and billing reports, and during the course of the three years, interviews were conducted by phone or in person with participants or proxies. Any evidence of seizure activity was documented and confirmed with an epileptologist who was part of the research team. A total of 115 patients developed PTE over the course of the three-year follow up. The frequency of EPTS was 3.1% and PTE was 6.2%. They also found that severity of injury greatly impacts the risk of developing PTE. The cumulative incidence over the three years of PTE was 4.4 per 100 persons for those hospitalized with mild TBI, 7.6 for moderate TBI and 13.6 for severe TBI, compared to those patients who did not develop PTE. In addition to injury severity, they also found that patients with early PTS, depression, and at least three other comorbid conditions were at higher risk for developing PTE [47].

These population-based studies confirm multiple hospital-based studies that have taken place over the years. In studies done on patients admitted for head trauma EPTS frequencies

range from 4-16% and LPTS frequencies range from 5-25% [33, 43, 45, 48, 84]. While these studies differed in how they identified severity, the ages of the population studied, and whether they were done in emergency departments or inpatient treatment facilities they all provide a general estimate of the frequency of both EPTS and LPTS in regards to TBI. Another feature of all the studies mentioned above is that they have helped determine that a key factor in the development in LPTS is the presence of EPTS. In terms of military populations the overall frequency of LPTS ranges from 35%-53% in studies of WWI, WWII, Korean and Vietnam veterans [35]. Presumably, the much higher frequency of LPTS in these groups is attributed to more severe injuries of a penetrating nature, that result in fracture of the skull, disruption of the dural linings, and the retention of bone and metal fragments in the brain [32]. The risk of developing LPTS in combat-associated, closed-head trauma with positive brain imaging is between about 10-25%, and about 5% without positive imaging findings [34]. While precise estimates of LPTS in the current conflicts in Afghanistan and Iraq are unknown, Chen and colleagues have estimated that the number of cases of PTE may be over 10,000 by using previous estimates of the risk of developing PTE for both penetrating and closed-head injuries and based upon the number of soldiers who have already been deployed in these conflicts [34].

2.2.4 Treatments for PTS

Antiepileptic drugs are often given prophylactically to PTS patients to prevent further secondary brain injury and possibly aid in improving their functional outcomes [5]. Chang and Lowenstein performed a meta-analysis of the evidence regarding antiepileptic therapy prophylaxis in patients with severe TBI by examining and grading prospective studies that compared PTS rates in patients receiving these medications compared to controls with severe

TBI who did not receive the medications. They pooled studies by their level of evidence based upon prospective study design, random or nonrandom level of assignment to AED therapy, reporting of PTS in both controls and cases, and publication in a peer reviewed journal. Studies were assigned a level of I-IV, with level I studies having a low risk of bias and level IV having significant bias. In four level I and II studies evaluating the risk of developing EPTS; patients receiving phenytoin had a significantly lower risk of developing EPTS (RR=0.37, 0.18-0.74) compared to controls that either received placebo or no antiepileptic therapy. In their pooled analysis of eight Level I and II studies they found that antiepileptic therapy had no significant effect in preventing LPTS in severe TBI patients compared to controls who either received placebo or no antiepileptic drug therapy. (RR=1.05, 0.82-1.35). The follow-up duration of these 8 studies ranged from 3 months to 8 years or more [81]. Anticonvulsant medications used to treat PTS can produce unwanted side effects, including further neurobehavioral impairment, and sedation [10]. Even though it appears that anti-epileptics decrease the rate of early seizures it doesn't appear that there is evidence that the prevention of early seizures affects mortality, morbidity, or the development of LPTS [12]. Further work needs to be done to understand the underlying risk factors and mechanisms of PTS in order to develop therapies that could identify targets for slowing, stopping and more effectively treating seizures [11].

2.2.5 Documented Risk Factors

As described above, one of the most consistent findings across both clinical and population based studies has been that injury severity is highly related to the risk of developing PTS of all types Many other studies have identified additional consistent risk factors for the development of both EPTS and LPTS.

In her excellent review of PTS, Frey identified the significant risk factors for both EPTS and LPTS from numerous previous studies. Important independent risk factors for EPTS include: acute intracerebral hematoma, acute subdural hematoma (in children), younger age, increased injury severity (including LOC, or PTA >30min), and chronic alcoholism. For LPTS the significant risk factors included: EPTS, acute intracerebral hematoma (especially subdural hematoma), brain contusion, increased injury severity (including LOC or PTA >24 hrs), and age >65 [32]. Temkin presented risk factor data from two of their previous studies on seizure prophylaxis at the University of Washington as well as other previously documented risk factors for PTS. The two clinical trials included an antiepileptic drug arm and a placebo arm, and were based on patients at high risk for developing PTS. The two studies enrolled a total of 783 patients within 24 hours of injury and almost all of them received CT scans soon after arrival. Patients had to be at least 14 years old, and have had at least one of the following: cortical contusion, hematoma (subdural, epidural or intracerebral), depressed skull fracture, penetrating brain injury, acute seizures, and for the earlier study a GCS<10. They followed the patients for up to two years after their injury as part of the trials. Among the 196 placebo-treated patients in their high seizure risk population they identified the following as risk factors associated with EPTS: depressed skull fracture (27%), intracerebral hematoma (23%), subdural hematoma (24%), penetrating head injury (20%), GCS<10 (20%), epidural hematoma (17%), cortical contusion (16%), and immediate seizures (28%). They only looked at the placebo arm for this analysis, as it has been documented that antiepileptic drugs are successful at preventing or suppressing EPTS. In terms of developing LPTS, Temkin identified the following subgroups with significantly elevated risk: those with subdural hematoma evacuation, surgery for an intracerebral hematoma, GCS between 3-8, early seizures, time to following commands of >1

week, depressed skull fracture, dural penetration, at least one non-reactive pupil, and parietal lesions on CT scans. She estimated that seizure risk in these subgroups were approximately 400 times that of the general population based upon data from the Rochester Epidemiology project that calculated the cumulative incidence of epilepsy in the general population over the course of 50 years to be 3% [38, 85].

2.2.6 Imaging Studies and Animal Models

As discussed in the previous section injury severity is one of the best predictors of who will go on to develop PTS. The presence of intracerebral contusions and subdural hematomas have also been identified as significant risk factors for the development of both early and late PTS [32, 38]. In a study performed of clinical and EEG factors on the development of PTE in civilians with moderate to severe closed-head injuries by da Silva and colleagues, they demonstrated that seizures were associated with both younger (<5) and older (>75) ages and that the level of neurological deficit (i.e. severity) and lesion location (focal vs. generalized) increased the risk of developing PTE [44]. In a recent prospective multicenter study of individuals admitted with TBI to four trauma centers within 24 hours of injury, Englander and colleagues assessed the natural history and stratified the risks of LPTS for individuals with moderate to severe TBI. They enrolled a total of 647 individuals older than 16 years with any of the following abnormalities documented by CT scan: extent of midline shift and/or cisternal compression or presence of any focal pathology (hemorrhage, contusions, extra-axial lesions) during the first 7 days post injury or best GCS score ≤ 10 during the first 24 hours post-injury. The subjects were enrolled over a period of three years and followed for up to 24 months, until

their death or their first LPTS. A total of 66 patients had late PTS and they found that the highest cumulative probability for LPTS were presence of biparietal contusions (66%), dural penetration with bone and metal fragments (62.5%), multiple intracranial operations (36.5%), multiple subcortical contusions (33.4%), subdural hematoma with evacuation (27.8%), midline shift greater than 5mm (25.8%), or multiple bilateral cortical contusions (25%). When they did risk stratification by the above factors and GCS scores, they found that those with an initial moderate GCS score (9-12) had a higher cumulative probability of developing LPTS than those with a more severe GCS scores (3-8). They concluded that certain types of TBI put individuals at a higher risk for late PTS, and that information from CT scan diagnosis that includes location of injury, lesion type, and extent of cortical damage are important factors in assessing patients most at risk for developing LPTS or eventual PTE [43]. Temkin also indicated that presence of parietal lesions on CT scan were a risk factor for early PTS, but were not an independent predictor of LPTS in the analysis of the 196 controls from her two earlier seizure prophylaxis studies [38]. A recent review of radiologic and neurophysiologic techniques used to study PTS done by Diaz-Arrastia and colleagues indicates that PTS often arises from mesial-temporal structures, presumably due to diffuse injury mechanisms that result in damage to vulnerable neuronal or axonal populations, or from focal scars in the frontal neocortex resulting from focal contusions and intracranial hemorrhages [86].

To better understand the underlying etiology of seizure activity that arises after closed-head injury, several animal models of TBI that use either a fluid percussion technique, or controlled cortical impact to simulate moderate to severe closed head injury have demonstrated that certain regions of the cerebrum, especially the frontal neocortex and temporal lobes, are more likely display seizure activity after brain injury. In a series of studies using fluid

percussion techniques in rats it was demonstrated that frontal parasagittal injuries of the moderate to severe category resulted in a high incidence of partial seizures that arose in the early weeks after the initial injury [41, 42, 87]. In a population of older rats, a more severe parietal fluid percussion injury had a lower incidence of overall epileptic activity and it did not spread to the frontal neocortex [40]. Curia et al., conducted a study of rats to examine the effects of location and severity of contusive closed-head injury to help elucidate the factors that may contribute to some of the heterogeneity seen in human PTS. They concluded that the location and severity of injury affects the incidence and severity of seizures, that the frontal cortex is more susceptible to PTS than the parietal and occipital lobes, and that there is a wide variation among animals in the severity and course of PTS [39].

2.2.7 Other Considerations from PTS Studies

Most studies of PTS have focused on either the natural history of seizure development, the relationship of TBI severity to the development of PTS, the use of antiepileptic drugs to prevent seizures, or how early seizures can be used to predict the onset of late seizures or PTE. Mechanism of injury is usually categorized as motor vehicle, fall, gunshot or other mechanism. However, one large prospective study from India followed 520 patients admitted for TBI for over a year from the time of injury and reported that of the 59 (11.4%) patients who had developed PTS, 6.5% developed immediate seizures, 2.1% developed EPTS and 2.7% LPTS. While these results are in agreement with many of the other studies described here, this study also reported that the risk of PTS was 3.7 times higher in patients who had fallen from height. The authors conclude that since the incidence of gunshot wounds were very low in this cohort (2 patients) and that most of the vehicular accidents involved pedestrians, which resulted in more

mild injuries that this may be an artifact, but the result did hold up in the multivariate analysis of their study. They also looked at a variety of outcome measures related to those who developed PTS and those who did not and found that those with PTS were more likely to have poorer behavioral outcomes and GOS scores at the end of follow up [46]. Other studies have also looked at outcome measures in those with and without PTS after head injury. In a prospective study of 210 patients, Marwitz and colleagues found there were no significant differences in outcomes at 1 year post-injury in those with PTS and those without [88]. A study done by Asikainen et al. followed 490 TBI patients for 5 years or more from the time of injury through a rehabilitation and reemployment program in Finland. They found that while LPTS did worsen functional outcome as measured by GOS, it had no significant influence on reemployment at the end of the follow-up period [48]. Additionally, late occurring seizures have also been associated with greater psychiatric problems, reduced general health and poorer functional and social outcomes [12].

The previous paragraphs have documented that PTS is highly heterogeneous in nature and has been proposed to depend on variation in the type, location, severity, number of injuries, secondary brain insults, ages, genders and different genetic backgrounds of the patients [36, 79]. One very important potential mechanism that may determine who does and does not develop PTS is the role of genetic susceptibility. Currently, it is far from understood and there is a wide range of variability in responses to similar injuries with some patients seizing frequently and others not at all. As early as 1979, Caveness wrote that there is likely to be a "constitutional tendency towards PTS (probably a multifactorial genetic trait) and brain damage" [79].

2.2.8 Genetic Susceptibility as a PTS Risk Factor

While the number of epidemiologic studies of PTS has grown substantially over the past several decades, there is still much work to be done to understand the risk factors and mechanisms that underlie PTS. One very important mechanism is the role of genetic susceptibility. Currently, it is far from understood and there is a wide range of variability found in responses to similar injuries with some patients seizing frequently and others not at all [32]. In the past two decades there have been several genes implicated as influencing outcomes after TBI (including, but not limited to PTS), and future studies will most likely identify even more genes that modulate recovery or provide neuroprotection from TBI [50]. Other recent studies have explored genetic susceptibility to seizure disorders [14, 15], and treatment outcomes after TBI [89].

APOE is by far the most widely studied in regards to TBI. APOE encodes for a cholesterol carrier lipoprotein and exists in three allelic forms E2, E3 and E4. APOE E4 in particular has been identified as a susceptibility gene for late onset and familial Alzheimer's disease [90]. Other established neurobiological functions of APOE include: amyloid plaque deposition, neurofibrillary tangle formation, antioxidant activity, neuronal repair, cholesterol transport, synaptic plasticity and neuroprotection [50]. There have been numerous studies to support the hypothesis that APOE allele status influences outcome after TBI, in particular that those individuals carrying at least one copy of the APOE E4 allele have poorer outcomes, longer hospital stays, and worse rehabilitation outcomes [50]. The adenosine A1 receptors (A1AR) are found primarily in neurons and are the high affinity target for adenosine and have been implicated in TBI neuroprotection by limiting excitability of neurons, and in traumatic and non/traumatic seizure development in multiple experimental models [19]. Haptoglobin (Hp) is

polymorphic and three major phenotypes exist. The primary job of haptoglobin is to bind free hemoglobin, and remove it from the damaged tissues after microhemorrhage events and its levels have been shown to increase after TBI [17]. The GRIN2a (glutamate receptor subunit epsilon 1) gene codes for a portion of a ligand gated channel and plays a role in excitatory synaptic transmission particularly after neuronal injury. The GAD1 and GAD2 genes code for enzymes that create two different isoforms of glutamic acid decarboxylase (GABA), which acts as an inhibitory neurotransmitter at the postsynaptic membrane.[18] The apolipoprotein E (APOE) gene is also one of the most studied in terms of PTS [16, 20, 50]. For example, the long term Vietnam Head Injury study (VHIS) screened for APOE E4 genotypes in regards to PTE [18]. Anderson et al, 2009 investigated relationships between PTS and APOE as well as PTS and haptoglobin phenotypes [17]. Even more recently, those carrying two copies of APOE 4/4 were more likely to have to late PTS (19). In the following section, 5 studies of nuclear genes and their realtionship to PTS will be reviewed.

Diaz-Arrastia et al. conducted a study to examine the association between a specific genetic variant and the development of PTS. They utilized a prospective design in which subjects were recruited from a level 1 urban trauma center between January 1999 and December 2001. Their purpose was to determine if inheritance of the APOE 4 allele was associated with the risk of developing LPTS in a population of patients who had suffered a moderate to severe brain injury. Inclusion criteria included any one or more of the following: evidence of cerebral contusion on CT, any intracranial hematoma, depressed skull fracture, penetrating brain injury, or EPTS occurring less than one week after injury. Patients with pre-injury epilepsy, previous neurologic conditions, nontraumatic hemorrhage, or major cortical infarction were excluded. A total of 204 patients met the criteria for enrollment and outcome questionnaires were collected 6

months after injury on 106 patients. The authors explained that this type of loss to follow-up is not uncommon in TBI studies and they found no differences in major demographic and clinical characteristics of the 98 patients lost to follow-up compared to those who went on to develop PTS in the study, but did note that there was a slight trend towards less severe injury in those who did not complete the 6 month follow-up questionnaire. Of the 20% (21/106) of patients who developed LPTS they found no significant differences due to age, sex, ethnicity, initial GCS scores, length of stay, discharge status or Glasgow Outcome Status (GOS) to those who did develop PTS. The only two significant covariates identified were the presence of EPTS (within 1 week of injury) and the presence of at least one copy of the APOE 4 allele. In their final adjusted model, the risk of developing PTS was 2.41 (1.15-5.07; p=0.03) in patients who carried at least one copy of the APOE 4 allele. Their sample size was too small to evaluate if carriage of two APOE 4/4 alleles conferred a greater risk [16].

Anderson and colleagues performed a matched case control on a group of patients who had previously taken part in a double blind controlled trial on the effectiveness of anti-epileptic drugs in the early 1990's [10]. The Diaz-Arrastia study mentioned in the previous paragraph modeled their inclusion and exclusion criteria on the patients from the original clinical trial; therefore, there were similarities in both of their cohorts. The purpose of this study was to investigate if there was any genetic predisposition to develop PTS in cases based upon their haptoglobin phenotype or their APOE allele status. For the APOE analysis they were seeking to expand upon the earlier work of Diaz-Arrastia and colleagues. Haptoglobin phenotype was chosen because its main biological function is to bind free hemoglobin and facilitate its excretion from the body and accumulation of hemoglobin and iron residues after TBI are known to be epileptogenic. The Haptoglobin gene codes for three haptoglobin genotype (Hp 1,1, Hp 2,2, and

Hp 2,1) that vary widely in their ability to bind free hemoglobin and they hypothesized that those with less ability to clear hemoglobin would be at higher risk for seizures. The original clinical trial examining the effects of antiepileptic drugs on PTS development had enrolled a total of 379 patients of whom 56 went on to develop late PTS. Samples used in this study were collected as part of the 10-year follow-up to the original trial or from frozen serum samples from when the trial took place. 25 subjects from the original late PTS group who returned for follow-up became the cases and were matched on age, sex and PTS risk factors with 26 controls with no evidence of late PTS from the original trial. In addition, they were able to obtain haptoglobin phenotypes from frozen serum samples of 25 additional patients who developed PTS and 32 matched controls with no evidence of PTS in the initial trial. For the APOE analyses they had a total of 25 cases and 26 controls and unlike the Diaz-Arrastia study they did not find any association between the carriage of the APOE 4 allele and greater risk of PTS. For the haptoglobin portion of their analyses they had a total of 50 cases of PTS and 58 matched controls. They found no significant difference in the odds of developing PTS based upon haptoglobin phenotypes in long term survivors of TBI [17].

Miller et al. performed a study on 322 patients with severe closed-head TBI who were enrolled as part of a larger cohort study at the University of Pittsburgh Medical Center studying the effects of APOE genotype on outcomes after TBI. Patients were enrolled into the cohort if they were between 18-75 years of age, had a GCS<8 with positive findings of trauma on CT scans and required extraventricular drainage and intracranial pressure (ICP) monitoring. Patients with penetrating head injury as well as those with prolonged cardiac or respiratory arrest were excluded. Information about PTS was collected through abstraction of medical records. The primary measure in this study was time to first seizure. PTS was divided into three categories,

early late and delayed onset. EPTS were those recorded within the first week of injury, LPTS occurred after 1 week, but before 6 months, and delayed onset PTS occurred 6 months or greater after the initial injury. There were a total of 235 subjects in the early PTS group who had both APOE genotype information and medical record information. Of this group, 16 developed seizures. Chi square analysis showed no significant association between APOE genotype and EPTS (p=0.479). Subjects with APOE 2 or APOE 3 alleles had no difference in EPTS rates compared to those who did not have the alleles, nor was the APOE 4 allele significantly associated with EPTS. There were 216 subjects in the LPTS group who had both APOE genotype information and medical record information. A total of 44 patients in this group developed seizures. None of the subjects who were E2/E2 or E2/E4 developed late PTS, and carriage of the E2 allele (p=0.126) or E3 allele (p=0.667) also did not predispose one to LPTS. Those carrying only one copy of the E4 allele did not show any increased odds of developing LPTS (p=0.430), however, the most intriguing finding was that they noted that half of patients who carried the APOE 4/4 developed late seizures. The authors do caution, however, that this was an extremely small group of subjects (n=5). There were a total of 196 patients in the delayed onset PTS group who had both APOE and medical record information. A total of 24 of these patients developed seizures. Similar to the LPTS group, those who were E2/E2 and E2/E4 had no evidence of PTS, and selecting for the E2 or E3 allele alone showed no greater odds of developing delayed onset PTS. There were also no differences in delayed onset PTS for subjects with or without the E4 allele (p=0.803) [20].

Working from the same population of patients, utilizing the same inclusion and exclusion criteria, and the same definitions of PTS as Miller et al., Wagner and colleagues studied whether variability in the adenosine A1 receptor (A1AR) gene was related to the development of PTS.

They identified 206 patients with both A1AR genotype information and medical record information regarding PTS. From this group, they removed those with evidence of premorbid seizure and non-caucasians (due to extremely small numbers), and analyzed a total of 187 patients to determine if genetic variability within the Adenosine A1 receptor (A1AR) gene and its flanking region were associated with the development of PTS. Adenosine and its receptor subtypes have been implicated as important factors in the secondary neurobiological cascade of injury that occurs after TBI. This study explored the A1AR gene by looking at tagging SNPs (tSNPs) that when analyzed are capable of capturing variability within the gene itself and the related flanking regions. 5 tSNPs were explored, and two of them, rs3766553 and rs10920573 of the A1AR gene were significantly associated the onset of PTS after adjusting for other covariates known to influence PTS. The AA genotype of rs3766553 was associated with EPTS (p=0.05) and the GG genotype of rs3766553 was associated with LPTS (p=0.019). The heterozygous CT genotype of rs10920573 was associated with LPTS (p=0.039). Those patients who had both the GG rs3766553 and CT rs10920573 genotypes had higher odds (OR = 13.124, p=0.001) of developing late PTS and higher odds (OR=28.869, p=0.005) of developing delayed-onset PTS [19].

Raymont et al. examined whether selected genetic markers from several candidate genes were associated with the development of seizure activity (defined as PTE in this study) in a long term follow-up of subjects enrolled in the Vietnam Head Injury Study (VHIS). Phase 3 follow-ups were conducted 35 years post injury on 199 veterans who had suffered primarily penetrating head injuries. 43.7% (87/199) reported seizure activity and 12.6% (11/87) of those developed PTE between the phase 2 visit 15 years earlier and the phase 3 follow-up. They screened for a number of genetic markers that had been associated with epilepsy in previous studies. The

markers selected for their analyses included: the presence of APOE 4 allele, glutamic acid decarboxylase (GAD), catechol-O-methyltransferase (COMT), GRIN (a glutamate receptor subunit), BDNF (brain derived neurotrophic factor) and DBH (dopamine B-hydroxlyase). In their initial analyses they found that the SNP rs11074504 of GRIN2a ($p=0.007$), SNP rs1330582 of GAD2 ($p=0.019$), and SNP rs769395 of GAD1 ($p=0.053$) were significantly associated with PTE. However, when the authors corrected for multiple comparisons, none of the SNPS retained their significance. They also found that carriage of the APOE E4 allele did not impact the development of PTE ($p=0.34$), nor did any of the variants in COMT, BDNF, or DBH [18].

The papers reviewed here show that there is a growing trend to investigate and elucidate the impact of genetic variants that are thought to underlie the development of PTS. A better understanding of the genetic contribution may lead to new treatments that could disrupt the mechanisms that contribute to PTS and result in more effective management of this common complication of TBI. These trends indicated by the papers reviewed here also highlight the need for investigation of the genetic variants in larger, multi-center cohorts that can achieve the sample sizes and power necessary to detect the true impact of these variants on the development of PTS.

2.3 MITOCHONDRIA AND SEIZURES

2.3.1 Mitochondria

Mitochondria are membrane bound organelles that lie within the cytoplasm of most eukaryotic cells. They are typically depicted as fusiform organelles that consist of a smooth outer membrane and a highly folded inner membrane that is referred to as the cristae. In reality they are much more than static organelles and can be thought of as a budding and fusing network similar to that of the endoplasmic reticulum [21]. Their shape varies in response to environmental demands and cellular differentiation [91]. Their numbers within cells vary based upon demand with high energy cells like muscle, kidney, liver and neurons having hundreds of copies and red blood cells having none [92]. In terms of function they are involved in cellular homeostasis where they play crucial roles in intracellular signaling, apoptosis, intermediary metabolism, and in the metabolism of amino acids, lipids, cholesterol, steroids, and nucleotides [21]. However, they are most often described as the power plant of the cell because they generate the majority of ATP needed to perform normal physiologic functions [92].

ATP is generated during cellular metabolism through the addition of a phosphate group to an adenosine diphosphate (ADP) molecule resulting in in a high energy molecule. When the terminal phosphate bond is transferred from ATP to other molecules, energy is released that can be used to do work within cells. Cells can create ATP through substrate level phosphorylation and oxidative phosphorylation (OXPHOS) [92]. Substrate phosphorylation occurs in situations such as glycolysis when glucose is cleaved into phosphorylated intermediaries that then transfer those phosphates to ADP molecules within the cell. Glycolysis is not very efficient as typically only two usable ATP are created for each molecule of glucose. Oxidative phosphorylation

(OXPHOS) is much more efficient, with electrons being harvested from nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) which are the intermediaries of glucose cleavage that occur within the mitochondrial membranes (Kreb's cycle and electron transport chain) and then used to generate much larger amounts of ATP for each glucose molecule. Most cell types utilize both types of energy production. The majority of the reactions necessary for OXPHOS occur on a group of enzyme complexes that lie on the inner mitochondrial membrane and are most often referred to as the mitochondrial respiratory chain or the electron transport chain (ETC) enzymes [21].

There are a total of five enzyme complexes (Complexes I – V) that are embedded into the inner mitochondrial membrane, and each one is composed of multiple polypeptide subunits. Complex I consists of over 40 subunits, Complex II has 4 subunits, Complex III has 11 subunits, Complex IV has 13 subunits, and Complex V has 16 subunits. In addition to these large enzymatic complexes, there are also two electron carriers, ubiquinone (coenzyme Q10) and cytochrome c [93]. The NADH and $FADH_2$ intermediaries created during aerobic respiration act as reduced cofactors that donate hydrogen to complexes I and II. As the hydrogen molecules pass through the complexes, their electrons are stripped and are shuttled down an electrochemical gradient via the mobile electron carriers to complexes III and IV and are eventually transferred to molecular oxygen where they will produce water. At the same time, the H^+ ions that have been stripped of their electrons are pumped into the intermembrane space creating the mitochondrial membrane potential. The accumulated H^+ ions move from the intermembrane space back into the inner matrix through complex V (ATP synthase), and as they do complex V rotates and adds inorganic phosphate to ADP to create ATP [93]. These enzymes

complexes are crucial to energy production throughout the body and mitochondria have unique characteristics not seen elsewhere.

2.3.2 Mitochondrial DNA (mtDNA)

The most unique aspect of mitochondria is that they contain their own DNA (mtDNA) that is separate from nuclear DNA (nDNA) [94]. The complete human mtDNA sequence was published in 1981 and is referred to as the Cambridge Reference Sequence [26]. The mitochondrial genome is very different than the nuclear genome in that it is a small 16.569kb circle of double stranded DNA with 37 genes that code for the transfer RNA (tRNA), ribosomal RNA (rRNA), and the polypeptide subunits of respiratory complex enzymes (Complexes I, III, IV and V). 13 genes from mtDNA code for the following subunits of complexes I, III, IV and V: Complex I has ND1, ND2, ND3, ND4, ND4L, ND5, and ND6. Complex III has the cytochrome b (cytb) component. Complex IV contains the COX I, II, and III subunits. Complex V contains the ATP6 and ATP8 units of the ATP synthase enzyme. The only respiratory chain enzyme complex with no contribution from mtDNA is complex II [21]. 22 genes code for transfer RNA molecules necessary to assemble the respiratory complex enzymes, and two genes code for rRNA molecules necessary for protein translation [26]. Mitochondria are the only organelles outside the nucleus that contain their own DNA and the ability to synthesize their own RNA and proteins. Depending on the cell type there can be hundreds to thousands of mitochondria per cell and each one contains approximately five mitochondrial genomes. It is known that there are approximately 900 gene products in the mitochondria, many more than is encoded by the 37 genes found in the mtDNA. The vast majority of the respiratory complex enzymes are encoded for by nDNA [93, 95].

A typical human cell can have 100 or more mitochondria, each with approximately five copies of their mtDNA. In a normal individual, these copies are identical (homoplastic). However, individuals with a mutation in their mtDNA often have a combination of both normal and mutated mtDNA in their cells and this is termed heteroplasmy [96]. The amount of mutated mtDNA can vary widely from person to person, organ to organ, or even between individual cells, causing a threshold effect in which a certain percentage of mutated mtDNA must be present before symptoms of mitochondrial disease are evident [93]. If the abnormal amount of mtDNA copies exceeds a certain level, then the normal mtDNA can no longer compensate and the symptoms of the mitochondrial disease are expressed [97]. This is particularly evident in organs that rely heavily on mitochondrial energy production such as the eyes and central nervous system [98].

mtDNA is maternally inherited with the oocyte passing their mitochondria to offspring [99]. If a mother contains a mutation in her mtDNA she will pass it on to all of her children, but it will only continue to be passed on by her daughters. This has allowed large family pedigrees to be created that have been helpful in understanding the clinical variability seen among siblings of an affected mother [100]. mtDNA is not tied to the cell cycle like nDNA and is able to replicate continuously, even in nonmitotic tissue like skeletal muscle and the brain. In a heteroplasmic cell this may allow mutated mtDNA to replicate at different rates than normal mtDNA either due to random chance or some selective pressure. [21]. This could account for some of the late onset and clinical variability seen in mtDNA diseases [101].

2.3.3 Mitochondrial Diseases

According to Dimauro, the term "mitochondrial disease" refers to disorders of the mitochondrial respiratory chain enzymes that are under the dual control of both mtDNA and nDNA. He states that genetic classifications of mitochondrial disease distinguish those disorders that are due to mutations in mtDNA from those that are due to defects in nDNA. The mtDNA mutations can be divided into those that impair one of the 13 respiratory chain proteins encoded by mtDNA or those that affect mitochondrial protein synthesis in total that also includes the 22 tRNA and 2 rRNA genes [102]. For the purpose of this review, the focus will be only on those diseases that are associated with alterations in mtDNA that affect the functioning of the respiratory complex proteins.

Mitochondrial diseases were once thought to be extremely rare, but in the past decade several population based studies have shed light on the incidence and prevalence of mitochondrial disease. Majamma and colleagues examined the medical records in northern Finland to identify a cohort of adults with clinical features suggestive of mitochondrial disease in population of 245,201. They did extensive genetic testing and family tree analysis and gave a minimum point prevalence of one of the most common mtDNA mutations, the A3243G transition. They estimated the prevalence of mutation to be 16.3/100,000 (95% CI 11.3-21.4/100,000) in the general population. Upon further analysis of those carrying the A3243G mutation they found a disease prevalence of 5.71/100,000 for conditions that included hypertrophic cardiomyopathy, deafness, and diabetes mellitus [103].

Chinnnery and colleagues based their study on adults referred with suspected mitochondrial disease over a 15 year period to their center in northeast England, which serves a population of over 2 million. Through clinical, biochemical, and genetic studies, they

determined the prevalence of mitochondrial diseases to be 6.57/100,000 (95% CI = 5.30-7.83), and through extended family studies of affected patients were able to determine a mutation prevalence of 12.48/100,000 (95% CI = 10.75-14.23) [104]. Darin et al. [105] conducted a study of 358,616 children over a 15 year period in Sweden and found a minimum prevalence of 4.7/100,000. In another study done in Australian children referred to Melbourne Children's hospital over a 10 year period, Skladal and colleagues estimated the minimum prevalence of childhood respiratory chain disease to be 4.7/100,000 (95% CI = 3.2-5.0) in a population of 1,710,000 [106]. In a recent review of the epidemiological literature regarding mitochondrial disease, Schaefer estimated the combined prevalence of childhood and adult mitochondrial disease to be as high as 20/100,000 across all known mtDNA mutations thus far studied. [107].

The first pathogenic mutations of mtDNA were reported in 1988 [108, 109]. Since that time over 200 different pathogenic point mutations of the mitochondrial genome have been identified as well as other large scale rearrangements of the mitochondrial genome [110]. One of the major databases for collecting and disseminating information regarding the mitochondrial genome and variation is MITOMAP: A Human Mitochondrial Genome Database (http://www.mitomap.org, 2011).

Diseases that are due to defects in mtDNA affect a cell's ability to produce energy through OXPHOS reactions and often result in proliferation of both normal and mutated mitochondria within the affected cells, which gives them a characteristic ragged red fiber look [111]. This is also often accompanied by increased production of lactic acid as the affected cells resort to substrate phosphorylation for energy production [112]. Additionally, mitochondria are known to produce the majority of reactive oxygen species (ROS) in cells [113]. Mitochondria are especially susceptible to damage due to ROS because unlike nDNA, mtDNA has limited

repair capabilities [114]. Damage to mtDNA creates a cycle of worsening mitochondrial dysfunction and increased ROS production [30]. According to Calabrese, mtDNA mutations lead to impairment of the respiratory chain enzyme functioning, which leads to decreased ATP production, increased formation of toxic free radicals, and altered calcium homeostasis [115].

Wallace states that mitochondrial diseases are heterogeneous, often multisystemic, and tend to affect tissues with high energy demands, such as the brain, muscle, heart and endocrine systems. The energetic deficits of altered mitochondrial function have been implicated in forms of blindness, deafness, movement disorders, dementias, cardiomyopathy, renal dysfunction and aging [116]. A comprehensive list of diseases associated with mtDNA mutations organized by phenotype can be found at MITOMAP (http://www.mitomap.org, 2011).

2.3.4 mtDNA and Seizures

According to Waldbaum and Patel, mitochondrial dysfunction and oxidative stress contribute to several neurologic disorders, and have been implicated in both inherited and acquired epilepsies [52]. Inherited epilepsies are those caused by mutations in either the nDNA or mtDNA and it is known that many inherited mitochondrial diseases include seizure as part of their pathogenesis [22, 117]. Acquired epilepsies are thought to account for up to 60% of all epilepsies and are typically initiated by a brain injury (trauma, ischemia, hypoxia, etc.) followed by a "latent period" in which molecular, biochemical and other cellular alterations occur leading to chronic epilepsy [52]. In one study it was found that seizures were the first recognized sign of a mitochondrial disorder in 53% of the patients they examined [23]. Two of the most studied mitochondrial diseases that exhibit seizure are Myoclonic Epilepsy with Ragged Red Fibers (MERRF) and Mitochondrial Myopathy Encephalopathy, Lactic Acidosis and Stroke (MELAS).

MERRF can be caused by several different point mutations in mtDNA, with clinical hallmarks that include progressive epilepsy and a distinct appearance of mitochondria in muscle fibers [24]. MELAS syndrome can be caused by multiple mtDNA point mutations and has clinical features that include seizures, hemiparesis, hemianopia, and blindness among others [25].

In preparation of this review, an initial search was performed on mtDNA and seizures, and returned several excellent reviews that have compiled tables of some of the more common mtDNA point mutations that have been associated with epileptic phenotypes [22, 53, 54]. In an effort to get the most complete picture of mtDNA point mutations that have been associated with seizure phenotypes, the MITOMAP database was accessed between 5/13/11 and 6/05/11 for the initial searches and again on 8/20/11 for updates (http://www.mitomap.org, 2011). There are two databases within MITOMAP that have a comprehensive list of all the mtDNA mutations, associated diseases, clinical phenotypes, and link to the original source articles.

1. Diseases associated with mtDNA mutations in the 22 tRNA and 2 rRNA genes (tRNA, rRNA link)
2. Diseases associated with mtDNA mutations in the 13 respiratory protein coding regions (coding region link).

There were a total of 257 articles in the two databases and the same review criteria were applied to both databases. In order to be included for this review, the articles had to include the following information: The affected patient(s) had to have seizures as part of their clinical presentation. The articles also had to include a discussion of the pathogenicity of the mutation.

There are well accepted criteria for pathogenicity of mtDNA mutations that include:

1. The mutation should not be found in normal individuals of the same ethnic group.
2. It should alter a site that has been conserved evolutionarily (i.e. it is functionally important and not novel).
3. It should cause single or multiple respiratory chain deficiencies.
4. There should be a correlation between the amount of mutant mtDNA and clinical severity (Adapted from Dimauro, 2010 [118], and Chinnery, 1999 [119]).

Articles were not included for review if seizures or epilepsy were not documented as part of the clinical presentation. If the clinical presentation was due to an insertion or deletion rather than a point mutation it was not included (there were a total of 3 articles excluded for this reason).

From the clinical phenotype database for the 22 tRNA and 2 rRNA genes responsible for protein assembly inside the mitochondrion, a total of 176 articles were reviewed and 31 different mtDNA point mutations met the criteria listed above. Mutations deemed to be pathologic in causing clinical phenotypes that included seizure were noted in 13 different genes. A list of the primary articles with the associated mutation in the tRNA or rRNA genes is included in Appendix A. The tRNA genes with identified mutations were: Asparagine (MT-TR), Glutamic Acid (MT-TE), Glutamate (MT-TQ), Glycine (MT-TG), Histidine (MT-TH), Isoleucine (MT-TI), Leucine (MT-TL), Lysine (MT-TK), Phenylalanine (MT-TF), Serine (MT-TS), Threonine (MT-TT) and Valine (MT-TV). The one rRNA assembly gene with an identified mutation was 16S (MT-RNR2). The tRNA regions with the most documented mutations related to seizure are the Leucine and Lysine genes, and the most common clinical conditions associated with them are MERRF and MELAS.

From the clinical phenotype database of mutations affecting respiratory enzyme coding region genes, a total of 81 articles were reviewed and there were 20 different mtDNA mutations that met the criteria of having seizure as part of the phenotype. Mutations were noted in 9 different genes of the 13 total genes in the mtDNA that code for proteins of the respiratory complex enzymes. In Complex I, mutations that associated with seizure were found in the ND1, ND3, ND4, ND5 and ND6 genes. In Complex III, mutations were found in the cytochrome b gene. In Complex IV, mutations were found in the COX I and COX III genes. In Complex V, mutations were found in the ATP6 gene that associated with seizure phenotypes. A list of the primary articles with the associated mutations in the respiratory protein coding genes is included in appendix B**.** Complex I subunits appear to be hotspots for mutation related to seizure activity. 10 different mutations with seizure phenotypes were noted in the ND1-ND6 subunits.

Seizures appear to be an extremely common component of many mitochondrially inherited diseases. This finding is not unexpected, given that mitochondrial diseases often manifest in those tissues with the highest energy demands (i.e. brain or muscle). However, the role that altered mtDNA and the associated mitochondrial dysfunction play in the 60% of acquired seizure disorders (i.e. PTS) remains to be explored [52].

2.3.5 mtDNA Variants as Risk Factors for PTS

Post-traumatic seizures are a form of acquired seizures that are a common and often debilitating consequence of TBI. Numerous risk factors have been identified in both clinical and population based studies of PTS, and recent work has begun to investigate the role that specific nuclear genetic variants may play in the risk of developing PTS. Over the past few decades, numerous variations in the mitochondrial genome have been identified that lead to disrupted

energy production, oxidative stress, and homeostatic imbalance. High energy tissues like the brain are particularly susceptible. Additionally, many mitochondrial disorders exhibit seizure phenotypes as part of their pathogenesis and mitochondrial variants have been implicated in contributing to both inherited and acquired forms of epilepsy.

To date no work has explored the possible association between mtDNA variants and the risk of developing PTS. In reviewing the current literature, only one other study was found that explored variation in the mtDNA genome as a risk factor for acquired injury. Canter and colleagues performed a prospective study on 745 patients admitted to the trauma intensive care unit at Vanderbilt University Medical Center between April 2005 and February 2007. All patients admitted to their level 1 trauma center were potentially eligible for the study. However, those younger than 18, prisoners, those with known pregnancy, and those that expired before admission into the trauma ICU were excluded from the study. All eligible patients had blood drawn within 24 hours of admission to the trauma ICU. A total of 666 patients had DNA extracted from a blood sample. Detailed demographic and clinical covariates were obtained at the time of admission. They sought to determine whether specific genetic variations in mtDNA known to impact energy production were associated with the risk of in-hospital mortality after severe trauma as assessed by Injury Severity Scores (ISS) They selected three mtDNA polymorphisms at positions 4216, 10398, and 4917 because variants are known to alter the amino acid sequence of key subunits of the Complex I enzyme of the respiratory chain. They demonstrated that one mtDNA variant (the 4216 T allele) was an independent predictor of increased mortality in the multivariate model [30]. While the study did not focus on TBI or PTS, their findings imply that mtDNA variants can be useful as predictors of outcome after injury.

2.3.6 Summary

Traumatic brain injury (TBI) represents a major public health issue with some 1.7 million people sustaining TBI annually in the United States [1]. Between 3.2-5.3 million people suffer from the long term sequelae of TBI that include reduced quality of life, and the loss of one or more physical or mental functions [3], and up to $60 billion is spent yearly to cover health expenses and lost wages due to TBI [31].

One common and potentially debilitating consequence of TBI are post-traumatic seizures (PTS), which are seizures that occur after head injury and thought to be causally related to the trauma itself [32]. The probability of developing PTS is significantly correlated to the type (closed versus penetrating) and severity of TBI (mild, moderate and severe). The prevalence of PTS ranges from as high as 53% in military populations with penetrating TBI [35], to approximately 10-15% among civilians with severe TBI [9].

PTS is a heterogeneous condition and it has been proposed to depend on variation in the type, and severity of injuries as well as on the different genetic backgrounds, ages and genders of the patients [36, 37]. Injury severity is by far the best predictor of who will go on to develop PTS [32, 38]. Lesion type and lesion location have also been associated with the development of PTS in several human studies [38, 43-45]. PTS may also play a role in moderating functional outcomes after TBI. PTS has been associated with poorer GOS scores and higher incidence of behavioral abnormality in patients with severe TBI [46]. Late PTS has also been associated with a higher incidence of depression [47]. A 5 year prospective study of rehabilitation and reemployment in Finland showed that late PTS was associated with poorer functional and social outcomes and was a considerable factor in hospital readmission [48]. Overall, it appears that PTS

can result in greater psychiatric problems as well as reduced general health and poorer functional and social outcomes [12].

Studies exploring genetic susceptibility to seizure disorders have expanded significantly the number of potential candidate genes involved with seizures [14, 15, 49], while others have begun to expand the concept of genetic susceptibility to TBI outcomes [50, 51]. Only recently have genetic studies revealed that several nuclear genetic variants (APOE, A1AR, GAD) may act as risk factors in the development of PTS [16-20]. While no studies have yet to focus on the role of mitochondrial DNA in the development of PTS, strong evidence has emerged that variations in the mitochondrial genome are linked to mitochondrial encephalopathies that have disrupted cellular metabolism and seizure as part of their phenotype [52-54].

The goals of this proposed project are to use data from our cohort of severe TBI patients to explore roles that mtDNA variants may play as independent risk factors for the development of both early PTS (EPTS) and late PTS (LPTS) (**Aim 1**). To explore whether mtDNA variants act as effect modifiers of other injury related factors (mechanism, lesion type) in relation to the development of EPTS or LPTS (**Aim 2**), and to explore if mtDNA variants affect the relationship between LPTS and functional outcome measures (GOS) at 6 months post-injury (**Aim 3**).

3.0 METHODS

3.1 STUDY POPULATION AND VARIABLE SELECTION

3.1.1 Setting

Subjects included in this research were recruited at the University of Pittsburgh Medical Center (UPMC), a Level I accredited regional trauma center with 816 beds and an NIH (NS: 30318) funded Head injury Clinical Research Center (Brain Trauma Research Center: BTRC). The BTRC has worked closely with the neurotrauma intensive care unit (NTICU) of UPMC since 1991 collecting data on severe head injury patients. This close partnership has led to a cohort of well characterized TBI patients with extensive biological, genetic, demographic and functional outcome data available through the BTRC databases.

3.1.2 Subjects

All subjects who were admitted to the Emergency Department of UPMC Presbyterian were considered for inclusion if they met the following criteria of severe TBI:

Inclusion criteria:
1. Severe TBI (Glasgow Coma Scale $\leq$ 8).
2. Age 18-75 years old.
3. Positive findings on head CT scan.
4. Required extra-ventricular catheter for drainage and intracranial pressure monitoring.

Exclusion criteria:
1. Any pre-existing neurologic diseases or deficits
2. Significant cardiac or respiratory arrest before hospital admission.
3. Severe TBI due to penetrating injury.

3.1.3 Critical Care Management

All subjects admitted to the BTRC through the NTICU received treatment as defined by *The Guidelines for Management of Severe Head Injury* [66]. CT scans were performed to identify intracranial lesions or hemorrhages. Each patient had an extra-ventricular catheter, a venous catheter and arterial catheter placed upon admission. If necessary, decompressive craniotomy was performed. Any elevation in intracranial pressure (ICP) was treated to maintain normal levels (<20mmHg) and cerebral perfusion pressure (CPP) was maintained at >60mmHg. However, if CPP remained low, then mean arterial pressure (MAP) was raised with appropriate medications to maintain a MAP >90mmHg. Standard EEG monitoring was ordered

intermittently for patients with suspicion of seizure activity. Most patients enrolled received anti-seizure prophylaxis for 1 week post-injury [20].

3.1.4 Sample Size

Sample Size: (n=336 for 6 SNPs, n=136 for 19 SNPs):

The samples for this study were collected from patients enrolled with additional consent for genetic analysis into the BTRC as part of a study that was conducted to assess relationships between genetic variants and TBI outcomes. The BTRC typically enrolls 40 patients per year under their criteria for severe TBI. With few exceptions (consent refused, patient or proxy unavailable to give consent) genetic consent was acquired on almost all patients who met the criteria. Genetic data was obtained from blood or CSF samples in 336 patients.

3.1.5 MitoChip Sampling

In the initial phase of the research, mitochondrial genotype data was collected using the MitoChip V2 array **[120]** in 136 patients. The MitoChip V2 allows for genotyping of the entire 16,569 base positions of the human mitochondrial genome. The probes on the MitoChip match the revised Cambridge Reference Sequence (CRS) for human mtDNA, but also contain probes for the 500 most common mutations across haplotypes of the CRS as well [120]. Any position that was not monomorphic and had call rates above 95% from the MitoChip assays were included in the initial sample. There were a total of 19 mtDNA polymorphisms that met these criteria and were included in the primary sample: T146C, T195C, T477C, C3494T, T4216C,

A4917G, C7028T, A8701G, A10398G, A10550G, T10873C, A11947G, A12308G, C12705T, G13708A, T13789C, A14233G, C14766T, and T16519C.

3.1.6 Additional Genotype Collection

In a preliminary analysis of mitochondrial variants and functional outcomes (Dr. Yvette Conley, personal discussion), four of the mtDNA variants (T195C, T4216C, A4917G, and A10398G) from the original sample of 136 had the strongest relationship to other TBI outcomes and were therefore collected in an additional 200 patients using Taqman® customized SNP assays (Applied Biosystems Inc., Carlsbad, CA) or RFLP techniques bringing the sample size for those four variants to 336, However, there were four subjects in which genotypes were unable to be collected using this method, so the total sample dropped to 332.

During the course of the univariate analysis for this study of the original 19 mtDNA variants in 136 individuals to PTS, two variants (T477C and T16519C), were found to have the strongest associations with LPTS. The original cases were revaluated using Taqman® SNP assays to ensure accurate genotype call rates and additional samples for both variants were added to the analysis.

For each variant, the Wild Type (WT) genotype was taken from the most up to date Revised Cambridge Reference Sequence (rCRS) found on MitoMap.org (Last Access, July 2012). For this analysis WT = 0 and the Variant = 1. Call rates for genotypes were above 90% for all the variants used in this analysis. Each variant was examined among those who have EPTS and those who have LPTS. For the last two variants added to this analysis (T477C and T16519C), banked samples were no longer available for 38 cases, hence the smaller n's for those

two variants compared to the others. However, without those missing samples, the call rates for T477C and T16519 were at or above 90% and deemed acceptable for analysis.

3.1.7 Power and Odds Ratio Estimates

As the sample size was fixed at 136 for 13 of the variants and 332 for 6 of the variants, estimates of power and effect sizes able to be detected were created using the PASS 11.0 (NCSS, Kaysville UT) software. A two proportion odds ratio (mtDNA variant present/absent vs. PTS Y/N) analysis was done to determine odds ratio and power for both the smaller and larger subsets of variants.

In the smaller subset (n=136) there were two variants in the 1-2% range, seven in the 3-9% range and four in the 10-40% range for minor allele frequencies in this study. For the variants with minor allele frequencies between 1-2%, odds ratios of 1.5, 2.0, and 3.0 were able to be detected with power between 2-3%. For the variants with a minor allele frequency between 3-9%, odds ratios of 1.5, 2.0 and 3.0 were able to be detected with power between 10-35%. For those variants with minor allele frequencies between 10-40%, odds ratios of 1.5, 2.0 and 3.0 were able to be detected with power between 15-70%.

In the larger subset (n=332) five of the variants had minor allele frequencies between 10-35% and only one of the variants had a minor allele frequency of less than 10%. For the one variant with a minor allele frequency of less than 10%, odds ratios of 1.5, 2.0 and 3.0 were able to be detected with power between 4-7%. For those variants with minor allele frequencies between 10-35%, odds ratios of 1.5, 2.0 and 3.0 were able to be detected with power between 10% and 85%.

3.1.8 Genotyping

Blood Samples:

Upon admission, a blood sample was collected in a 3-ml EDTA vacutainer tube and delivered to the genetics lab in the School of Nursing within 48 hours for DNA extraction. All samples were processed to remove the buffy coat and then stored in an -80ºC freezer in the School of Nursing laboratory until analyzed. All blood was drawn as per hospital policy using an existing arterial or central venous catheter

CSF Samples:

CSF samples were collected every 12 hours for the first the first 5 days post-injury using passive drainage from a ventriculostomy placed by the neurosurgeon on call and verified by x-ray as part of the standard of care. The ventriculostomy bag was changed using sterile technique, and the CSF is removed from the collection system. Three milliliters of CSF were aliquoted into a cryogenic tube, labeled with the date and time and identification number and stored in a -20C freezer. After 5 days of samples were collected, the samples were transferred to -80ºC freezers in the School of Nursing for long-term storage. Some mtDNA samples were obtained from this banked CSF for the current analysis.

mtDNA Extraction:

DNA was extracted from one of two sources for each subject, whole blood or CSF. Whole blood was collected in EDTA vacutainer tubes, processed to retrieve the buffy coat and DNA was extracted using a simple salting out procedure. CSF was collected by passive drainage as part of clinical care and DNA was extracted using the Qiamp DNA extraction protocol for

extraction from bodily fluids (Qiagen Corp, Valencia, California) [19]. DNA collection from whole blood was the preferred source when available.

3.1.9 Demographic and Injury Data

Demographic Data:

Demographic information including age, sex, race, and ethnicity was recorded from medical records upon admission by the BTRC staff. Medical history; including a history of premorbid seizures, were recorded upon admission or from the patient's medical records. Information regarding anti-seizure medication usage during the ICU and hospital stays was also extracted from medical records. Each patient's age was calculated in years using their date of birth from their records. Length of hospital stay was calculated from the date of injury to their indicated discharge date in the medical records.

Injury Data:

The BTRC staff collected information on mechanism of injury (fall, motor vehicle accident, etc.). Injury severity (as defined by admission GCS score) was coded by the neurosurgeon responsible for clinical care of the BTRC after the patient was stabilized and not under the influence of any paralytic drugs. For this analysis GCS scores were categorized into the most severe group (i.e. GCS 3-4) and a less severe group (i.e. GCS 5-8). Injury data collected from the Trauma Registry included type of lesion (intracerebral hematoma, subdural hematoma, extradural hematoma, etc.) as indicated by the patients ICD 9 code in their medical record.

Evidence of depressed skull fractures and whether the patient had cranial surgery during their hospital stay were recorded. Some of the patients included in this analysis were previously enrolled in a larger prospective hypothermia trial within the BTRC. Information on whether a person received cooling during their hospital stay was also abstracted from their medical records.

Originally, it was planned to include data on injury location as evidenced by CT scan as one of the injury factors that may relate to PTS. During the course of data collection and abstraction, it was found that the CT scan data was not consistently reported over time and different data collection methods made it difficult to choose which information to use. The decision was made to use lesion types, mechanism, skull fracture, and cranial surgery as injury factors.

3.1.10 Mortality

Mortality data was collected using death reports from medical record or in the case of late occurring death from the social security death index. Deaths were coded as occurring during the first week, during the first month and during six months. To avoid the confounding effects of death in this population of severe TBI, those who died in the first week without having seizure were excluded from analysis of EPTS. If a subject had EPTS and then died, they were included in the analysis group. To avoid the confounding effects of death in the LPTS group, anyone who died without evidence of seizure was removed from the analysis.

3.1.11 PTS Data

To assess PTS activity, all electronic inpatient and outpatient medical records available were reviewed to document the first documented PTS. Time to first seizure in days was the primary measure abstracted from the medical records. Inpatient notes used to determine time to first seizure included ambulance emergency room reports, progress notes, nursing notes, EEG notes, patient history and physical reports and discharge or transfer summaries. Determining late PTS required at least one discharge/death summary or inpatient/outpatient note related to the TBI and referencing the appropriate PTS time period. Availability of late PTS information ranged from 6 months to 6 years post injury [19]. Time to first seizure was recorded in days from injury. PTS was coded into three different categories: early, late and delayed-onset. EPTS were those that occurred between 1-7 days post injury, LPTS occurred after one week and before 6 months, and delayed-onset seizures occurred after 6 months. Any premorbid history of seizures, and any information about the use of anti-epileptic drugs during the acute phase of the hospital stay was also abstracted from the electronic medical records. Information regarding additional seizures was not available.

For this analysis, due to the small sample sizes of the SNP data after adjusting for mortality in the population, the decision was made to condense the late and delayed-onset groups into a single group. For this analysis, EPTS is considered to be any PTS activity that happens within the first week of injury and LPTS is considered to be any PTS that occurs beyond the seventh day post-injury. This is in agreement with how EPTS and LPTS are considered in much of the PTS literature.

3.1.12 Functional Outcome Data

Different functional outcome variables were available via the BTRC database for most of the patients who were genotyped at a variety of time points. Glasgow Outcome Scale (GOS) data were available for many subjects at time periods of 6 and 12 months post-injury. The GOS is a clinical observation scale that categorizes functional outcomes into five levels: (1) Death, (2) Persistent vegetative state, (3) Severe disability, (4) Moderate disability, and (5) Good recovery. [121]. Interrater reliability has been reported from 68-95% [122]. GOS also correlates well with severity of injury and other measures of function such as the Disability Rating Scale [123]. The GOS has wide acceptance and established validity. Each outcome was reclassified into one of three groups; GOS 1=0, GOS 2-3=1, GOS 4-5=2. In this analysis, since deaths were removed (see Figures below) there were essentially the two categories of GOS, with the first representing those with poor or severely impaired outcomes and the second with those who have better outcomes.

3.1.13 Study Inclusion: Smaller Subset

For 13 of the mitochondrial variants, 136 subjects met the inclusion criteria to be enrolled into the BTRC and had mtDNA genotyping done. To reduce the potential confounding effect of the existing seizure disorders on the development of PTS, 7 subjects with evidence of premorbid seizures were immediately excluded.

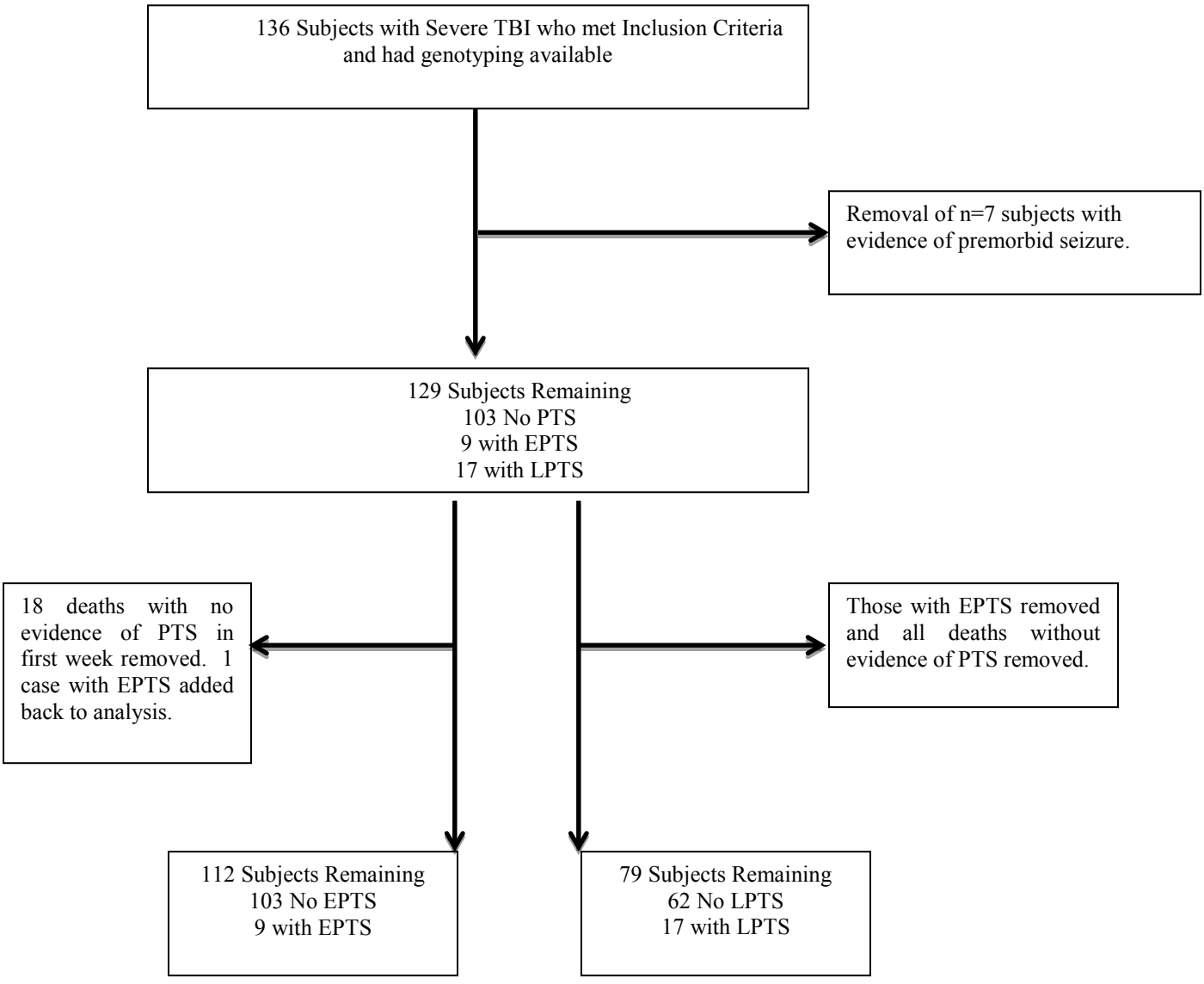


Figure 3: Sample Selection - Smaller Subset of Populations (n=136)

3.1.14 Study Inclusion: Larger Subset

For 6 of the mitochondrial variants there were a total of 336 subjects who met the inclusion criteria for the BTRC and had mitochondrial genotyping done. 17 subjects with evidence of premorbid seizure were excluded from the final sample. In addition, there were 3 subjects in which no genotypes were able to be collected for any of the SNPs and they were excluded from the sample. The total sample size for the larger subset of data is 312.

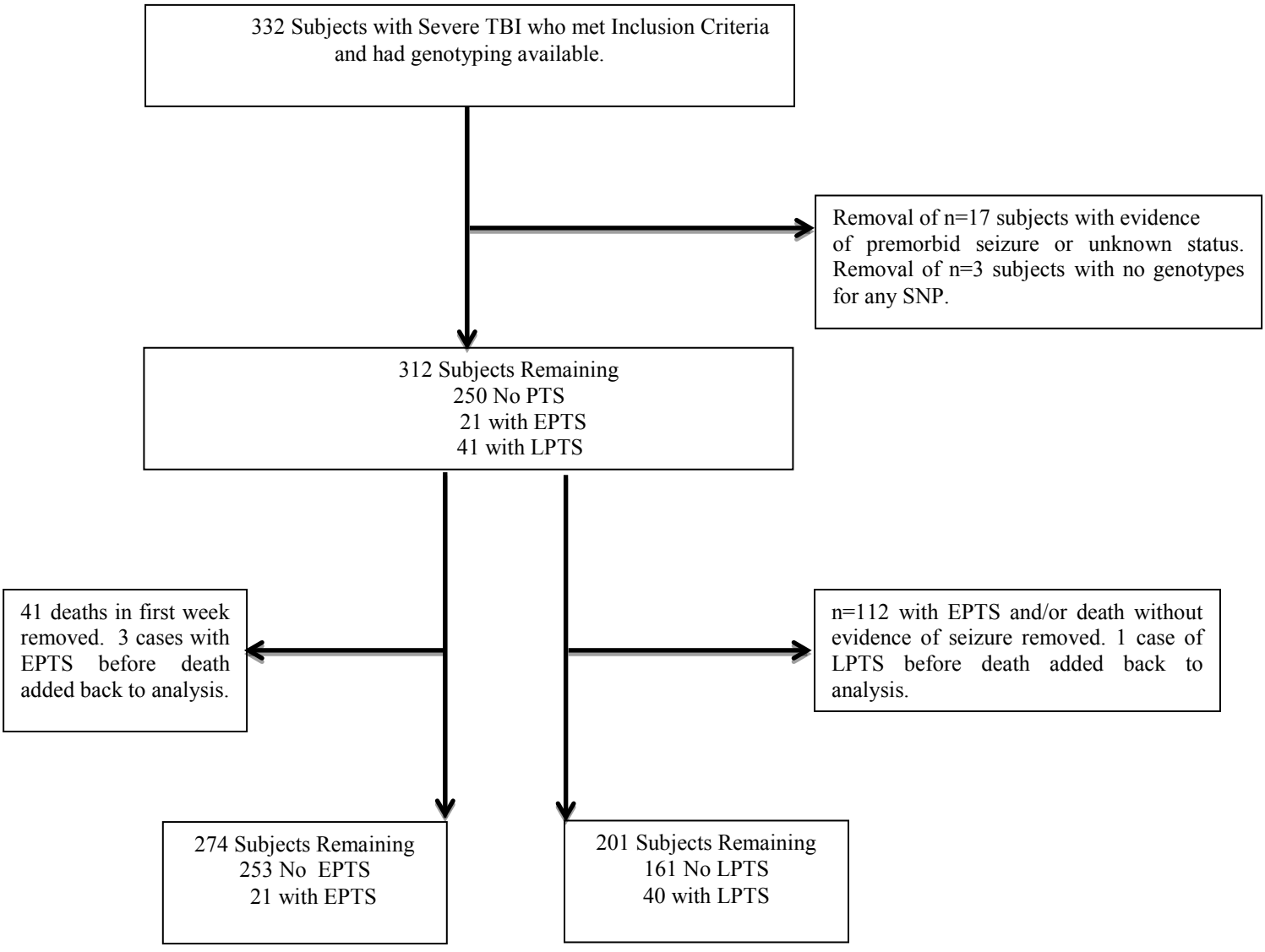


Figure 4: Sample Selection – Larger Subset of Population (n=332)

3.2 ANALYSIS PLAN – AIM 1

3.2.1 Specific Aim 1

Examine the contribution of mtDNA variation to the development of both EPTS and LPTS in this cohort of severe TBI patients.

3.2.2 Descriptive Variables

For descriptive purposes, measures of central tendency and dispersion will be obtained for all continuous variables at all available time points. Exploratory data analytic techniques and visual examination of trends and patterns will be utilized to become familiar with the data and to identify any potential outliers.

3.2.3 Univariate Analysis

Chi-squared analysis of each mtDNA variant to compare allele frequencies between individuals who did and did not have EPTS and LPTS was performed. If any cell was too small for Chi-square analysis, Fisher's exact tests were used. The data were checked for excessive outliers and multiple missing values using visual inspection, as well as using the Data Validation and Missing Value Analysis functions in SPSS 20 (Chicago, IL).

3.2.4 Data Coding

Each of the 19 mtDNA genotypes in this analysis are coded such that the Wild Type = 0 and the Variant =1.

3.2.5 EPTS vs. mtDNA Variants

EPTS was coded as a binary variable in which 0 = no EPTS and 1 =EPTS. When doing the analysis of variants vs. EPTS any subjects who died in the first week without evidence of seizure were excluded from the analysis. There were 3 subjects with EPTS who later died in the first week. Since these subjects had evidence of EPTS before their death, they were added back into the analysis of the variants to EPTS. In the smaller subset (n-136) – A total of 112 subjects were in the EPTS analysis. In the larger subset (n=332) – A total of 274 subjects were in the EPTS analysis.

3.2.6 LPTS vs. mtDNA Variants

LPTS was coded as a binary variable in which 0=no LPTS and 1 = LPTS. As the focus for this analysis was on subjects whose first seizure occurred after 7 days post-injury, any subject who had EPTS was removed from the analysis. Any subject who died without evidence of PTS was also removed from the analysis. While this decreases the sample number, it eliminates the potential confounding effect of death. In the smaller subset (n-136) – A total of 79 subjects were in the LPTS analysis. In the larger subset (n=332) – A total of 201 subjects were in the LPTS analysis.

3.2.7 Demographic and Injury Data vs. PTS

The majority of demographic and injury variables in this study were coded in a binary fashion. Each of these factors was examined among individuals with and without EPTS/LPTS using Chi-squared tests or Fishers Exact test where appropriate to look for an association. Age and Length of Hospital Stay were continuous variables and Independent samples t-tests were used to assess these variables for descriptive and comparative purposes.

3.2.8 Multivariate Analysis

To ensure that no potential associations between variants and the outcomes of EPTS or LPTS were being overlooked, any variant with a univariate test statistic of $p<0.25$ was entered into a binary logistic regression model. In the case of EPTS, there were a total of 4 variants that showed an association to EPTS in the Chi-Square analysis at this p-value level. A series of models were built to test the individual effects of each variant on EPTS. Forward and backward stepwise procedures were used to assess which covariates assert influence in the model. An odds-ratio and 95% confidence interval was calculated for each variant that had a univariate association of $p<0.25$ in both the smaller and larger samples.

For each multivariate model, the clinical covariates of Gender, Age, and GCS were entered regardless of their significance in the univariate models as these are known covariates in the literature with both EPTS and LPTS. Additionally, since SDH and Cranial Surgery associated strongly with LPTS in this analysis, these were added as additional covariates in the models of LPTS.

3.3 ANALYSIS PLAN – AIM 2

3.3.1 Specific Aim 2

Examine if selected injury factors associated with increased risk of both EPTS and LPTS, are affected by the presence of specific mtDNA variants in this cohort of severe TBI patients.

3.3.2 Stratified Analysis of Injury Factor by LPTS by Variant

To explore whether the mtDNA variants could potentially affect the relationship between injury factors and the outcome of LPTS, stratified analyses were done in which the injury factors that associated with LPTS at $p<0.05$ in the univariate analysis (SDH and Cranial Surgery) were compared to the outcome of LPTS, and each of the 19 mtDNA variants were then entered as stratifying variables. This generated an odds ratio and significance for each allele with the injury factor of interest on the outcome of LPTS.

In the next step, determination of which allele acted as the risk allele, the prevalence of each allele in those with LPTS (cases) was compared to the prevalence of the allele in those with no LPTS (controls). For each variant, the risk allele was the one with a higher frequency in the cases than in the controls.

In the final step to further explore whether there were any trends towards effect modification of mtDNA variants with the exposure variable (injury risk factors) on the outcome of PTS each of the variants was entered into a 2X4 table to assess their joint and individual effects on the outcome of LPTS (see figure 5 below). The first row of table identifies the combined effect of the gene and exposure, the second row identifies the effect of the risk allele

alone, the third row identifies the effect of the exposure (I.e. the opposite allele and exposure) only, and the fourth row is the reference category. The reference category is the control group (i.e. no risk allele and no exposure). Odds ratios, confidence intervals, and p-values can be calculated for each combination of genetic factor and exposure allowing for direct comparisons between groups. This model allows for risk estimates of gene environment interactions to be easily tested and visualized and allows for direct comparisons of the gene alone and exposure effect alone to be compared. It can also highlight issues related to small samples sizes, by providing confidence interval estimates for the risk estimates [124].

Strata	Cases (Affected)	Controls (Unaffected)	Odds Ratio	Information
Gene & Exposure G+ E+	a	b	ah/bg	Joint genotype and exposure factor compared to none.
Gene & No Exposure G+ E-	c	d	ch/dg	Genotype alone vs. none
No Gene & Exposure G- E+	e	f	eh/fg	Exposure alone vs. none
No Gene & No Exposure G- E-	g	h	1	Reference group

Figure 5: Testing Gene-Environment Interactions
Adapted from Botto et al., 2001[124]

3.4 ANALYSIS PLAN – AIM 3

3.4.1 Specific Aim 3

To examine if EPTS and LPTS are associated with functional outcomes and if this relationship is affected by mtDNA variants in this population of severe TBI patients.

3.4.2 Stratified Analysis of LPTS by GOS by Variant

To determine is EPTS or LPTS was associated with worse functional outcomes at 6 months post-injury, those with PTS were compared to those with worse outcomes (GOS 1-3) and those with better outcomes (GOS 4-5). EPTS was not associated with GOS at 6 months, so the remainder of this analysis focuses on those with LPTS. Also, as deaths were removed from the LPTS group, the outcomes at 6 months became those alive with worse outcomes (GOS 2-3) compared to those alive with better outcomes (GOS 4-5). Those with and without LPTS were stratified by mtDNA variants to look for possible associations. For the variants from the smaller subset (n=79), the cell sizes became too small to explore any further possible effect modification of the variant on outcome. For this reason, the analysis in this section was restricted to the variants from the larger subset. During the initial stratification, the variant T477C had cells with no subjects so further stratification was unable to be performed. During the initial stratification of the five remaining mitochondrial variants, and odds ratio and confidence interval was calculated for each variant.

In the next step, for each variant, the risk allele was the one with a higher frequency in the cases than in the controls. For further 2X4 analysis of LPTS to GOS, the determination of the risk allele for additional stratification was done by taking the prevalence of the allele in those with LPTS (cases) compared to the prevalence of the allele in those with no LPTS (controls).

In the final step, to explore possible trends of effect modification of the selected variants with the exposure (LPTS) on the outcome of GOS, they were entered into a 2X4 table to assess their joint and individual effects on the outcome of poorer GOS (see figure 5). Odds ratios, confidence intervals and p-values were computed for each row allowing for direct comparisons of allele & exposure, allele alone and exposure alone. This model allows for risk estimates of gene exposure interactions to be easily tested and visualized and allows for direct comparisons of the gene alone and exposure effect alone to be compared. It can also highlight issues related to small samples sizes, by providing confidence interval estimates for the risk estimates [124].

3.4.3 Statistical Analysis Resources

All primary statistical analyses were completed using SPSS version 20 (Chicago, IL) and additional online resources were used to aid in computation of Odds Ratios, confidence intervals and exact p-values for the 2X4 tables.

EpiMax for Calculating OR's and CI: **http://www.healthstrategy.com/epiperl/epiperl.htm**

Exact p-value calculator for RXC tables: **http://www.physics.csbsju.edu/cgi-bin/stats/exact**

Vassar p value calculator for RXC tables: **http://vassarstats.net/newcs.html**

4.0 RESULTS

4.1 GENERAL POPULATION DESCRIPTION

4.1.1 Description of Population

Nineteen mitochondrial variants were analyzed initially in a total of 136 subjects and 6 were analyzed in larger sample of 336. Descriptive analyses were done to compare the smaller subset (n=129) and larger subset (n=312) after removal of those with premorbid seizures and cases with no genotype information. No major differences were noted in any of the demographic, clinical, or injury characteristics between the two subsets (Tables 1 and 2). Since the original 136 subjects were included in the larger subset, the following descriptions of the sample are based upon the entire population.

Table 1: Population Characteristics in Large and Small Subests

Variable	n=312 Count (%)	n=129 Count (%)
Gender		
Male	245 (78.5)	99 (76.7)
Female	67 (21.5)	30 (23.3)
Race		
Caucasian	288 (92.3)	121 (93.8)
African American	18 (5.8)	7 (5.4)
Other/Biracial	3 (1.0)	1 (.8)
Missing	2 (.6)	-
Age	34.13+-14.72	35.02+-15.33
Length of Stay	20.54+-11.93	21.63+-11.85
AED		
Yes	293 (93.9)	123 (95.3)
No	18 (5.8)	6 (4.7)
Missing	1 (.3)	
Hypothermia		
Yes	76 (24.4)	20 (15.5)
No	236 (75.6)	109 (84.5)
GCS		
3-4	71 (22.8)	31 (24.0)
5-8	232 (74.4)	97 (75.2)
Missing	9 (2.9)	1 (.8)

Frequencies are after removal of those with premorbid seizure.

Table 2: Injury Characteristics Large and Small Subsets

VARIABLE	N=312 n (%)	N=129 n (%)
Mechanism		
Motor Vehicle	191 (61.2)	72 (55.8)
Motorcycle	47 (15.1)	24 (18.6)
Fall/ Sport	57 (18.3)	23 (17.8)
Hit/Assault	8 (2.6)	5 (3.9)
Other	5 (1.6)	3 (2.3)
Missing	4 (1.3)	2 (1.6)
ICD9 – SDH		
Yes	170 (54.5)	78 (60.5)
No	102 (32.7)	51 (39.5)
Missing	40 (12.8)	
ICD9 – SAH		
Yes	183 (58.7)	93 (72.1)
No	89 (28.5)	36 (27.9)
Missing	40 (12.8)	
ICD9 – DAI		
Yes	86 (27.6)	48 (37.2)
No	186 (59.6)	81 (62.8)
Missing	40 (12.8)	
ICD9 – EDH		
Yes	44 (14.1)	16 (12.4)
No	228 (73.1)	113 (87.6)
Missing	40 (12.8)	
ICD9 – CONTUSION		
Yes	127 (40.7)	61 (47.3)
No	145 (46.5)	68 (52.7)
Missing	40 (12.8)	
ICD9 – IVH		
Yes	81 (26.0)	44 (34.1)
No	191 (61.2)	85 (65.9)
Missing	40 (12.8)	
ICD9 – ICH		
Yes	97 (31.1)	40 (31.0)
No	175 (56.1)	89 (69.0)
Missing	40 (12.8)	
ICD9 – OTHER		
Yes	21 (6.7)	7 (5.4)
No	251 (80.4)	122 (94.6)
Missing	40 (12.8)	
SKULL FRACTURE		
Yes	15 (4.8)	8 (6.2)
No	271 (86.9)	107 (82.9)
Missing	26 (8.3)	14 (10.9)
CRANIAL SURGERY		
Yes	119 (38.1)	42 (32.6)
No	167 (53.5)	73 (56.6)
Missing	26 (8.4)	14 (10.9)

Frequencies are after removal of those with premorbid seizure.

4.1.2 Demographic and Clinical Characteristics

The population is largely male (78.5%), Caucasian (92.3%) and relatively young (34 years ±14). The average inpatient stay was almost 21 days (20.54±12) and the vast majority of subjects (93.9%) were given anti-epileptic drugs during at least the first week of their hospital stay. All patients had initial GCS scores in the 3-8 range, further stratification of GCS into more severe (GCS 3-4) and less severe categories (GCS 5-8), shows that the majority (74.4%) fell into the range of GCS 5-8. Some of the subjects from this sample were also enrolled in previous trials of induced hypothermia for TBI. However, more than 75% of the subjects in this sample were part of the normothermic control groups.

4.1.3 Race and Ethnicity

The vast majority of subjects were Caucasian (>92%) in both smaller and larger samples. While it is known that ancestral background can influence allele frequency, the MitoChip assay does account for the 500 most common SNPs from the major mtDNA haplogroups. During the initial analysis of the variants vs. PTS status and the demographic and injury variables vs. PTS, all races were left in and a second scenario was created using only Caucasians. In these samples, omitting race had no effect on any of the outcome measures, and due to the limited sample size of this study, it was decided to leave all races in the final analysis. For ethnicity, there was a tremendous amount of nonresponses (>60%), and in those who did respond, over 99% reported as non-Hispanic so it was decided to not try and analyze based upon ethnicity.

4.1.4 Injury Characteristics

Motor vehicle accidents were the primary mechanism of injury (61.2%), followed by injuries due to falls (18.3%), motorcycle accidents (15.1%), being hit by objects (2.6%) and other unidentified causes (1.6%). It should be noted that in the smaller subset, injuries due to motorcycle accidents were slightly higher than in the larger subset.

Injury types were classified according to the ICD 9 codes abstracted from the Trauma Registry. The two most common injury categories were Subdural Hematoma (SDH) and Subarachnoid Hemorrhage (SAH). When missing data was accounted for SAH was present in 67% and SDH was present in 62.5% of the subjects. Skull fractures were present in only about 5% of the subjects and cranial surgical procedures were performed on 41.6% of the subjects.

4.1.5 Mortality

Mortality was above 32%, with approximately 13% of deaths occurring in the first week and 19% of deaths occurring after the first week. None of the 19 variants were associated with increased mortality.

4.2 POPULATION DESCRIPTIONS FOR EPTS AND LPTS

4.2.1 PTS Status

As a preliminary analysis, each of the 19 mtDNA variants were compared initially to all PTS (i.e. PTS Y/N). The overall frequency of PTS in both the smaller and larger subsets was approximately 26% after the removal of those with premorbid seizures and deaths without evidence of seizures. There were no associations between the 19 variants and all PTS (Appendix C). Subjects were then categorized into either EPTS or LPTS for the remainder of the study.

After adjustments were made for early mortality in the EPTS group, 8% had seizures during the first week after injury and 92% had no evidence of seizure. In the LPTS group after adjustments were made to remove those who died and/or had evidence of early seizures, 19.9% had LPTS and 80.1% had no evidence of LPTS. In the first stage of the univariate analysis, EPTS and LPTS were examined to assess potential differences in demographic, clinical and injury covariates in the both the small and large subsets of the population. Demographic and injury comparisons for the smaller subset of the population are found in Tables 3 and 4. For the larger subset they are found in Tables 5 and 6.

Table 3: Demographic and Clinical vs. EPTS and LPTS in Smaller Subset

Variable	NO EPTS Count (%) n=112+	EPTS Count (%) n=112+	p-value*	NO LPTS Count (%) n=79#	LPTS Count (%) n=79#	p-value*
Gender						
Male	82 (91.1)	8 (8.9)	p=0.686	50 (78.1)	14 (21.9)	p=1.000
Female	21 (95.5)	1 (4.5)		12 (80.0)	3 (20.0)	
Race						
Caucasian	97 (93.3)	7 (6.7)	p=0.172	58 (77.3)	17 (22.7)	p=0.679
Black	5 (71.4)	2 (28.6)		3 (100.0)	0 (0.0)	
Other/Biracial	1 (100.0)	0 (0.0)		1 (100.0)	0 (0.0)	
AEDs in week 1						
Yes	99 (91.7)	9 (8.3)	p=1.000	60 (77.9)	17 (22.1)	p=1.000
No	4 (100.0)	0 (0.0)		2 (100.0)	0 (0.0)	
Hypothermia						
Yes	17 (100.0)	0 (0.0)	p=0.350	11 (73.3)	4 (26.7)	p=0.728
No	86 (90.5)	9 (9.50)		51 (79.7)	13 (20.3)	
Age						
years	34.36±15.0	34.89±14.5	p=0.919	30.76±12.6	30.65±11.6	p=0.974
LOSH						
Days	24.04±10.6	21.67±14.1	p=0.533	24.54±10.4	26.88±11.2	p=0.421

***** - Chi-squared analysis, Fisher's Exact test or independent samples t-test where appropriate.
+ - Deaths with no evidence of seizure in the first week removed.
# - Those with EPTS and/or death without evidence of seizure removed.

Table 4: Injury Variables vs. EPTS and LPTS in Smaller Subset

Variable	No EPTS Count (%) N=112+	EPTS Count (%) N=112+	p-value*	NO LPTS Count (%) n=79#	LPTS Count (%) n=79#	p-value*
GCS						
3-4	24 (96.0)	1 (4.0)	p=0.470	11 (73.3)	4 (26.7)	p=0.728
5-8	79 (90.8)	8 (9.2)		51 (79.7)	13 (20.3)	
MECHANISM						
Motor Vehicle	63 (96.9)	2 (3.1)	p=0.062	42 (82.4)	9 (17.6)	p=0.738
Motorcycle	18 (78.3)	5 (21.7)		9 (69.2)	4 (30.8)	
Fall/Sport	14 (93.3)	1 (6.7)		5 (71.4)	2 (28.6)	
Hit By/Assault	4 (80.0)	1 (20.0)		3 (75.0)	1 (25.0)	
Other	3 (100.0)	1 (0.0)		3 (100.0)	0 (0.0)	
ICD9 – SDH						
Yes	61 (89.7)	7 (10.3)	p=0.318	33 (75.0)	11(25.0)	p=0.399
No	42 (95.5)	2 (4.5)		29 (82.9)	6 (17.1)	
ICD9 – SAH						
Yes	72 (93.5)	5 (6.5)	p=0.457	40 (81.6)	9 (18.4)	p=0.384
No	31 (88.6)	4 (11.4)		22 (73.3)	8 (26.7)	
ICD9 – DAI						
Yes	43 (93.5)	3 (6.5)	p=0.735	27 (87.1)	4 (12.9)	p=0.134
No	60 (90.9)	6 (9.1)		35 (72.9)	13 (27.1)	
ICD9 – EDH						
Yes	15 (100.0)	0 (0.0)	p=0.360	12 (85.7)	2 (14.3)	p=0.517
No	88 (90.7)	9 (9.3)		50(76.9)	15 (23.1)	
ICD9 –Contusion						
Yes	44 (91.7)	4 (8.3)	p=1.000	26 (78.8)	7 (21.2)	p=0.955
No	59 (92.2)	5 (7.8)		36 (78.3)	10 (21.7)	
ICD9 – IVH						
Yes	33 (91.7)	3 (8.3)	p=1.000	19 (82.6)	4 (17.4)	p=0.765
No	70 (92.1)	6 (7.9)		43 (76.8)	13 (23.3)	
ICD9 – ICH						
Yes	33 (91.7)	3 (8.3)	p=1.000	20 (80.0)	5 (20.0)	p=0.823
No	70 (92.1)	6 (7.9)		42 (77.8)	12 (22.2)	
ICD9 – Other						
Yes	3 (100.0)	0 (0.0)	p=1.000	1 (100.0)	0 (0.0)	p=1.000
No	100 (91.7)	9 (8.3)		61 (78.2)	17 (21.5)	
Skull Fx						
Yes	7 (100.0)	0 (0.0)	p=1.000	5 (83.3)	1 (16.7)	p=1.000
No	87 (93.5)	6 (6.5)		51 (76.1)	16 (23.9)	
Cranial Surgery						
Yes	35 (92.1)	3 (7.9)	p=0.671	15 (53.6)	13 (46.4)	**p=0.000**
No	59 (95.2)	3 (4.8)		41 (91.1)	4 (8.9)	

***** - Chi-squared analysis, Fisher's Exact test and independent samples t-test where appropriate.
+ - Deaths with no evidence of seizure in the first week removed.
# - Those with EPTS and/or death without evidence of seizure removed.
Bold values in the table indicate a significant test statistic (p<0.05).

Table 5: Demographic and Clinical vs. EPTS and LPTS in Larger Subset

Variable	NO EPTS Count (%) n=274+	EPTS Count (%) n=274	p-value*	NO LPTS Count (%) n=201#	LPTS Count (%) n=201	p-value*
Gender						
Male	200 (91.7)	18 (8.3)	p=0.583	125 (80.1)	31 (19.9)	p=0.985
Female	53 (94.6)	3 (5.4)		36 (80.0)	9 (20.0)	
Race						
Caucasian	234 (92.9)	18 (7.1)	p=0.322	146 (78.9)	39 (21.1)	p=0.092
Black	14 (82.4)	3 (17.6)		12 (100.0)	0 (0.0)	
Other/Biracial	3 (100.0)	0 (0.0)		1 (50.0)	1 (50.0)	
AEDs in week 1						
Yes	241 (92.0)	21 (8.0)	p=0.608	151 (79.1)	40 (20.9)	p=0.216
No	12 (100.0)	0 (0.0)		10 (100.0)	0 (0.0)	
Hypothermia						
Yes	67 (98.5)	1 (1.5)	**p=0.027**	42 (77.8)	12 (22.2)	p=0.617
No	186 (90.3)	20 (9.7)		119 (81.0)	28 (19.0)	
Age						
years	32.94±14.0	36.14±15.5	p=0.317	30.22±12.2	31.10±10.9	p=0.677
LOSH						
Days	22.76±10.8	21.43±13.2	p=0.593	22.66±10.4	25.30±12.6	p=0.171

***** - Chi-squared analysis, Fisher's Exact test and independent samples t-test where appropriate.
+ - Deaths with no evidence of seizure in the first week removed.
# - Those with EPTS and/or death without evidence of seizure removed.
Bold values in the table indicate a significant test statistic (p<0.05).

Table 6: Injury Variables vs. EPTS AND LPTS in Larger Subset

Variable	No EPTS Count (%) N=274+	EPTS Count (%) N=274+	p-value*	NO LPTS Count (%) n=201#	LPTS Count (%) n=201#	p-value*
GCS						
3-4	53 (96.4)	2 (3.6)	p=0.265	25 (75.8)	8 (24.2)	p=0.549
5-8	194 (91.1)	19 (8.9)		131 (80.4)	32 (19.6)	
MECHANISM						
Motor Vehicle	167 (94.9)	9 (5.1)	p=0.231	113 (82.5)	24 (17.5)	p=0.256
Motorcycle	37 (86.0)	6 (14.0)		23 (74.2)	8 (25.8)	
Fall/Sport	35 (87.5)	5 (12.5)		17 (85.0)	3 (15.0)	
Hit By/Assault	6 (85.7)	1 (14.3)		4 (66.7)	2 (33.3)	
Other	5 (100.0)	0 (0.0)		3 (75.0)	1 (25.0)	
Missing	3 (100.0)	0 (0.0)		1 (33.3)	2 (66.7)	
ICD9 – SDH						
Yes	130 (89.0)	16 (11.0)	p=0.157	72 (72.7)	27(27.3)	**p=0.004**
No	85 (94.4)	5 (5.6)		66 (90.4)	7 (9.6)	
ICD9 – SAH						
Yes	136 (90.1)	15 (9.9)	p=0.456	81 (78.6)	22 (21.4)	p=0.522
No	79 (92.9)	6 (7.1)		57 (82.6)	12 (17.4)	
ICD9 – DAI						
Yes	76 (93.8)	5 (6.2)	p=0.288	52 (85.2)	9 (14.8)	p=0.238
No	139 (89.7)	16 (10.3)		86 (77.5)	25 (22.5)	
ICD9 – EDH						
Yes	36 (90.0)	4 (10.0)	p=1.000	23 (71.9)	9 (28.1)	p=0.188
No	179 (91.3)	17 (8.7)		115 (82.1)	25 (17.9)	
ICD9 –Contusion						
Yes	95 (90.5)	10 (9.5)	p=0.763	60 (82.2)	13 (17.8)	p=0.580
No	120 (91.6)	11 (8.4)		78 (78.8)	21 (21.2)	
ICD9 – IVH						
Yes	66 (91.7)	6 (8.3)	p=0.840	38 (79.2)	10 (20.8)	p=0.827
No	149 (90.9)	15 (9.1)		100 (80.6)	24 (19.4)	
ICD9 – ICH						
Yes	75 (89.3)	9 (10.7)	p=0.466	46 (80.7)	11 (19.3)	p=0.913
No	140 (92.1)	12 (7.9)		92 (80.0)	23 (20.0)	
ICD9 – Other						
Yes	12 (80.0)	3 (20.0)	p=0.137	7 (87.5)	1 (12.5)	p=0.701
No	203 (91.9)	18 (8.1)		131 (79.9)	33 (20.1)	
Skull Fx						
Yes	14 (100.0)	0 (0.0)	p=0.608	8 (80.0)	2 (20.0)	p=1.000
No	221 (92.9)	17 (7.1)		139 (78.5)	38 (21.5)	
Cranial Surgery						
Yes	96 (91.4)	9 (8.6)	p=0.329	48 (63.2)	28 (36.8)	**p=0.000**
No	139 (94.6)	8 (5.4)		99 (89.2)	12 (10.8)	

***** - Chi-squared analysis, Fisher's Exact test and independent samples t-test where appropriate.
+ - Deaths with no evidence of seizure in the first week removed.
# - Those with EPTS and/or death without evidence of seizure removed.
Bold values in the table indicate a significant test statistic (p<0.05).

4.2.2 Demographic and Clinical Associations with EPTS

Gender was not significantly associated with EPTS, but men were about one and half times (OR=1.59, .451-5.60) likely to develop EPTS than women in both the smaller and larger subsets of the population. Less than 15% of EPTS cases were races other than Caucasian.

Antiepileptic drug (AED) usage was considered in regards to EPTS. All of those with EPTS were also treated with AEDs during at least the first week after injury. Some of the subjects for this study were also enrolled in an earlier trial on hypothermia and TBI outcomes. Only 1 subject who received cooling developed EPTS (p=0.027). Those with EPTS were slightly older than those without (36 vs. 33), but the difference was not significant (p=0.317). There was less than a one day difference (20 vs. 21) in the length of hospital stay between the EPTS and No EPTS groups.

4.2.3 Injury Associations with EPTS

Although GCS was not significantly associated with EPTS in either the smaller or larger subsets approximately 90% of those with EPTS fell into the less severe category compared to 78% of those without EPTS. Mechanism of injury was not significantly associated with EPTS (p=0.231), but there appears to be a trend towards more injuries due to motorcycle and falls in the EPTS group. In the smaller subset, motorcycle injuries were significantly associated (p=0.018) with EPTS when grouped vs. all other injury types. A comparison of GCS scores to motorcycle injuries shows that 24% of those with motorcycle injury are in the lower GCS strata

(3-4) compared to 18% in the higher GCS strata (5-8). This is not a significant association, but may lend credence to the idea that those with EPTS and motorcycle injury have more severe injuries than those with LPTS.

None of the lesion types as evidenced by ICD9 codes were significantly associated with EPTS in either the smaller or larger subsets of the population. The lesion type with the strongest association to EPTS was SDH (p=0.157). None of the EPTS subjects had skull fractures, and having a cranial surgical procedure did not associate (p=0.329) with the development of EPTS.

4.2.4 Demographic and Clinical Associations with LPTS

There were no gender differences between those with LPTS and those without (p=0.985). The slight gender difference between males and females found in the EPTS group were not observed in the LPTS group. At first glance, race appears to trend toward significance, but greater than 95% of those with LPTS were Caucasian in the larger subset and all of those in the smaller subset were Caucasian.

AED usage was considered in regards to LPTS. All of the subjects with LPTS were treated with AEDs during at least the first week post-injury. While more of those with LPTS were also included in the earlier hypothermia trials, there was no association between being cooled and LPTS (p=0.617). Those with LPTS were slightly older than those without (31 vs. 30), but the difference was not significant (p=0.677). While direct univariate comparisons were not made, those with LPTS were almost 5 years younger than those with EPTS. Those with LPTS had an average stay of approximately 26 days compared to 23 days in those without LPTS. However, the difference was not significant (p=0.171). Those with LPTS generally had longer hospital stays than those with EPTS as well.

4.2.5 Injury Associations with LPTS

Having a cranial surgical procedure is strongly associated with the development of LPTS (p=0.000) in both the smaller and larger subsets of the population. Those who had cranial surgery were almost 5 times more likely to develop LPTS (OR=4.813, 2.25-10.28) compared to those who did not have any cranial surgeries. In the larger subset of cases, the injury lesion with the strongest association to LPTS was subdural hematoma (p=0.004). Those with a diagnosis of SDH were 3.5 times more likely to develop LPTS (OR=3.53, 1.44-8.66) than those without the diagnosis. Skull fractures did not associate with the development of LPTS (p=1.000) nor did any other lesion types in either the small or large subsets of the population.

4.3 AIM 1

4.3.1 mtDNA Variants and EPTS

In the univariate analysis no variant was significantly associated with EPTS or LPTS in either the smaller subset that included 13 variants or the larger subset that contained 6 variants (see Table 7). Originally it was planned that any variant with a significant ($p<0.05$) association to either EPTS or LPTS would be entered into a binary logistic regression model with the most common demographic and clinical covariates that have been used in this population in previous

reports. The multivariate model that is typically built includes gender, age and GCS as covariates regardless of their univariate significance. Since no variants associated at $p<0.05$, it was decided to construct the same models using all 19 variants paying particular attention to those variants that had at least a marginal association with EPTS ($p<0.250$) in the univariate analysis to ensure that no potential associations are being overlooked in the data. The number of covariates entered into the models is deliberately kept small due to the overall small number of cases of EPTS and LPTS, particularly in the smaller subset.

In the smaller subset, four variants were associated with EPTS at $p<0.250$. C3494T ($p=0.080$), A8701G ($p=0.084$), A10550G ($p=0.215$) and T10873C ($p=0.154$) all showed some association with EPTS. As seen in Table 7, for most variants there were very few cells that were positive for the variant and EPTS. T146C, A11947G, G13708A, and T13789A had no cases of EPTS and the variant of interest. Additionally, T477C, C3494T, and A14233G each only had one case of that was positive for variant and EPTS. These extremely small numbers present difficulties when trying to analyze any multivariate models or perform any stratified analyses.

4.3.2 mtDNA Variants and LPTS

Two variants associated with LPTS at $p<0.250$, one from the smaller subset (G13708A) and one from the larger subset (T16519C). For G13708A ($p=0.152$) approximately 40% of those with the A allele had LPTS. For T16519C ($p=0.053$), approximately 25% of those with the T allele were in the LPTS group compared to only 12% with the C allele. While not significant, the odds of having the T allele and LPTS were more than 2 times greater than having the C allele and LPTS for T16519C.

As with the EPTS group there were very few cells that were positive for both the variant and LPTS. C3494T, T10873C, and T13789C had no cases of LPTS and the variant. Additionally, A8701G and A14233G each only had one case that was positive for the variant and LPTS. These extremely small numbers present difficulties when trying to analyze any multivariate models or perform any stratified analyses in the LPTS group.

Table 7: mtDNA Variants vs. EPTS and LPTS

mtDNA Variant	No EPTS + Count (%)	EPTS+ Count (%)	p-value* (n)	NO LPTS# Count (%)	LPTS# Count (%)	p-value* (n)
T146C						
T	92 (91.1)	9 (8.9)	p=0.595	56 (78.9)	15 (21.1)	p=1.000
C	11 (100.0)	0 (0.0)	(112)	6 (75.0)	2 (25.0)	(79)
T195C ¢						
T	193 (92.3)	16 (7.7)	p=1.000	123 (80.4)	30 (19.6)	p=0.408
C	49 (92.5)	4 (7.5)	(262)	29 (74.4)	10 (25.6)	(192)
T477C ¢						
T	202 (91.0)	20 (9.0)	p=1.000	130 (81.2)	30 (18.8)	p=0.380
C	9 (90.0)	1 (10.0)	(232)	6 (66.7)	5 (33.3)	(171)
C3494T						
C	103 (92.8)	8 (7.2)	p=0.080	62 (78.5)	17 (100.0)	----------
T	0 (0.0)	1 (100.0)	(112)	0 (0.0)	0 (0.0)	(79)
T4126C¢						
T	193 (91.9)	17 (8.1)	p=0.792	121 (79.6)	31 (20.4)	p=0.804
C	59 (93.7)	4 (6.3)	(273)	39 (81.2)	9 (18.8)	(200)
A4917G¢						
A	227 (92.7)	18 (7.3)	p=0.709	147 (80.8)	35 (19.2)	p=0.545
G	26 (89.7)	3 (10.3)	(274)	14 (73.7)	5 (26.3)	(201)
C7028T						
C	40 (95.2)	2 (4.8)	p=0.480	21 (75.0)	7 (25.0)	p=0.577
T	63 (90.0)	7 (10.0)	(112)	41 (80.4)	10 (19.6)	(79)
A8701G						
A	92 (93.9)	6 (6.1)	p=0.084	55 (77.5)	16 (22.5)	p=0.680
G	11 (78.6)	3 (21.4)	(112)	7 (87.5)	1 (12.5)	(79)
A10398G¢						
A	176 (92.6)	14 (7.4)	p=0.279	110 (79.1)	29 (20.9)	p=0.992
G	50 (87.7)	7 (12.3)	(247)	34 (79.1)	9 (20.9)	(182)
A10550G						
A	94 (93.1)	7 (6.9)	p=0.215	57 (79.2)	15 (20.8)	p=1.000
G	9 (81.8)	2 (18.2)	(112)	5 (71.4)	2 (28.6)	(79)
T10873C						
T	96 (93.2)	7 (6.8)	p=0.154	58 (77.3)	17 (2.7)	p=0.572
C	7 (77.8)	2 (22.2)	(112)	4 (100.0)	0 (0.0)	(79)
A11947G						
A	100 (91.7)	9 (8.3)	p=1.000	59 (77.6)	17 (22.4)	p=0.593
G	3 (100.0)	0 (0.0)	(112)	3 (100.0)	0 (0.0)	(79)
A12308G						
A	77 (92.8)	6 (7.2)	p=0.693	45 (77.6)	13 (22.4)	p=0.773
G	26 (89.7)	3 (10.3)	(112)	17 (81.0)	4 (19.0)	(79)
C12705T						
C	90 (92.8)	7 (7.2)	p=0.605	53 (77.9)	15 (22.1)	p=1.000
T	13 (86.7)	2 (13.3)	(112)	9 (81.8)	2 (18.2)	(79)
G13708A						
G	92 (91.1)	9 (8.9)	p=0.608	57 (81.4)	13 (18.6)	p=0.152
A	9 (100.0)	0 (0.0)`	(112)	4 (57.1)	3 (42.9)	(79)
T13789C						
T	100 (91.7)	9 (8.3)	p=1.000	59 (77.6)	17 (16.6)	p=1.000
C	2 (100.0)	0 (0.0)		2 (100.0)	0 (0.0)	(79)
A14233G						
A	96 (92.3)	8 (7.7)	p=1.000	58 (78.4)	16 (21.6)	p=1.000
G	7 (87.5)	1 (12.5)	(112)	4 (80.0)	1 (20.0)	(79)
C14766T						
C	43 (93.5)	3 (6.5)	p=0.733	24 (77.4)	7 (22.6)	p=0.713
T	59 (90.8)	6 (9.2)	(112)	38 (80.9)	9 (19.1)	(79)
T16519C¢						
T	129 (90.8)	13 (9.2)	p=0.672	77 (75.5)	25 (24.5)	p=0.053
C	74 (92.5)	6 (7.5)	(222)	52 (88.1)	7 (11.9)	(161)

¢ - mtDNA variants with additional samples.
***** - Chi-squared analysis and Fisher's Exact test.
+ - Deaths with no evidence of seizure in the first week removed.
# - Those with EPTS and/or death without evidence of seizure removed.

4.3.3 Multivariate Models: EPTS

Despite the limited cell sizes, each variant with a $p<0.250$ in the univariate analysis was entered into a multivariate model with EPTS as the dependent variable and the variant, age, gender, and GCS entered as covariates. Previous univariate analyses of variants by gender revealed possible associations for T195C, A8701G, T10873C, C12705T, and T13789C. Additionally, T4216C showed a possible association with GCS scores in a similar univariate analysis so particular attention was paid to this variant as well.

Of the variants entered into the multivariate model that controlled for the effects of gender, age and GCS, only A8701G showed an association with EPTS (Table 8). With other clinical covariates included in the model, those with the G allele appear almost 6 times more likely to have EPTS. To determine if this was a valid association, variables were added and removed in a stepwise fashion to determine if they changed the association between the SNP and EPTS. Gender is the one clinical factor that drives the association between A8701G and EPTS. Removal of gender at any step of the model has the most impact on the relationship and caused the association to return to its borderline significance from the univariate analysis. Addition and removal of other covariates has no impact on the association. To further explore the relationship between the SNP and gender, stratified analysis was performed and it was found that no women had the A variant and EPTS and only 1 woman had the G variant and EPTS. The paucity of cases in the female subgroup appears to be what is driving the association seen in the multivariate model.

Table 8: Multivariate Model of A8701G vs. EPTS

Variable	*Exp(B) OR*	*95% CI*	*p-value*
A8701G (Odds of G/A)	5.994	1.076-33.395	**0.041**
Gender (Odds of F)	3.769	.368-39.284	.263
GCS (Odds of Lower)	2.160	.246-18.959	.487
Age	1.012	.966-2.060	.616

Bolded values significant at p<0.05

4.3.4 Multivariate Models: LPTS

For LPTS, each variant from the univariate tests with p<0.250 was entered into a multivariate model with LPTS as the dependent variable. In addition to common covariates of age, gender, and GCS, the presence of SDH and Cranial surgery were also entered as covariates because of their significance in the univariate models. Previous univariate analyses of variants by gender revealed possible gender associations for T195C, A8701G, T10873C, C12705T, and T13789C. Additionally, T4216C showed a possible association with GCS scores in a similar univariate analysis so particular attention was paid to these variants as well. Of the 19 variants entered into the multivariate model that controlled for the effects of Gender, Age, GCS, SDH and Cranial Surgery, none showed any potential associations with LPTS, although T16519C was of borderline significance (p=0.095).

In the case of T16519C the presence of the T allele was approaching significance (p= 0.053) in the univariate model, but when clinical and significant injury covariates were

accounted for in the multivariate model (Table 9) the slight association from the univariate model became less significant. Removal of other covariates without significant associations to LPTS also had no effect on the relationship between T16519C and LPTS.

Table 9: Multivariate model of T16519C vs. LPTS

Variable	*OR*	*95% CI*	*p-value*
T16519C	2.376	.860-6.568	.095
Gender	.798	.275-2.316	.678
Age	.983	.947-1.020	.359
GCS	.780	.209-2.914	.711
Cranial Surgery	3.909	1.599-9.557	**.003**
SDH	2.687	.991-7.281	.052

Bolded values significant at p<0.05

4.4 AIM 2

4.4.1 Injury Factors in LPTS

In the initial univariate analysis of injury factors (mechanism, lesion type, cranial surgery), there were no significant associations between any of the factors and the development of EPTS. However, in the LPTS group, having a subdural hematoma (SDH) was significantly associated with LPTS (p=0.004) as was having a cranial surgical procedure (p=0.000). Those with SDH were almost 4 times more likely to have LPTS than those who didn't (Table 10). Those who had a cranial surgery were almost 5 times more likely to have LPTS than those who didn't (Table 11**).** These results are similar to what was shown in the multivariate model above (Table 9).

Table 10: SDH vs. LPTS

Variable	No LPTS	LPTS	TOTAL	p-value*	OR (CI)
NO SDH count %	 66 90.4	 7 9.6	73	0.004	3.54 (1.44-8.66)
SDH count %	 72 72.7	 27 27.3	99		
TOTAL	138	34	172		

*Chi-square test of independence.

Table 11: Cranial Surgery vs. LPTS

Variable	No LPTS	LPTS	TOTAL	p-value*	OR (CI)
NO SURGERY count %	 99 89.2	 12 10.8	111	0.004	4.81 (2.25-10.28)
SURGERY count %	 48 63.2	 28 36.8	76		
TOTAL	138	34	172		

*Chi-square test of independence.

4.4.2 SDH Stratified Analysis

As there were no direct univariate associations between variants and the outcome of LPTS or with the injury factors of SDH and Cranial surgery (see Appendix D Table 1) a series of stratified analyses were done to highlight the possible effects of specific alleles in combination with injury factors on the outcome of LPTS. The initial stratification consisted of the injury factor compared to the outcome of LPTS, and each of the 19 variants was entered as stratifying variables (see Appendix D Table 2). This generated an odds ratio and significance for each allele with the injury factor of interest on the outcome of LPTS. For SDH, of the 19 variants entered only the 6 with additional samples from the larger subset had enough cases to allow for

further stratification (see Appendix D Tables 3-8). For the 2X4 analysis of SDH by LPTS, determination of the risk allele for additional stratification was done by taking the prevalence of the allele in those with LPTS (cases) compared to the prevalence of the allele in those with no LPTS (controls) (Table 12).

Table 12: Risk Allele selection SDH by Variant by LPTS

mtDNA Variants (n)	Allele - Cases (%) – (count)	Allele - Controls (%) – (count)
T195C (165)	T: 76% - (26/34) **C: 24% - (8/34)**	T: 79% - (104/131) C: 21% - (27/131)
T477C (167)	T: 91% - (29/32) **C: 9% - (3/32)**	T: 95% - (129/135) C: 4% - (6/136)
T4216C (171)	**T: 79% - (27/34)** C: 21% - (7/34)	T: 77% - (106/138) C: 23% - (32/138)
A4917G (172)	**A: 91% - (31/34)** G: 9% - (3/34)	A: 90% - (124/138) G: 10% - (14/138)
A10398G (165)	A: 76% - (26/34) **G: 24% - (8/34)**	A: 77% - (101/131) G: 23% - (30/131)
T16519C (160)	**T: 81% - (25/31)** C: 19% - (6/31)	T: 60% - (77/129) C: 40% - (52/129)

Risk alleles are bolded in the table.

4.4.3 Summary: SDH by Variant by LPTS

The 2X4 tables presented in this section are meant for descriptive purposes and to explore whether any of the mtDNA variants and injury variables may affect the outcome of LPTS. Many of the models are influenced by very small cell sizes, which are easily visualized. Trends do seem to appear and possible allele and exposure effects could be borne out with a larger sample of cases and controls. In general if the odds ratios for the first row (risk allele + exposure) and the third row (non risk allele + exposure) were both increased, compared to the reference group, then it can be assumed that the exposure variable is causing the increased risk. T16519C showed the most likely trend towards a combined effect of the risk allele and SDH. T16519C was also the variant that had the strongest univariate association with LPTS ($p=0.053$). Those with the T allele and SDH (row 1) have a 6 times greater odds of developing LPTS than those than in the reference group, while those with the C allele and SDH (row 3) only have a 2 times greater odds of LPTS compared to the reference group (Table 18). In contrast, in A10398G, the combined risk of the G allele and SDH (row 1) is similar to those with the A allele and SDH (row 3) compared to the reference group, therefore, the exposure of SDH is most likely driving the association (Table 17).

Table 13: T195C

Strata	LPTS	NO LPTS	Odds Ratio	p-value*
C AND SDH	7	10	5.36 (1.25-23.75)	0.012
C AND NO SDH	1	17	0.45 (.01-4.34)	0.668
T AND SDH	20	58	2.64 (.90-8.07)	0.072
T AND NO SDH	6	46	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 14: T477C

Strata	LPTS	NO LPTS	Odds Ratio (CI)	p-value*
C AND SDH	2	5	4.2 (.45-34.71)	0.156
C AND NO SDH	1	1	10.5 (.247-458.51)	0.189
T AND SDH	23	66	3.66 (1.30-10.80)	0.006
T AND NO SDH	6	63	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 15: T4216C

Strata	LPTS	NO LPTS	Odds Ratio (CI)	p-value*
T AND SDH	24	54	1.67 (.45-6.68)	0.574
T AND NO SDH	3	52	0.216 (.03-1.33)	0.067
C AND SDH	3	17	0.661 (.095-4.38)	0.695
C AND NO SDH	4	15	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 16: A4917G

Strata	LPTS	NO LPTS	Odds Ratio (CI)	p-value*
A AND SDH	26	64	1.21 (.201-9.40)	1.00
A AND NO SDH	5	60	0.250 (.033-1.33)	0.167
G AND SDH	1	8	0.375 (.010-7.73)	0.577
G AND NO SDH	2	6	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 17: A10398G

Strata	LPTS	NO LPTS	Odds Ratio (CI)	p-value*
G AND SDH	6	14	4.11 (.922-18.89)	0.060
G AND NO SDH	2	16	1.20 (.144-8.15)	1.000
A AND SDH	21	53	3.80 (1.23-12.57)	0.013
A AND NO SDH	5	48	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 18: T16519C

Strata	LPTS	NO LPTS	Odds Ratio	p-value
T AND SDH	20	38	6.31 (1.24-43.07)	0.014
T AND NO SDH	5	39	1.53 (.234-12.57)	1.000
C AND SDH	4	28	1.71 (.237-14.96)	0.681
C AND NO SDH	2	24	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

4.4.4 Cranial Surgery Stratified Analysis

A similar approach was taken to explore potential modifying effects of the variants with the presence of cranial surgery on the outcome of LPTS (see appendix D Table 9). For Cranial Surgery, of the 19 variants entered, only 5 of the variants with additional samples from the larger subset had enough cases to allow for further stratification (see Appendix D Tables 10-14). For the analysis of Cranial surgery by LPTS, determination of the risk allele for additional stratification was done by taking the prevalence of the allele in those with LPTS (cases) was compared to the prevalence of the allele in those with no LPTS (controls). The results are presented in Table 19.

Table 19: Risk Allele Selection Cranial Surgery by Variant by LPTS

mtDNA Variants (n)	Allele - Cases (%) – (count)	Allele - Controls (%) – (count)
T195C (179)	T: 76% - (30/40) **C: 25% - (10/40)**	T: 83% - (115/139) C: 17% - (24/139)
T4216C (187)	**T: 78% - (31/40)** C: 23% - (9/40)	T: 76% - (111/147) C: 25% - (36/147)
A4917G (187)	A: 88% - (35/40) **G: 13% - (5/40)**	A: 90% - (133/147) G: 10% - (14/147)
A10398G (170)	A: 76% - (29/38) **G: 24% - (9/38)**	A: 80% - (106/132) G: 20% - (26/132)
T16519C (150)	**T: 78% - (25/32)** C: 22% - (7/32)	T: 61% - (72/118) C: 39% - (48/118)

Risk alleles are bolded in table.

4.4.5 Summary: Cranial Surgery by Variant by LPTS

As anticipated none of the 2X4 models were significant considering the main effects models were not significant for variant by Cranial Surgery or variant by PTS status. Many of the models are influenced by very small cell sizes in either the cases or controls. While it appears at first glance that the effect of the allele plus the presence of cranial surgery has an effect on the outcome of LPTS, a closer inspection shows that for all the variants selected, the presence of cranial surgery itself is likely to be what is driving the apparent associations. The odds ratio of the risk allele and cranial surgery (row 1) compared to the reference group is highly significant for all the models. The odds of the other allele and cranial surgery (row 3) were also highly significant in all but T16519C (Tables 20-24).

T16519C was the only allele from the initial analysis that was approaching significance with LPTS. The combination of the T allele and brain surgery (row 1) is greater than the reference group. While the combination of the C allele and cranial surgery also increased the odds of developing LPTS, the effect is not significant compared to the reference group.

Table 20: T195C

Strata	LPTS	NO LPTS	Odds Ratio	p-value*
C AND SURGERY	8	7	9.02 (2.33-36.23)	0.000
C AND NO SURGERY	2	17	0.929 (.128-5.209)	1.000
T AND SURGERY	20	36	4.39 (1.73-11.30)	0.000
T AND NO SURGERY	10	79	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 21: T4216C

Strata	LPTS	NO LPTS	Odds Ratio	p-value*
T AND SURGERY	21	36	7.00 (1.38-47.69)	0.007
T AND NO SURGERY	10	75	1.60 (.295-11.42)	0.729
C AND SURGERY	7	12	7.00 (1.06-58.30)	0.024
C AND NO SURGERY	2	24	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 22: A4917G

Strata	LPTS	NO LPTS	Odds Ratio	p-value*
G AND SURGERY	4	2	15.82 (2.11-143.88)	0.003
G AND NO SURGERY	1	12	0.659 (.029-5.81)	1.000
A AND SURGERY	24	46	4.13 (1.74-9.92)	0.000
A AND NO SURGERY	11	87	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 23:A10398G

Strata	LPTS	NO LPTS	Odds Ratio	p-value*
G AND SURGERY	5	11	4.21 (.975-18.03)	0.048
G AND NO SURGERY	**1**	12	2.47 (.539-10.81)	1.000
A AND SURGERY	24	46	6.07 (2.25-16.83)	0.000
A AND NO SURGERY	11	87	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 24: T16519C

Strata	LPTS	NO LPTS	Odds Ratio	p-value*
T AND SURGERY	18	22	8.73 (2.06-42.63)	0.000
T AND NO SURGERY	7	50	1.43 (.314-7.94)	0.737
C AND SURGERY	4	14	3.05 (.482-20.45)	0.211
C AND NO SURGERY	3	32	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

4.5 AIM 3

4.5.1 GOS at 6 months by PTS

When EPTS was evaluated as potential risk factor for GOS, there were no significant associations between having EPTS and worse outcomes (p=1.000). Since there were no main effect between EPTS and GOS it was decided that to add the mitochondrial variants would not be productive in further exploring whether the variants were effect modifiers (see Table 25).

When LPTS was evaluated as a potential risk factors for GOS, there was a significant association between LPTS and worse outcomes (p=0.002). Those with LPTS were almost 3 times more likely to fall into the lower GOS categories than those without LPTS (Table 26). Therefore, for the stratified models, LPTS will be the exposure variable and the outcome of interest will be the GOS 2-3 category and the variants were included to look for trends towards effect modification.

Table 25: EPTS vs. GOS at 6 months

Variable	GOS 2-3	GOS 4-5	TOTAL	p-value*	OR (CI)
EPTS count exp	 8 9.2	 6 7.8	 14	 0.175	 1.14 (.35-3.89)
NO EPTS count exp	 107 105.8	 92 90.2	 199		
TOTALS	115	98	213		

* Chi-square test of independence.

Table 26: LPTS vs. GOS at 6 months

Variable	GOS 2-3	GOS 4-5	TOTAL	p-value*	OR (CI)
LPTS					
count	27	10	37	0.004	3.33 (1.41-8.01)
exp	18.7	18.3			
NO LPTS					
count	64	79	143		
exp	72.3	70.7			
TOTALS	91	89	180		

* - Chi-square test of independence

4.5.2 Stratified Analysis of LPTS by GOS 6 months by Variant

Initially all 19 variants were entered into a stratified analysis of LPTS by GOS with the outcome of interest being the lower GOS group (GOS 2-3). Those with and without LPTS were stratified by mtDNA variants to look for possible associations. For the variants from the smaller subset (n=79), the cell sizes became too small to explore any further possible effect modification of the variant on outcome. For this reason, the analysis in this section will be restricted to the variants from the larger subset. During the initial stratification (see Appendix E Tables 15-19), the variant T477C had multiple cells with no subjects so further stratification was unable to be performed. The five remaining variants are shown below (Table 27).

Table 27: Stratified analysis of LPTS by GOS 6 Months by Variant

mtDNA Variants (n)+	**OR (95% CI)**	**p- value ***
T195C (172)	T – 4.48 (1.21-16.68) C – 1.31 (0.16-10.56)	0.025
T4216C (179)	T – 3.50 (0.92-13.32) C – 2.38 (0.38-14.70)	0.035
A4917G (180)	A – 2.87 (0.87-9.41) G – 5.40 (0.44-66.67)	0.033
A10398G (163)	A – 2.89 (0.86-9.75) G – 4.00 (0.40-39.83)	0.030
T16519C (142)	T – 9.75 (1.19-79.81) C – 0.86 (0.073-10.18)	0.018

+ - Differing n's due to missing cases in the GOS or Variant groups.
* Chi-Square test of independence or Fisher's exact test where appropriate.

4.5.3 Risk Allele Selection

For further 2X4 analysis of LPTS to GOS, the determination of the risk allele for additional stratification was done by taking the prevalence of the allele in those with LPTS (cases) compared to the prevalence of the allele in those with no LPTS (controls). The results are presented in Table 28.

Table 28: Risk Allele Selection for LPTS by GOS at 6 Months

mtDNA Variants (n)	Allele - Cases (%) – (count)	Allele - Controls (%) – (count)
T195C (172)	T: 79% - (15/19**)** **C: 21% - (4/19)**	T: 80% - (123/153) C: 20% - (30/153)
T4216C (171)	T: 68% - (13/19) **C: 32% - (20/91)**	T: 78% - (125/160) C: 22% - (35/160)
A4917G (180)	A: 79% - (15/19) **G: 21% - (4/19)**	A: 91% - (147/161) G: 9% - (14/161)
A10398G (165)	A: 74% - (14/19) **G: 26% - (5/19)**	A: 78% - (112/144) G: 22% - (32/144)
T16519C (142)	**T: 79% - (11/14)** C: 21% - (3/14)	T: 62% - (79/128) C: 38% - (49/128)

Risk alleles are bolded in table.

4.5.4 Summary: LPTS by GOS 6 months by Variant

The combination of the T allele and LPTS for T195C appears to increase the risk of a worse outcome (GOS 2-3) compared to those who have the C allele and LPTS (Table 29). The combination of the T allele and LPTS in 16519C appears to increase the risk of a poorer outcome at 6 months as well (Table 33). For A10398G, it appears the LPTS is driving the increases in the odds ratios in rows 1 and 3 (Table 32). LPTS appears to be strongly affecting the odds ratios in T4216C (Table 30) and A4917G (Table 31) as well. While this analysis descriptive in nature, it may bear out with increased genotype samples, that certain mtDNA variants interact with LPTS to increase the risk of poorer functional outcomes in severe TBI patients.

Table 29: T195C

Strata	GOS 2-3	GOS 4-5	Odds Ratio	p-value*
C AND LPTS	2	2	1.12 (0.11-11.59)	1.000
C AND NO LPTS	13	17	0.85 (0.36-2.06)	0.865
T AND LPTS	12	3	4.48 (1.10-21.15)	0.033
T AND NO LPTS	58	65	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 30: T4216C

Strata	GOS 2-3	GOS 4-5	Odds Ratio	p-value*
C AND LPTS	4	2	2.10 (0.31-17.21)	0.662
C AND NO LPTS	16	19	0.88 (0.39-2.00)	0.896
T AND LPTS	10	3	3.50 (0.83-16.92)	0.101
T AND NO LPTS	61	64	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 31: A4917G

Strata	GOS 2-3	GOS 4-5	Odds Ratio	p-value*
G AND LPTS	3	1	3.13 (0.28-78.53)	0.602
G AND NO LPTS	5	9	0.58 (0.16-2.01)	0.503
A AND LPTS	11	4	2.86 (0.79-11.24)	0.127
A AND NO LPTS	72	75	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 32: A10398G

Strata	GOS 2-3	GOS 4-5	Odds Ratio	p-value*
G AND LPTS	10	4	2.50 (0.55-12.06)	0.305
G AND NO LPTS	52	60	0.87 (0.37-2.04)	0.876
A AND LPTS	4	1	4.00 (0.34-103.67)	0.442
A AND NO LPTS	16	16	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

Table 33: T16519C

Strata	GOS 2-3	GOS 4-5	Odds Ratio	p-value
T AND LPTS	10	1	17.22 (1.95-396.32)	0.004
T AND NO LPTS	40	39	1.77 (0.89-2.23)	0.563
C AND LPTS	1	2	.861 (0.03-13.57)	0.641
C AND NO LPTS	18	31	1	

* - Chi-square test of independence or Fisher's exact test where appropriate.

5.0 DISCUSSION

5.1 GENERAL FINDINGS

PTS is a heterogeneous condition and it has been proposed to depend on variation in the injury type and severity as well as on the different genetic backgrounds, ages and genders of the patients [36, 37]. More recently, genetic studies have revealed that several nuclear genetic variants (APOE, A1AR, and GAD) may act as risk factors in the development of PTS [16-20].

Over the past few decades, numerous variations in the mitochondrial genome have been identified that lead to disrupted energy production, oxidative stress, and homeostatic imbalance. Many mitochondrial disorders exhibit seizure phenotypes as part of their pathogenesis and mitochondrial variants have been implicated in contributing to both inherited and acquired forms of epilepsy. This finding is not unexpected, given that mitochondrial diseases often manifest in those tissues with the highest energy demands (i.e. brain or muscle). However, the role that altered mtDNA and the associated mitochondrial dysfunction play in acquired seizure disorders (i.e. PTS) remains to be explored [52].

The goals of this study were to explore whether mtDNA variants act as potential risk factors of the clinical phenotypes of PTS. To examine if any of the variants may act as effect modifiers between injury factors and PTS and if mtDNA variants may modify the relationship between PTS and functional outcomes (GOS) at 6 months post-injury.

The overall frequency of EPTS in this population of severe TBI patients was found to be 8% and LPTS was found to be 19.9% after adjustments were made for mortality. These finding are in agreement with previous work done in this population [19, 20] and with other studies done on patients admitted for head trauma where EPTS frequencies range from 4-16% and LPTS frequencies range from 5-25% [33, 43, 45, 48, 84]. In many population based studies, EPTS is often a risk factor for the prediction of LPTS; however, as the primary measure of seizure in this study was time to first seizure, and not number of subsequent seizures, that temporal relationship was unable to be tested. Some of the patients in this sample were enrolled as part of an earlier hypothermia trial as well and only one patient of the 68 (1.5%) who received cooling in the larger subset developed EPTS. This is in agreement with earlier works done on PTS in this population [19, 20] and with recent work done on seizure prevention in an animal model [125]. The vast majority of the patients (95%) in this sample were treated with anti-epileptic drugs during their acute stay. Antiepileptic drugs are often given prophylactically to PTS patients to prevent further secondary brain injury and possibly aid in improving their functional outcomes [5]. Chang and Lowenstein performed a meta-analysis of the evidence regarding antiepileptic therapy prophylaxis in patients with severe TBI by examining and grading prospective studies that compared PTS rates in patients receiving these medications compared to controls with severe TBI who did not receive the medications. Patients receiving phenytoin had a significantly lower risk of developing EPTS (RR=0.37, 0.18-0.74) compared to controls that either received placebo or no antiepileptic therapy. AED's did not seem to have the same effect on preventing LPTS in severe TBI patients compared to controls. (RR=1.05, 0.82-1.35)[81]. Even though it appears that anti-epileptics decrease the rate of early seizures it doesn't appear that there is evidence that the prevention of early seizures affects mortality, morbidity, or the development of

LPTS[12]. Further work needs to be done to understand the underlying risk factors and mechanisms of PTS in order to develop therapies that could identify targets for slowing, stopping and more effectively treating seizures[11].

In regards to LPTS, the two factors with the strongest associations were the presence of a subdural hematoma (p=0.004) and cranial surgery (p=0.000). Cranial surgeries took place during the acute hospital stay and are most often done within the first few days after injury (Dr. Amy Wagner personal communication). These two injury related factors will be discussed in more detail below in regards to the mtDNA variants.

5.2 MTDNA VARIANTS AND PTS

Overall, no significant univariate associations were found between the 19 mtDNA variants and the outcomes of EPTS or LPTS. Variant T477C highlights the issue of exploratory analysis. In the initial sample of 136 subjects it had a strong association with LPTS in both univariate (p=0.038) and multivariate models adjusted for age, GCS, and gender (p=0.044), but with the addition of more individuals, the association disappeared.

In regards to EPTS only one variant showed a potential association and had enough cases to evaluate with a limited multivariate model. The A8701G variant showed the strongest association with EPTS in the univariate analysis (p=0.084) so it was entered a multivariate model along with age, gender and GCS status. This model showed a significant effect compared to the univariate analysis (p=0.041). It appeared that those who carried the G allele at 8701 were almost 6 times more likely to have EPTS than those without the G allele. In further analysis using a 2X4 stratified table it was found that of the 9 cases of EPTS 8 were male and 1 was female, which

may seem abnormally high (88%), but the ratio of men to women in the No EPTS group was above 80% as well. Additionally none of the women carried the A allele, which may explain stronger level of significance in the logistic model. While unable to draw definite conclusions from this small sample, it should be noted that the A to G transition in 8701 results in an amino acid substitution in the ATP6 gene that has been identified as altering mitochondrial matrix pH and intracellular calcium functions [126]. Also, in a recent case control study done in China, A8701G was found to associate in both univariate and multivariate models with a greater risk of sporadic hypertension [127]. Since this variant is known to cause an amino acid change and has been implicated in mitochondrial energy dynamics, it may warrant further consideration as a biologically plausible candidate in future studies.

Of all the variants examined, T16519C had the strongest association with the outcome of LPTS (p=0.053) in the initial analysis and after the inclusion of additional genotyped individuals. The T allele appears to be the risk allele as over 70% of those with LPTS carried this allele compared to the C allele. The odds of having the T allele and LPTS were about two times greater (2.07, 0.95-4.48) than having the C allele. When a multivariate model was created that adjusted for the most relevant clinical covariates and injury associations and the association between the T allele and LPTS was weakened somewhat (OR=2.38, 0.86-6.57, p=0.095). While adjustments for other covariates lowered the association of T16519C to LPTS, some of this may be due the relatively small sample size. In a larger sample T16519C may provide some information about the overall risk of developing LPTS.

T16519C has been reported as one of the most frequent variants across haplogroups worldwide [128] and is located in the D-loop region of the mitochondrial genome, The D-loop doesn't code for any of the mitochondrial complex proteins or t-RNA's needed for their

assembly, although some regions of the D-loop are thought to control and regulate transcription and replication of the mitochondrial genome. However, no reports in the literature were found that this variant is directly associated with that process. Higher frequencies of D-loop mutations (including T16519C) have been reported in tumor cells and it has been speculated that this might reflect increased oxidative damage in diseased cells compared to healthy cells [129].

A review of the literature relevant to T16519C found associations of both the T allele and C allele as possible markers for clinical conditions. T6519C has been associated with a higher risk of being on chronic dialysis [130], migraine headaches and cyclic vomiting syndrome [131], increased oxygen consumption during exercise [132] and nominal associations have been found linking bipolar and schizophrenia to mutations in T6519C and T195C [133]. There seems to be a building trend toward associations of this variant with clinical conditions and diseases involving high energy consumption tissues**.** While not a direct causative link to LPTS, further analysis of the role of T16519C and other D-loop variants may prove useful information regarding the long-term outcomes after TBI.

The second aim of this study was to explore whether mtDNA variants acted as effect modifiers on the relationship between injury factors and the outcome of PTS. While no other studies exist of how mtDNA variants may act as effect modifiers on the phenotype of TBI, the concept of a variant acting to modify a phenotype is not unusual in the literature. For example, Warfarin is prescribed to prevent blood clotting and prevent strokes, but patients vary widely in how much initial dose they need. Warfarin dosing is correlated with relatively common SNPs in the VKORC1 and CYP2C9 nuclear genes that are thought to account for 30-40% of the variability seen in a patient's response to the drug[134, 135].

However, as there were no direct associations between the mtDNA variants and the outcome of LPTS; the original intent of testing whether the variants were moderators of the relationship between injury factors and the outcome of LPTS had to be reconsidered given the constraints of the data. The intent was to describe whether any trends exist that might be validated in larger, prospective studies. The injury factors that associate with PTS will be discussed first and then the process undertaken to try and describe how mtDNA variants might impact the relationship between those factors and PTS will be described.

Injury severity is a consistently documented risk factor for both EPTS and LPTS with those falling in the GCS 3-8 category having the highest risk of seizures [9, 32, 38]. Since all of the subjects in this study were severe TBI patients, it is not unexpected that GCS did not associate with PTS. Multiple lesion types have been associated with both EPTS and LPTS in previous works, including intracerebral hematoma (ICH), subdural hematoma (SDH) and cortical contusions [32, 43]. Cranial surgery and the presence of depressed skull fractures (especially in penetrating head injuries) have also been consistently associated with the development of EPTS and LPTS [32, 33]. In this analysis, none of the commonly reported injury risk factors were significantly associated with the development of EPTS, although, there does seem to be a trend towards more injuries from motorcycle accidents and falls in this group compared to the LPTS group. In the LPTS group the two factors with the strongest univariate associations were SDH ($p=0.004$) and cranial surgery ($p=0.000$).

For both SDH and cranial surgery, the 6 variants with enough cases to evaluate were entered into a 2X4 stratified analysis (see Tables 13-18 and 20-24) to explore for possible modifying effects of the variants on the outcome of LPTS. The only variant to show a strong trend towards increasing the outcome of LPTS in regards to both SDH and cranial surgery was

the T allele of T16519C, which is not unexpected since it had the highest univariate association with LPTS as well. These findings are also in line with the results of the multivariate model in which both SDH and cranial surgery were adjusted for and the risk of LPTS was still elevated for the T allele of 16519. The trends shown here suggest that T16519C may play some role in conjunction with injury or clinical factors in modifying the outcome of LPTS.

In the final part of this analysis, possible trends linking mtDNA variants and the phenotypes of PTS to functional outcomes were explored. In the univariate analysis of LPTS to GOS, it was found that those with LPTS had a greater likelihood (OR=3.33, 1.41-8.01) of having a poorer outcome at 6 months post-injury. This is in agreement with much of the PTS literature. For example, LPTS has been associated with poorer GOS scores and higher incidence of behavioral abnormality in patients with severe TBI in India [46], and has also been associated with a higher incidence of depression [47]. A 5 year prospective study of rehabilitation and reemployment in Finland showed that LPTS was associated with poorer functional and social outcomes and was a considerable factor in hospital readmission [48]. Overall, it appears that PTS can result in greater psychiatric problems as well as reduced general health and poorer functional and social outcomes [12].

The 5 variants with enough cases to evaluate were entered into a 2X4 stratified analysis (see Tables 29-33) to explore for possible modifying trends of the variants on the outcome of LPTS. Overall, it appears that T16519C and T195C have the strongest trends towards lower functional outcomes in those with LPTS.

While unclear what the exact mechanism may be for the role of T16519C, it does lie very close to the beginning of the control region of the mtDNA (bp16160-570) in the D-loop region, which helps to control mitochondrial transcription and replication and multiple mutations in

control regions variants (T477C, T414C, T195C, T146C) have been found in the brains of patients with Alzheimer's disease compared to controls [136]. Taken with the recent findings of increased mutations in both T16519C and T195C in those with other psychiatric diseases [133], it may be plausible to hypothesize that like nuclear DNA, certain blocks of mtDNA are consistently inherited and evolutionarily conserved. While T16519C may not itself alter mitochondrial function, it may be consistently associated with other regions within the D-loop that are involved in the replication of the genome itself or are involved with the production of transcription products necessary to build the respiratory complex proteins.

5.3 WEAKNESSES

Due to the exploratory nature of this study, predetermined power and sample size calculations were not done to maximize the chance of finding an association between the mtDNA variants and PTS and overall this study was under powered. All possible mitochondrial polymorphisms were selected in the original sample of 136 patients via the MitoChip V2 assay; however, there were several with very little variation in regard to outcomes of EPTS and LPTS which made any further analysis impossible. In order to maximize sample size, additional analyses were conducted of the variants vs. all PTS, however, the slight associations shown here for EPTS and LPTS were not apparent. Additionally, McRae et al. have done power analysis of whole mitochondrial genome association studies and they have estimated that approximately 900 cases and controls would be needed to detect an allele with a relative risk of 3 at a power of 80%. They further estimated that number would rise to 3000 to detect a relative risk of 2.0 and 10,000

to detect a relative risk of 1.5. They do note, however, that sample sizes of 1000-10,000 are not uncommon in genome wide association studies of nuclear SNPs, and that adding in mitochondrial genotyping with tools such as the MitoChip V2 assay would only trivially increase the overall cost of the study [137].

Also, due to the retrospective nature of this study, PTS information was abstracted from medical charts rather than from direct monitoring or patient interviews and could have led to an underestimation of the true seizure risk. Additionally, time to first seizure was the primary measure of PTS so there was no ability to assess recurrent seizure risks [20]. In many studies of mitochondrial polymorphisms, race is typically removed as certain haplogroups are known to have much higher proportions of certain mutations due to their ancestral lineage. In the initial stages of this study analyses of all injury, clinical and mtDNA variants to PTS categories were done limited only to Caucasians and with all races left in. There were no differences found between any of the potential associations presented here between those analyses. In total there were 15 subjects (13 No PTS, 2 PTS) who did not self-identify as Caucasian. The ultimate decision to leave these subjects in was based upon the limited sample size and the results of my initial analyses.

5.4 STRENGTHS

While no significant independent associations were found between the 19 mitochondrial variants and the outcome of PTS, some of the findings from this study may help guide future research in this area. This study was able to provide some limited evidence that mitochondrial polymorphisms may in fact play a role in unraveling the complex phenotype that is PTS.

5.5 CONCLUSION

From the outset of this dissertation project, my interest in PTS has been in the heterogeneous nature of the condition. While we know that those with the most severe injuries are most likely to develop PTS, and that certain injuries (particularly penetrating head injuries) are more likely to cause seizures, we still lack reliable means of determining who will go on to develop PTS or intractable lifelong epilepsy (PTE). I've also come to think (although I'm certainly not the first one to do so) that EPTS and LPTS may represent different biological pathways with their own unique environmental and genetic markers. Over the course of this research I have poured over the literature regarding PTS and PTE and one theme that continues to emerge is a lack of reliable biomarkers that could help provide clues as to who might develop PTS/PTE. While at first I had hoped to find the "smoking gun" in the mitochondrial genome in the form of a functional polymorphism, what I've learned is that there are possible clues hidden in areas that are most often overlooked (the D-loop). Perhaps the answer to a reliable biomarker lies buried in that short sequence of base pairs. A study that looks at the mtDNA profile of those with TBI compared to healthy controls may reveal fundamental differences between their mitochondrial genomes.

APPENDIX A: Mitochondrial Diseases with Seizure Phenotypes: tRNA/rRNA Mutations

Table A 1

Condition	Population	Gene	Mutation Location	References
MERRF/ KSS	42yo Caucasian male	tRNA Leucine	G3255A	Nishigaki, 2003, [138]
MELAS	Family study, n=4	tRNA Leucine	A3243G	Enter, 1991, [139]
MELAS	Case Report	tRNA Leucine	G3244A	Kirino, 2005, [140]
MELAS	Japanese MELAS patients, n=94	tRNA Leucine	A3252G	Goto, 1995, [141]
MELAS	45yo male Caucasian	tRNA Leucine	C3256T	Morares, 1993, [142]
MELAS	166 MELAS patients screened, n=5 with this mutation	tRNA Leucine	T3258C	Sternberg, 2001, [143]
MELAS	N=3 of 41 MELAS patients	tRNA Leucine	T3271C	Goto, 1991, [144]
MELAS	N=1 of 46 MELAS patients	tRNA Leucine	T3291C	Goto, 1994, [145]
MELAS/ MERRF	N=1, patient also had 3243 mutation	tRNA Leucine	A12308G	Pulkes, 2000, [146]
MERRF	Family study (n=3)	tRNA Lysine	A8344G	Shoffner, 1990, [24] Noer, 1991, [147]
MERRF	n=5	tRNA Lysine	T8356C	Silvestri, 1992, [148]
MERRF	Two Japanese families	tRNA Lysine	G8363A	Ozawa, 1997,[149]
MERRF	7yo Austrian boy	tRNA Lysine	G8361A	Rossmanith, 2003, [150]
CPEO/ MYO	39yo female	tRNA Lysine	G8342A	Tiranti, 1998, [151]
MINGIE	7yo male developed seizures	tRNA Lysine	G8313A	Verma, 1997, [152]
PEM	33yo female	tRNA Glycine	T10010C	Bidooki, 1997, [153]
GER/SIDS/ ENC	N=8, siblings	tRNA Glycine	A10044G	Santorelli,1996, [154]
ENC	7mo, female, generalized seizures	tRNA Isoleucine	C4320T	Santorelli, 1995, [155]
Familial ENC	N=3, mother and 2 sons	tRNA Isoleucine	G4284A	Corona, 2002, [156]
MERRF	42yo Italian female	tRNA Phenylalanine	G611A	Mancuso, 2004, [157]
MELAS	1 of 5 MELAS patients	tRNA Phenylalanine	G583A	Hanna, 1998, [158]
ENC/Ataxia	n=1	tRNA Valine	G1606A	Tiranti, 1998, [151]
MELAS	1 of 22 MELAS patients	tRNA Valine	G1642A	De Coo, 1998, [159]
ENC/MYO	11yo female	tRNA Asparagine	A7543G	Shtilbans, 1999, [160]
ENC	5yo female, family hx of seizures.	tRNA Cysteine	T5814C	Manfredi, 1996, [161]
MELAS	n=1	tRNA Glutamine	G4332A	Bataillard, 2000, [162]
MERRF/ MELAS	26yo Italian male	tRNA Histidine	G12147A	Melone, 2004, [163]
MERRF/ MELAS	N=3, Japanese Family members	tRNA Serine	T7512C	Nakamura 1995, [164] Jaksch, 1998, [165]
ENC	16yo male	tRNA Threonine	G15915A	Seki, 1997, [166]
MELAS	35yo female, also had A3243G	16S – RNA	C3093G	Hsieh, 2004, [167]
RETT/ENC	N=15 Chinese children with RETT	16S – RNA	C2835T	Tang, 1997, [168]

CPEO, Chronic Progressive External Ophthalmoplegia; ENC, Encephalopathy; GER/SIDS, GastroEsophageal Reflux/Sudden Infant Death Sydrome; KSS, Kearns-Sayre Syndrome; MELAS, Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like episodes; MERRF, Myoclonus Epilepsy with Ragged Red Fibers; MINGIE, Myo-Neuro GastroIntestinal Encephalopathy; MYO, Myoclonus; PEM, Progressive Myoclonic Epilepsy; RETT Syndrome

APPENDIX B: Mitochondrial Diseases with Seizure Phenotypes: Coding Region Mutations

Table B 1

Condition	**Population**	**Complex / Gene**	**Mutation Location**	**References**
MELAS/ BSN	22yo Spanish female	Complex I / ND1	T3308C	Campos, 1997, [169]
MELAS/ LHON	43yo female	Complex I / ND1	G3376A	Blakely, 2005, [170]
MELAS	Pt. 1 – 7yo Caucasian male Pt. 2 – 14yo Caucasian female Pt. 3 - 14yo Chinese male	Complex I / ND1 Complex I / ND1 Complex I / ND1	G3697A G3946A T3949C	Kirby, 2004, [171]
LS	42yo male	Complex I / ND3	T10191C	Taylor, 2001, [172]
LS	67yo male	Complex I / ND4	C11777A	Deschauer, 2003, [173]
MELAS	19yo Caucasian female	Complex I / ND4	A11084G	Lertrit, 1992, [174]
MELAS/ LHON	Pt. 1 – 8.5yo male Pt. 2 – 9yo male	Complex I / ND5 Complex I / ND5	A12770G A13045C	Liolitsa, 2003, [175]
LS/MELAS	16yo Italian male	Complex I / ND5	A13084T	Crimi, 2003, [176]
MELAS	43yo male	Complex I / ND5	G13513A	Santorelli, 1997, [177]
MELAS/ ENC	1 male and 1 female, Italian	Complex I / ND5	A13514G	Corona, 2000, [178]
LS	N=3, 2 brothers and 1 unrelated female	Complex I / ND6	G14459A	Kirby, 2000, [179]
MELAS	7yo female, Danish	Complex I / ND6	G14453A	Ravn, 2000, [180]
MULTI	19yo female	Complex III / cyt b	A15579G	Wibrand, 2001, (157)
EXIT/ENC	34yo female	Complex III / cyt b	G15242A	Keightley, 2000, [181]
HCM	N=5 with mutation, 1 female with seizures	Complex III / cyt b	G15243A	Valnot, 1999, [182]
ENC / EPI	17yo female, German	Complex IV / COX I	C6489C	Varlamov, 2002, [183]
ENC / MULTI	21yo female	Complex IV / COX I	G6930A	Bruno, 1999, [184]
MELAS/ ENC	11yo male	Complex IV / COX III	G9952A	Manfredi, 1995, [185]
LS	5yo	Complex V / A6	T8993C	Santorelli, 1994, [186]
LS/NARP	N=4, Family members all shared same mutation	Complex V / A6	T8993G	Harding, 1992, [187]

BSN-Bilateral Striatal Necrosis; CPEO-Chronic Progressive External Ophthalmoplegia; ENC-Encephalopathy; EXIT-Exercise Intolerance; GER/SIDS-GastroEsophageal Reflux/Sudden Infant Death Sydrome; HCM-Hypertrophic Cardiomyopathy KSS-Kearns-Sayre Syndrome; LHON-Leiber's Hereditary Optic Neuropathy; LS-Leigh Syndrome; MELAS-Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like episodes; MERRF-Myoclonus Epilepsy with Ragged Red Fibers; MINGIE-Myo-Neuro Gastro GastroIntestinal Encephalopathy; MYO-Myoclonus; MULTI-Multisystem Disease; NARP- Neurogenic muscle weakness Ataxia and Retinitis Pigmentosa; PEM-Progressive Myoclonic Epilepsy; RETT Syndrome

APPENDIX C: Aim 1 Additional Tables

Table C 1: mtDNA Variants vs. All PTS

mtDNA Variant	No PTS + Count (%)	PTS+ Count (%)	p-value* (n)
T146C			
T	56 (71.8)	22 (28.2)	p=1.000
C	6 (75.0)	2 (25.0)	(86)
T195C ¢			
T	122 (75.8)	39 (24.2)	p=0.428
C	29 (69.0)	13 (31.0)	(203)
T477C ¢			
T	129 (75.9)	41 (24.1)	p=0.260
C	6 (60.0)	4 (40.00)	(180)
C3494T			
C	62 (72.1)	24 (27.9)	---
T	0 (0.0)	0 (0.0)	(86)
T4126C¢			
T	121 (75.6)	39 (24.4)	p=0.902
C	39 (76.5)	12 (23.5)	(211)
A4917G¢			
A	146 (76.4)	45 (23.6)	p=0.323
G	14 (66.7)	7 (33.3)	(212)
C7028T			
C	21 (70.0)	9 (30.0)	P=0.751
T	41 (73.2)	15 (26.8)	(86)
A8701G			
A	55 (72.4)	21 (27.6)	P=1.000
G	7 (70.0)	3 (30.0)	(86)
A10398G¢			
A	110 (76.4)	34 (23.6)	p=0.331
G	34 (69.4)	15 (30.6)	(193)
A10550G			
A	57 (74.0)	20 (26.0)	P=0.434
G	5 (55.6)	4 (44.4)	(86)
T10873C			
T	58 (71.6)	23 (28.4)	P=1.000
C	4 (80.0)	1 (20.0)	(86)
A11947G			
A	59 (71.1)	24 (28.9)	p=0.557
G	3 (100.0)	0 (0.0)	(86)
A12308G			
A	45 (72.6)	17 (27.4)	p=0.871
G	17 (70.8)	7 (29.2)	(86)
C12705T			
C	53 (71.6)	21 (28.4)	p=1.000
T	9 (75.0)	2 (25.0)	(86)
G13708A			
G	57 (74.0)	20 (26.0)	p=0.386
A	4 (57.1)	3 (42.9)	(86)

mtDNA Variant	No PTS + Count (%)	PTS+ Count (%)	p-value* (n)
A14233G			
A	58 (72.5)	22 (27.5)	p=1.000
G	4 (66.7)	2 (33.3)	(86)
C14766T			
C	24 (72.7)	9 (27.3)	p=0.972
T	38 (73.1)	14 (26.9)	(85)
T16519C¢			
T	77 (71.3)	31 (28.7)	p=0.160
C	51 (81.0)	12 (19.0)	(171)

¢ - mtDNA variants with additional samples
***** - Chi-squared analysis and Fisher's Exact
+ - Deaths with no evidence of seizure removed

APPENDIX D: Aim 2 Additional Tables

Table D 1 – Comparison of Variants to SDH and Cranial Surgery in LPTS

mtDNA Variant (n)	NO SDH Count (%)	SDH Count (%)	p-value		NO Cranial Surgery Count (%)	Cranial Surgery Count (%)	p-value
T146C (79)							
T	31 (43.7)	40 (56.3)	1.000		41 (61.2)	26 (38.8)	1.000
C	4 (50.0)	4 (50.0)			4 (66.7)	2 (33.3)	
T195C (165)							
T	52 (40.0)	78 (60.0)	0.225		89 (61.4)	56 (38.6)	0.555
C	18 (51.4)	17 (48.6)			19 (55.9)	15 (44.1)	
T477C (167)							
T	69 (43.7)	89 (56.3)	0.304		91 (62.3)	55 (37.7)	0.732
C	2 (22.2)	7 (77.8)			5 (55.6)	4 (44.4)	
C3494T (79)							
C	35 (44.3)	44 (55.7)	---		45 (61.6)	28 (38.4)	---
T	0 (0)	0			0 (0.0)	0 (0.0)	
T4216C (171)							
T	54 (40.9)	78 (59.1)	0.462		85 (59.9)	57 (40.1)	0.804
C	19 (48.7)	20 (51.3)			26 (57.8)	19 (42.2)	
A4917G (172)							
A	65 (41.9)	90 (58.1)	0.685		98 (58.3)	70 (41.7)	0.396
G	8 (47.1)	9 (52.9)			13 (68.4)	6 (40.6)	
C7028T (79)							
C	14 (50.0)	14 (50.0)	0.450		17 (60.7)	11 (39.3)	0.897
T	21 (41.2)	30 (58.8)			28 (62.2)	17 (37.8)	
A8701G (79)							
A	30 (42.3)	41 (57.7)	0.455		42 (60.9)	27 (39.1)	0.656
G	5 (62.5)	3 (6.8)			3 (75.0)	1 (25.0)	
A10398G (165)							
A	53 (41.7)	74 (58.3)	0.538		82 (60.7)	53 (39.3)	0.488
G	18 (47.4)	20 (21.3)			19 (54.3)	16 (45.7)	
A10550G (79)							
A	33 (45.8)	39 (54.2)	0.454		42 (62.7)	25 (37.3)	0.669
G	2 (28.6)	5 (71.4)			3(50.0)	3 (50.)	
T10873C (79)							
T	33 (44.0)	42 (56.0)	1.000		45 (61.6)	28 (38.4)	---
C	2 (50.0)	2 (50.0)			0 (0.0)	0 (0.0)	
A11947G (79)							
A	34 (44.7)	42 (55.3)	1.000		43 (61.4)	27 (38.6)	1.000
G	1 (33.3)	2 (66.7)			2 (66.7)	1 (33.3)	
A12308G (79)							
A	27 (46.6)	31 (53.4)	0.504		33 (61.1)	21 (38.9)	0.875
G	8 (38.1)	13 (61.9)			12 (63.2)	7 (36.8)	
C12705T (79)							
C	30 (44.1)	38 (55.9)	1.000		40 (60.6)	26 (39.4)	0.700
T	5 (45.5)	6 (54.4)			5 (71.4)	2 (28.6)	
G13708A (79)	29 (41.4)	41 (58.6)					
G	5 (71.4)	2 (28.6)	0.230		40 (61.5)	25 (38.5)	0.704
A					5 (71.4)	2 (28.6)	

mtDNA Variant (n)	NO SDH Count (%)	SDH Count (%)	p-value		NO Cranial Surgery Count (%)	Cranial Surgery Count (%)	p-value
T13789C (79) T C	32 (42.1) 2 (100.0)	44 (57.9) 0 (0.0)	0.187		43 (61.4) 2 (100.0)	27 (38. 6) 0 (0.0)	0.525
A14233G (79) A G	33 (44.6) 2 (40.)	41 (55.4) 3 (60.)	1.000		42 (61.8) 3 (60.0)	26 (38.2) 2 (40.0)	1.000
C14766T (79) C T	15 (48.4) 19 (40.4)	16 (51.6) 28 (59.6)	0.488		18 (58.1) 27 (65.9)	13 (41.9) 14 (34.1)	0.333
T16519C (160) T C	44 (43.1) 26 (44.8)	58 (56.9) 32 (55.2)	0.836		57 (58.8) 35 (66.0)	40 (41.2) 18 (34.0)	0.382

***** Chi-square test of independence, or Fisher's exact test
--- Cells with 0 counts.

Table D 2 – Initial Stratification of SDH by Variant by LPTS

Variants (n)+	OR (95% CI)	p- value*
T146C (79)	T – 1.210 (.379-3.857) C - ---	0.399
T195C (165)	**T – 2.64 (.981-7.122)** **C – 11.90 (1.27-111.35)**	**0.004**
T477C (167)	**T – 3.66 (1.39-9.58)** **C - .400 (.01-10.01)**	**0.009**
C3494T (79)	C – 1.61 (.529-4.90) T - ---	0.399
T4216C (171)	**T – 7.56 (2.14-26.63)** **C - .662 (.127 – 3.45)**	**0.004**
A4917G (172)	**A – 4.87 (1.75-13.51)** **G - .375 (.027-5.16)**	**0.004**
C7028T (79)	C – 3.33 (.522-21.27) T – 1.06 (.260-4.35)	0.399
A8701G (79)	A – 1.83 (.562-5.98) G - ---	0.399
A10398G (165)	**A – 3.80 (1.33-10.87)** **G – 3.42 (.594-19.80)**	**0.003**
A10550G (79)	A – 1.35 (.425-4.29) G - ---	0.399
T10873C (79)	T – 1.59 (.521-4.89) C - ---	0.399
A11947G (79)	A – 1.65 (.541-5.06) G – 1.61 (.529-4.90)	0.399
A12308G (79)	A – 1.02 (.296-3.52) G - ---	0.399
C12705T (79)	C – 1.78 (.537-5.93) T - .800 (.037-17.19)	0.399
G13708A (77)	G – 1.75 (.484-6.37) A – 1.50 (.055-40.63)	0.547
T13789C (78)	T – 1.44(.471-4.42) C - ---	0.435
A14233G (79)	A – 2.05 (.634-6.65) G - ---	0.399
C14766T (78)	C – 2.95 (.476-18.34) T – 1.45 (.316-6.70)	0.264
T16519C (160)	**T – 4.10 (1.39-12.05)** **C – 1.71 (.288-10.19)**	**0.008**

+ - Note the differing n's for the 6 variants from the larger subset of samples.

Tables D 3-8 - Stratification of SDH by Variant by LPTS

Table 3 - T195C

T195C (n=165)		LPTS	No LPTS	OR (95% CI)	p-value
T	**SDH** obs exp	 20 15.6	 58 62.4	2.64(.981-7.12)	0.049
	No SDH obs exp	 6 10.4	 46 41.6		
C	**SDH** obs exp	 7 3.9	 10 13.1	11.90(1.27-111.35)	0.018
	No SDH obs exp	 1 4.1	 17 13.9		

Table D 4 - T477C

T477C (n=165)		LPTS	No LPTS	OR (95% CI)	p-value
T	**SDH** obs exp	 23 16.3	 66 72.7	3.66(1.39-9.58)	0.006
	No SDH obs exp	 6 12.7	 63 56.3		
C	**SDH** obs exp	 2 2.3	 5 4.7	0.400(.016-10.01)	1.000
	No SDH obs exp	 1 .7	 1 1.3		

Table D 5 – T4216C

T4216C (n=171)		LPTS	No LPTS	OR (95% CI)	p-value
T	**SDH** obs exp	 24 16.0	 54 62.0	7.56(2.14-26.63)	0.000
	No SDH obs exp	 3 11.0	 51 43.0		
C	**SDH** obs exp	 3 3.6	 17 16.4	0.662(.127-3.45)	0.695
	No SDH obs exp	 4 3.4	 15 15.6		

Table D 6 – A4917G

A4917G (n=172)		LPTS	No LPTS	OR (95% CI)	p-value
A	**SDH** obs exp	 26 18.0	 64 72.0	4.87(1.76-13.52)	0.001
	No SDH obs exp	 5 13.0	 60 52.0		
G	**SDH** obs exp	 1 1.6	 8 7.4	0.375(.027-5.17)	0.576
	No SDH obs exp	 2 1.4	 6 6.6		

Table D 7 – A10398G

A10398G (n=165)		LPTS	No LPTS	OR (95% CI)	p-value
A	**SDH** obs exp	 21 15.1	 53 58.9	3.80(1.33-10.88)	0.009
	No SDH obs exp	 5 10.9	 48 42.1		
G	**SDH** obs exp	 6 4.2	 14 15.8	3.42(.594-19.80)	0.238
	No SDH obs exp	 2 3.8	 16 14.2		

Table D 8 – T16519C

T16519C (n=165)		LPTS	NO LPTS	OR (95% CI)	p-value
T	**SDH** obs exp	 20 14.2	 38 43.8	4.10(1.39-12.05)	0.007
	No SDH obs exp	 5 10.8	 39 33.2		
C	**SDH** obs exp	 4 3.3	 28 28.7	1.71(.288-10.19)	0.681
	No SDH obs exp	 2 2.7	 24 23.3		

Table D 9 – Initial Stratification of Cranial Surgery by Variant by LPTS

Variant (n)+	OR (95% CI)	p-value*
T146C (73)	T – 10.85 (2.66-44.28) C - 3.00 (.084-107.44)	0.000$
T195C (179)	**T – 4.38 (1.86-10.32)** **C – 9.71 (1.63-57.72)**	0.000
T477C (155)	T – 3.83 (1.65-8.89) C - ---	---
C3494T (73)	C – 8.88 (2.50-31.54) T - ---	---
T4216C (187)	**T – 4.37 (1.86-10.25)** **C – 7.00 (1.25-38.99)**	0.000
A4917G (187)	**A – 4.12 (1.85-9.16)** **G – 24.00(1.68-340.99)**	0.000
C7028T (73)	C – --- T – 3.27 (.766-13.98)	---
A8701G (73)	A – 7.60 (2.11-27.32) G - ---	---
A10398G (170)	**A – 6.07 (2.43-15.13)** **G – 1.70 (.370-7.85)**	0.000
A10550G (73)	A – 12.00(2.92-49.26) G – 1.00 (.034-29.80)	0.000$
T10873C (73)	T – 8.88 (2.50-31.54 C - ---	---
A11947G (73)	A – 9.05 (2.52-32.44) G – ---	---
A12308G (73)	A – 17.05 (3.22-90.27) G – 2.00 (.214-18.68)	0.000$
C12705T (73)	C – 10.57 (2.58-43.15) T – 4.00 (.117-136.95)	0.000$
G13708A (72)	G – 8.22 (1.98-34.11) A – ---	---
T13789C (72)	T – 9.05 (2.52-32.44) C - ---	---
A14233G (73)	A – 8.14 (2,24-29.48) G - ---	---
C14766T (72)	C – --- T – 3.19 (.696-14.66)	---
T16519C (150)	**T – 5.84 (2.13-15.99)** **C – 3.04 (.601-15.45)**	0.000

+ - Note the differing n's for the 6 variants from the larger subset of samples.

***** Chi-square test of independence or Fisher's Exact test where appropriate. (bold: $p<.05$)

$ - Not enough cases with risk allele and Cranial Surgery to evaluate, odds ratio and confidence intervals become unstable.

Tables D 10-14 - Stratification of Cranial Surgery by Variant by LPTS

Table 10 - T195C

T195C (n=179)		LPTS	No LPTS	OR (95% CI)	p-value
T	**Surgery**				
	obs	20	36	4.39(1.86-10.32)	0.000
	exp	11.6	44.4		
	No Surgery				
	obs	10	79		
	exp	18.4	70.6		
C	**Surgery**				
	obs	8	7	9.71(1.64-57.72)	0.010
	exp	4.4	10.6		
	No Surgery				
	obs	2	17		
	exp	5.6	13.4		

Table D 11 – T4216C

T4216C (n=187)		LPTS	No LPTS	OR (95% CI)	p-value
T	**Surgery**				
	obs	21	36	4.38(1.86-10.25)	0.000
	exp	12.4	44.6		
	No Surgery				
	obs	10	75		
	exp	18.6	66.4		
C	**Surgery**				
	obs	7	12	7.00(1.26-38.99)	0.024
	exp	3.8	15.2		
	No Surgery				
	obs	2	24		
	exp	5.2	20.8		

Table D 12 – A4917G

A4917G (n=187)		LPTS	No LPTS	OR (95% CI)	p-value
A	**Surgery**				
	obs	24	46	4.13(1.86-9.17)	0.000
	exp	14.6	55.4		
	No Surgery				
	obs	11	87		
	exp	20.4	77.6		
G	**Surgery**				
	obs	4	2	24.0(1.69-340.99)	0.017
	exp	1.6	4.4		
	No Surgery				
	obs	1	12		
	exp	3.4	9.6		

Table D 13 – A10398G

A10398G (n=170)		LPTS	No LPTS	OR (95% CI)	p-value
A	**Surgery** obs exp	 21 11.4	 32 41.6	6.07(2.43-15.14)	0.000
	No Surgery obs exp	 8 17.6	 74 64.4		
G	**Surgery** obs exp	 5 4.1	 11 11.9	1.71(.370-7.85)	0.700
	No Surgery obs exp	 4 4.9	 15 14.1		

Table D 14 – T16519C

T16519C (n=150)		LPTS	No LPTS	OR (95% CI)	p-value
T	**Surgery** obs exp	 18 10.3	 22 29.7	5.84(2.14-15.99)	0.000
	No Surgery obs exp	 7 14.7	 50 42.3		
C	**Surgery** obs exp	 4 2.4	 14 15.6	3.05(.601-15.45)	0. 211
	No Surgery obs exp	 3 4.6	 32 30.4		

APPENDIX E: Aim 3 Additional Tables

Tables E 15-19- Stratification of LPTS by GOS at 6 months by Variant

Table E 15 – T195C

T195C (n=172)		LPTS	No LPTS	OR (95% CI)	p-value
T	**GOS 2-3** obs exp	 12 7.6	 58 62.4	4.48(1.21-16.68)	0.016
	GOS 4-5 obs exp	 3 7.4	 65 60.6		
C	**GOS 2-3** obs exp	 2 1.8	 13 13.2	1.31(0.16-10.56)	1.000
	GOS 4-5 obs exp	 2 2.2	 17 16.8		

Table E 16 – T4216C

T4216C (n=179)		LPTS	No LPTS	OR (95% CI)	p-value
T	**GOS 2-3** obs exp	 10 6.7	 61 64.3	3.50(.92-13.32)	0.053
	GOS 4-5 obs exp	 3 6.3	 64 60.7		
C	**GOS 2-3** obs exp	 4 2.9	 16 17.1	2.38(.38-14.70)	0.410
	GOS 4-5 obs exp	 2 3.1	 19 17.9		

Table E 17 – A4917G

A4917G (n=180)		LPTS	No LPTS	OR (95% CI)	p-value
A	**GOS 2-3** obs exp	 11 7.7	 72 75.3	2.87(.87-9.41)	0.072
	GOS 4-5 obs exp	 4 7.3	 75 71.7		
G	**GOS 2-3** obs exp	 3 1.8	 5 6.2	5.40(.44-66.67)	0.275
	GOS 4-5 obs exp	 1 2.2	 9 7.8		

Table E 18 – A10398G

A10398G (n=163)		LPTS	No LPTS	OR (95% CI)	p-value
A	**GOS 2-3** obs exp	 10 6.9	 52 55.1	2.89(.86-9.75)	0.078
	GOS 4-5 obs exp	 4 7.1	 60 56.9		
G	**GOS 2-3** obs exp	 4 2.7	 16 17.3	4.00(.40-39.83)	0.348
	GOS 4-5 obs exp	 1 2.3	 16 14.7		

Table E 19 – T16519C

T16519C (n=142)		LPTS	No LPTS	OR (95% CI)	p-value
T	**GOS 2-3** obs exp	 10 6.1	 40 43.9	9.75(1.19-79.81)	0.012
	GOS 4-5 obs exp	 1 4.9	 39 35.1		
C	**GOS 2-3** obs exp	 1 1.1	 18 17.9	.861(.073-10.18)	1.000
	GOS 4-5 obs exp	 2 1.9	 31 31.1		

BIBLIOGRAPHY

1. Coronado, V.G., et al., *Surveillance for traumatic brain injury-related deaths--United States, 1997-2007.* MMWR Surveill Summ, 2011. **60**(5): p. 1-32.

2. Faul M, X.L., Wald MM, Coronado VG., *Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths 2002–2006.*, 2010, National Center for Injury Prevention and Control: Atlanta (GA): Centers for Disease Control and Prevention.

3. Zaloshnja, E., et al., *Prevalence of long-term disability from traumatic brain injury in the civilian population of the United States, 2005.* J Head Trauma Rehabil, 2008. **23**(6): p. 394-400.

4. Finkelstein, E., P.S. Corso, and T.R. Miller, *The incidence and economic burden of injuries in the United States*2006, Oxford ; New York: Oxford University Press. xiii, 187 p.

5. Haltiner, A.M., N.R. Temkin, and S.S. Dikmen, *Risk of seizure recurrence after the first late posttraumatic seizure.* Arch Phys Med Rehabil, 1997. **78**(8): p. 835-40.

6. Hauser, W.A., J.F. Annegers, and L.T. Kurland, *Prevalence of epilepsy in Rochester, Minnesota: 1940-1980.* Epilepsia, 1991. **32**(4): p. 429-45.

7. Willmore, L.J., *Posttraumatic epilepsy.* Neurol Clin, 1992. **10**(4): p. 869-78.

8. Marshall, L.F., *Head injury By Louis Bakay and Franz E. Glasauer Little, Brown and Company, Boston, 1980 445 Pages, Illustrated, $28.95.* Ann Neurol, 1982. **11**(1): p. 108-108.

9. Annegers, J.F., et al., *A population-based study of seizures after traumatic brain injuries.* N Engl J Med, 1998. **338**(1): p. 20-4.

10. Dikmen, S.S., et al., *Neurobehavioral effects of phenytoin prophylaxis of posttraumatic seizures.* JAMA, 1991. **265**(10): p. 1271-7.

11. Temkin, N.R., *Preventing and treating posttraumatic seizures: the human experience.* Epilepsia, 2009. **50 Suppl 2**: p. 10-3.

12. Agrawal, A., et al., *Post-traumatic epilepsy: an overview.* Clin Neurol Neurosurg, 2006. **108**(5): p. 433-9.

13. Noebels, J.L., *Exploring new gene discoveries in idiopathic generalized epilepsy.* Epilepsia, 2003. **44 Suppl 2**: p. 16-21.

14. Cavalleri, G.L., et al., *A multicenter study of BRD2 as a risk factor for juvenile myoclonic epilepsy.* Epilepsia, 2007. **48**(4): p. 706-12.

15. Cavalleri, G.L., et al., *Multicentre search for genetic susceptibility loci in sporadic epilepsy syndrome and seizure types: a case-control study.* Lancet Neurol, 2007. **6**(11): p. 970-80.

16. Diaz-Arrastia, R., et al., *Increased risk of late posttraumatic seizures associated with inheritance of APOE epsilon4 allele.* Arch Neurol, 2003. **60**(6): p. 818-22.

17. Anderson, G.D., et al., *Haptoglobin phenotype and apolipoprotein E polymorphism: relationship to posttraumatic seizures and neuropsychological functioning after traumatic brain injury.* Epilepsy Behav, 2009. **16**(3): p. 501-6.

18. Raymont, V., et al., *Correlates of posttraumatic epilepsy 35 years following combat brain injury.* Neurology, 2010. **75**(3): p. 224-9.

19. Wagner, A.K., et al., *Adenosine A1 receptor gene variants associated with post-traumatic seizures after severe TBI.* Epilepsy Res, 2010. **90**(3): p. 259-72.

20. Miller, M.A., et al., *APOE genetic associations with seizure development after severe traumatic brain injury.* Brain Inj, 2010. **24**(12): p. 1468-77.

21. Chinnery, P.F. and E.A. Schon, *Mitochondria.* J Neurol Neurosurg Psychiatry, 2003. **74**(9): p. 1188-99.

22. Debray, F.G., M. Lambert, and G.A. Mitchell, *Disorders of mitochondrial function.* Curr Opin Pediatr, 2008. **20**(4): p. 471-82.

23. Canafoglia, L., et al., *Epileptic phenotypes associated with mitochondrial disorders.* Neurology, 2001. **56**(10): p. 1340-6.

24. Shoffner, J.M., et al., *Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation.* Cell, 1990. **61**(6): p. 931-7.

25. Pavlakis, S.G., et al., *Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: a distinctive clinical syndrome.* Ann Neurol, 1984. **16**(4): p. 481-8.

26. Anderson, S., et al., *Sequence and organization of the human mitochondrial genome.* Nature, 1981. **290**(5806): p. 457-65.

27. DiMauro, S., *Mitochondrial DNA medicine.* Biosci Rep, 2007. **27**(1-3): p. 5-9.

28. McDonald, R.P., et al., *Mitochondrial DNA deletions in acute brain injury.* Neuroreport, 1999. **10**(9): p. 1875-8.

29. Lifshitz, J. and T.K. McIntosh, *Age-associated mitochondrial DNA deletions are not evident chronically after experimental brain injury in the rat.* J Neurotrauma, 2003. **20**(2): p. 139-49.

30. Canter, J.A., et al., *Specific polymorphic variation in the mitochondrial genome and increased in-hospital mortality after severe trauma.* Ann Surg, 2007. **246**(3): p. 406-11; discussion 411-4.

31. Corso, P., et al., *Incidence and lifetime costs of injuries in the United States.* Inj Prev, 2006. **12**(4): p. 212-8.

32. Frey, L.C., *Epidemiology of posttraumatic epilepsy: a critical review.* Epilepsia, 2003. **44 Suppl 10**: p. 11-7.

33. Jennett, B., *Epilepsy and acute traumatic intracranial haematoma.* J Neurol Neurosurg Psychiatry, 1975. **38**(4): p. 378-81.

34. Chen, J.W., et al., *Posttraumatic epilepsy and treatment.* J Rehabil Res Dev, 2009. **46**(6): p. 685-96.

35. Salazar, A.M., et al., *Epilepsy after penetrating head injury. I. Clinical correlates: a report of the Vietnam Head Injury Study.* Neurology, 1985. **35**(10): p. 1406-14.

36. Caveness, W.F., A.E. Walker, and P.B. Ascroft, *Incidence of posttraumatic epilepsy in Korean veterans as compared with those from World War I and World War II.* J Neurosurg, 1962. **19**: p. 122-9.

37. Caveness, W.F., *Incidence of craniocerebral trauma in the United States in 1976 with trend from 1970 to 1975.* Adv Neurol, 1979. **22**: p. 1-3.

38. Temkin, N.R., *Risk factors for posttraumatic seizures in adults.* Epilepsia, 2003. **44 Suppl 10**: p. 18-20.

39. Curia, G., et al., *Impact of injury location and severity on posttraumatic epilepsy in the rat: role of frontal neocortex.* Cereb Cortex, 2011. **21**(7): p. 1574-92.

40. Kharatishvili, I., et al., *A model of posttraumatic epilepsy induced by lateral fluid-percussion brain injury in rats.* Neuroscience, 2006. **140**(2): p. 685-97.

41. D'Ambrosio, R., et al., *Post-traumatic epilepsy following fluid percussion injury in the rat.* Brain, 2004. **127**(Pt 2): p. 304-14.

42. D'Ambrosio, R., et al., *Functional definition of seizure provides new insight into post-traumatic epileptogenesis.* Brain, 2009. **132**(Pt 10): p. 2805-21.

43. Englander, J., et al., *Analyzing risk factors for late posttraumatic seizures: a prospective, multicenter investigation.* Arch Phys Med Rehabil, 2003. **84**(3): p. 365-73.

44. da Silva, A.M., et al., *Posttraumatic epilepsy in civilians: clinical and electroencephalographic studies.* Acta Neurochir Suppl (Wien), 1992. **55**: p. 56-63.

45. Angeleri, F., et al., *Posttraumatic epilepsy risk factors: one-year prospective study after head injury.* Epilepsia, 1999. **40**(9): p. 1222-30.

46. Thapa, A., et al., *Post-traumatic seizures-A prospective study from a tertiary level trauma center in a developing country.* Seizure, 2010. **19**(4): p. 211-6.

47. Ferguson, P.L., et al., *A population-based study of risk of epilepsy after hospitalization for traumatic brain injury.* Epilepsia, 2010. **51**(5): p. 891-8.

48. Asikainen, I., M. Kaste, and S. Sarna, *Early and late posttraumatic seizures in traumatic brain injury rehabilitation patients: brain injury factors causing late seizures and influence of seizures on long-term outcome.* Epilepsia, 1999. **40**(5): p. 584-9.

49. Noebels, J.L., *The biology of epilepsy genes.* Annu Rev Neurosci, 2003. **26**: p. 599-625.

50. Jordan, B.D., *Genetic influences on outcome following traumatic brain injury.* Neurochem Res, 2007. **32**(4-5): p. 905-15.

51. Dardiotis, E., et al., *Genetic association studies in patients with traumatic brain injury.* Neurosurg Focus, 2010. **28**(1): p. E9.

52. Waldbaum, S. and M. Patel, *Mitochondrial dysfunction and oxidative stress: a contributing link to acquired epilepsy?* J Bioenerg Biomembr, 2010. **42**(6): p. 449-55.

53. Zsurka, G. and W.S. Kunz, *Mitochondrial dysfunction in neurological disorders with epileptic phenotypes.* J Bioenerg Biomembr, 2010. **42**(6): p. 443-8.

54. Kunz, W.S., *The role of mitochondria in epileptogenesis.* Curr Opin Neurol, 2002. **15**(2): p. 179-84.

55. Thurman, D.J., et al., *Traumatic brain injury in the United States: A public health perspective.* J Head Trauma Rehabil, 1999. **14**(6): p. 602-15.

56. Waxweiler, R.J., et al., *Monitoring the impact of traumatic brain injury: a review and update.* J Neurotrauma, 1995. **12**(4): p. 509-16.

57. Binder, S., J.D. Corrigan, and J.A. Langlois, *The public health approach to traumatic brain injury: an overview of CDC's research and programs.* J Head Trauma Rehabil, 2005. **20**(3): p. 189-95.

58. Langlois, J.A., W. Rutland-Brown, and M.M. Wald, *The epidemiology and impact of traumatic brain injury: a brief overview.* J Head Trauma Rehabil, 2006. **21**(5): p. 375-8.

59. Horner, M.D., et al., *Patterns of alcohol use 1 year after traumatic brain injury: a population-based, epidemiological study.* J Int Neuropsychol Soc, 2005. **11**(3): p. 322-30.

60. Holsinger, T., et al., *Head injury in early adulthood and the lifetime risk of depression.* Arch Gen Psychiatry, 2002. **59**(1): p. 17-22.

61. Plassman, B.L., et al., *Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias.* Neurology, 2000. **55**(8): p. 1158-66.

62. Selassie, A.W., et al., *Risk of posthospitalization mortality among persons with traumatic brain injury, South Carolina 1999-2001.* J Head Trauma Rehabil, 2005. **20**(3): p. 257-69.

63. Sherer, M., et al., *Race and productivity outcome after traumatic brain injury: influence of confounding factors.* J Head Trauma Rehabil, 2003. **18**(5): p. 408-24.

64. Okie, S., *Traumatic brain injury in the war zone.* N Engl J Med, 2005. **352**(20): p. 2043-7.

65. Warden, D., *Military TBI during the Iraq and Afghanistan wars.* J Head Trauma Rehabil, 2006. **21**(5): p. 398-402.

66. Narayan, R.K., *Development of guidelines for the management of severe head injury.* J Neurotrauma, 1995. **12**(5): p. 907-12.

67. Wojcik, B.E., et al., *Traumatic brain injury hospitalizations of U.S. army soldiers deployed to Afghanistan and Iraq.* Am J Prev Med, 2010. **38**(1 Suppl): p. S108-16.

68. Saatman, K.E., et al., *Classification of traumatic brain injury for targeted therapies.* J Neurotrauma, 2008. **25**(7): p. 719-38.

69. Teasdale, G. and B. Jennett, *Assessment of coma and impaired consciousness. A practical scale.* Lancet, 1974. **2**(7872): p. 81-4.

70. Narayan, R.K., et al., *Clinical trials in head injury.* J Neurotrauma, 2002. **19**(5): p. 503-57.

71. Thurman, D.J., et al., *Surveillance of traumatic brain injuries in Utah.* West J Med, 1996. **165**(4): p. 192-6.

72. Langlois, J.A., et al., *Traumatic brain injury-related hospital discharges. Results from a 14-state surveillance system, 1997.* MMWR Surveill Summ, 2003. **52**(4): p. 1-20.

73. Institute of Medicine, I., *Gulf War and Health, Volume 7: Long-term Consequences of Traumatic Brain Injury.* . Vol. 7. 2009, Washington, DC: The National Academies Press.

74. Helmick, K., *Cognitive rehabilitation for military personnel with mild traumatic brain injury and chronic post-concussional disorder: Results of April 2009 consensus conference.* NeuroRehabilitation, 2010. **26**(3): p. 239-55.

75. Cooper, P.R. and J. Golfinos, *Head injury*. 4th ed2000, New York: McGraw-Hill, Medical Publishing Division. x, 598 p.

76. Heegaard, W. and M. Biros, *Traumatic brain injury.* Emerg Med Clin North Am, 2007. **25**(3): p. 655-78, viii.

77. Gaweda-Walerych, K., et al., *Mitochondrial DNA haplogroups and subhaplogroups are associated with Parkinson's disease risk in a Polish PD cohort.* J Neural Transm, 2008. **115**(11): p. 1521-6.

78. Lowenstein, D.H., *Epilepsy after head injury: an overview.* Epilepsia, 2009. **50 Suppl 2**: p. 4-9.

79. Caveness, W.F., et al., *The nature of posttraumatic epilepsy.* J Neurosurg, 1979. **50**(5): p. 545-53.

80. Yablon, S.A., *Posttraumatic seizures.* Arch Phys Med Rehabil, 1993. **74**(9): p. 983-1001.

81. Chang, B.S. and D.H. Lowenstein, *Practice parameter: antiepileptic drug prophylaxis in severe traumatic brain injury: report of the Quality Standards Subcommittee of the American Academy of Neurology.* Neurology, 2003. **60**(1): p. 10-6.

82. Kollevold, T., *Immediate and early cerebral seizures after head injuries. Part III.* J Oslo City Hosp, 1978. **28**(6): p. 77-86.

83. Annegers, J.F., et al., *Seizures after head trauma: a population study.* Neurology, 1980. **30**(7 Pt 1): p. 683-9.

84. Jennett, W.B. and W. Lewin, *Traumatic epilepsy after closed head injuries.* J Neurol Neurosurg Psychiatry, 1960. **23**: p. 295-301.

85. Hauser, W.A., J.F. Annegers, and W.A. Rocca, *Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester, Minnesota.* Mayo Clin Proc, 1996. **71**(6): p. 576-86.

86. Diaz-Arrastia, R., et al., *Posttraumatic epilepsy: the endophenotypes of a human model of epileptogenesis.* Epilepsia, 2009. **50 Suppl 2**: p. 14-20.

87. D'Ambrosio, R., et al., *Progression from frontal-parietal to mesial-temporal epilepsy after fluid percussion injury in the rat.* Brain, 2005. **128**(Pt 1): p. 174-88.

88. Marwitz, J.H., et al., *A multi-center analysis of rehospitalizations five years after brain injury.* J Head Trauma Rehabil, 2001. **16**(4): p. 307-17.

89. Hung, C.C., et al., *Complex haplotypic effects of the ABCB1 gene on epilepsy treatment response.* Pharmacogenomics, 2005. **6**(4): p. 411-7.

90. Corder, E.H., et al., *Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families.* Science, 1993. **261**(5123): p. 921-3.

91. Taguchi, N., et al., *Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission.* J Biol Chem, 2007. **282**(15): p. 11521-9.

92. Marieb, E.N. and K. Hoehn, *Human anatomy & physiology*. 8th ed2010, San Francisco: Benjamin Cummings. xxxii, 1114, 28, 23, 3, 61 p.

93. DiMauro, S. and E.A. Schon, *Mitochondrial respiratory-chain diseases.* N Engl J Med, 2003. **348**(26): p. 2656-68.

94. Nass, S. and M.M. Nass, *Intramitochondrial Fibers with DNA Characteristics. Ii. Enzymatic and Other Hydrolytic Treatments.* J Cell Biol, 1963. **19**: p. 613-29.

95. Shoubridge, E.A., *Nuclear gene defects in respiratory chain disorders.* Semin Neurol, 2001. **21**(3): p. 261-7.

96. Holt, I.J., et al., *A new mitochondrial disease associated with mitochondrial DNA heteroplasmy.* Am J Hum Genet, 1990. **46**(3): p. 428-33.

97. Edmond, J.C., *Mitochondrial disorders.* Int Ophthalmol Clin, 2009. **49**(3): p. 27-33.

98. Finsterer, J., *Central nervous system manifestations of mitochondrial disorders.* Acta Neurol Scand, 2006. **114**(4): p. 217-38.

99. Giles, R.E., et al., *Maternal inheritance of human mitochondrial DNA.* Proc Natl Acad Sci U S A, 1980. **77**(11): p. 6715-9.

100. Chinnery, P.F., *Mitochondrial disorders come full circle.* Neurology, 2003. **61**(7): p. 878-80.

101. Chinnery, P.F. and D.C. Samuels, *Relaxed replication of mtDNA: A model with implications for the expression of disease.* Am J Hum Genet, 1999. **64**(4): p. 1158-65.

102. DiMauro, S. and M. Mancuso, *Mitochondrial diseases: therapeutic approaches.* Biosci Rep, 2007. **27**(1-3): p. 125-37.

103. Majamaa, K., et al., *Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population.* Am J Hum Genet, 1998. **63**(2): p. 447-54.

104. Chinnery, P.F., et al., *The epidemiology of pathogenic mitochondrial DNA mutations.* Ann Neurol, 2000. **48**(2): p. 188-93.

105. Darin, N., et al., *The incidence of mitochondrial encephalomyopathies in childhood: clinical features and morphological, biochemical, and DNA anbormalities.* Ann Neurol, 2001. **49**(3): p. 377-83.

106. Skladal, D., J. Halliday, and D.R. Thorburn, *Minimum birth prevalence of mitochondrial respiratory chain disorders in children.* Brain, 2003. **126**(Pt 8): p. 1905-12.

107. Schaefer, A.M., et al., *The epidemiology of mitochondrial disorders--past, present and future.* Biochim Biophys Acta, 2004. **1659**(2-3): p. 115-20.

108. Wallace, D.C., et al., *Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy.* Science, 1988. **242**(4884): p. 1427-30.

109. Holt, I.J., A.E. Harding, and J.A. Morgan-Hughes, *Mitochondrial DNA polymorphism in mitochondrial myopathy.* Hum Genet, 1988. **79**(1): p. 53-7.

110. DiMauro, S. and E.A. Schon, *Mitochondrial disorders in the nervous system.* Annu Rev Neurosci, 2008. **31**: p. 91-123.

111. Zeviani, M. and V. Carelli, *Mitochondrial disorders.* Curr Opin Neurol, 2003. **16**(5): p. 585-94.

112. DiMauro, S., *Mitochondrial encephalomyopathies: what next?* J Inherit Metab Dis, 1996. **19**(4): p. 489-503.

113. St-Pierre, J., et al., *Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators.* Cell, 2006. **127**(2): p. 397-408.

114. Wallace, D.C., M.D. Brown, and M.T. Lott, *Mitochondrial DNA variation in human evolution and disease.* Gene, 1999. **238**(1): p. 211-30.

115. Calabrese, V., et al., *Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity.* Neurochem Res, 2001. **26**(6): p. 739-64.

116. Wallace, D.C. and W. Fan, *Energetics, epigenetics, mitochondrial genetics.* Mitochondrion, 2010. **10**(1): p. 12-31.

117. Chinnery, P.F. and D.M. Turnbull, *Mitochondrial DNA mutations in the pathogenesis of human disease.* Mol Med Today, 2000. **6**(11): p. 425-32.

118. DiMauro, S. and C. Garone, *Historical perspective on mitochondrial medicine.* Dev Disabil Res Rev, 2010. **16**(2): p. 106-13.

119. Chinnery, P.F., et al., *Mitochondrial DNA analysis: polymorphisms and pathogenicity.* J Med Genet, 1999. **36**(7): p. 505-10.

120. Thieme, M., et al., *ReseqChip: automated integration of multiple local context probe data from the MitoChip array in mitochondrial DNA sequence assembly.* BMC Bioinformatics, 2009. **10**: p. 440.

121. Jennett, B., *Outcome of severe damage to the central nervous system. Scale, scope and philosophy of the clinical problem.* Ciba Found Symp, 1975(34): p. 3-21.

122. Gennarelli, T.A. and L.E. Thibault, *Biomechanics of acute subdural hematoma.* J Trauma, 1982. **22**(8): p. 680-6.

123. Hall, K., D.N. Cope, and M. Rappaport, *Glasgow Outcome Scale and Disability Rating Scale: comparative usefulness in following recovery in traumatic head injury.* Arch Phys Med Rehabil, 1985. **66**(1): p. 35-7.

124. Botto, L.D. and M.J. Khoury, *Commentary: facing the challenge of gene-environment interaction: the two-by-four table and beyond.* Am J Epidemiol, 2001. **153**(10): p. 1016-20.

125. Fujii, M., et al., *Cooling of the epileptic focus suppresses seizures with minimal influence on neurologic functions.* Epilepsia, 2012. **53**(3): p. 485-93.

126. Kazuno, A.A., et al., *Identification of mitochondrial DNA polymorphisms that alter mitochondrial matrix pH and intracellular calcium dynamics.* PLoS Genet, 2006. **2**(8): p. e128.

127. Zhu, H.Y., et al., *The role of mitochondrial genome in essential hypertension in a Chinese Han population.* Eur J Hum Genet, 2009. **17**(11): p. 1501-6.

128. Soares, P., et al., *Correcting for purifying selection: an improved human mitochondrial molecular clock.* Am J Hum Genet, 2009. **84**(6): p. 740-59.

129. Kirches, E., et al., *High frequency of mitochondrial DNA mutations in glioblastoma multiforme identified by direct sequence comparison to blood samples.* Int J Cancer, 2001. **93**(4): p. 534-8.

130. Chen, J.B., et al., *Sequence-based polymorphisms in the mitochondrial d-loop and potential SNP predictors for chronic dialysis.* PLoS One, 2012. **7**(7): p. e41125.

131. Zaki, E.A., et al., *Two common mitochondrial DNA polymorphisms are highly associated with migraine headache and cyclic vomiting syndrome.* Cephalalgia, 2009. **29**(7): p. 719-28.

132. Murakami, H., et al., *Polymorphisms in control region of mtDNA relates to individual differences in endurance capacity or trainability.* Jpn J Physiol, 2002. **52**(3): p. 247-56.

133. Sequeira, A., et al., *Mitochondrial mutations and polymorphisms in psychiatric disorders.* Front Genet, 2012. **3**: p. 103.

134. Cooper, G.M., et al., *A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose.* Blood, 2008. **112**(4): p. 1022-7.

135. Takeuchi, F., et al., *A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose.* PLoS Genet, 2009. **5**(3): p. e1000433.

136. Coskun, P.E., M.F. Beal, and D.C. Wallace, *Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication.* Proc Natl Acad Sci U S A, 2004. **101**(29): p. 10726-31.

137. McRae, A.F., et al., *Power and SNP tagging in whole mitochondrial genome association studies.* Genome Res, 2008. **18**(6): p. 911-7.

138. Nishigaki, Y., et al., *A novel mitochondrial tRNA(Leu(UUR)) mutation in a patient with features of MERRF and Kearns-Sayre syndrome.* Neuromuscul Disord, 2003. **13**(4): p. 334-40.

139. Enter, C., et al., *A specific point mutation in the mitochondrial genome of Caucasians with MELAS.* Hum Genet, 1991. **88**(2): p. 233-6.

140. Kirino, Y. and T. Suzuki, *Human mitochondrial diseases associated with tRNA wobble modification deficiency.* RNA Biol, 2005. **2**(2): p. 41-4.

141. Goto, Y., *Clinical features of MELAS and mitochondrial DNA mutations.* Muscle Nerve, 1995. **3**: p. S107-12.

142. Moraes, C.T., et al., *Two novel pathogenic mitochondrial DNA mutations affecting organelle number and protein synthesis. Is the tRNA(Leu(UUR)) gene an etiologic hot spot?* J Clin Invest, 1993. **92**(6): p. 2906-15.

143. Sternberg, D., et al., *Mitochondrial DNA transfer RNA gene sequence variations in patients with mitochondrial disorders.* Brain, 2001. **124**(Pt 5): p. 984-94.

144. Goto, Y., I. Nonaka, and S. Horai, *A new mtDNA mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS).* Biochim Biophys Acta, 1991. **1097**(3): p. 238-40.

145. Goto, Y., et al., *A new point mutation at nucleotide pair 3291 of the mitochondrial tRNA(Leu(UUR)) gene in a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS).* Biochem Biophys Res Commun, 1994. **202**(3): p. 1624-30.

146. Pulkes, T., M.G. Sweeney, and M.G. Hanna, *Increased risk of stroke in patients with the A12308G polymorphism in mitochondria.* Lancet, 2000. **356**(9247): p. 2068-9.

147. Noer, A.S., et al., *A tRNA(Lys) mutation in the mtDNA is the causal genetic lesion underlying myoclonic epilepsy and ragged-red fiber (MERRF) syndrome.* Am J Hum Genet, 1991. **49**(4): p. 715-22.

148. Silvestri, G., et al., *A new mtDNA mutation in the tRNA(Lys) gene associated with myoclonic epilepsy and ragged-red fibers (MERRF).* Am J Hum Genet, 1992. **51**(6): p. 1213-7.

149. Ozawa, M., et al., *Myoclonus epilepsy associated with ragged-red fibers: a G-to-A mutation at nucleotide pair 8363 in mitochondrial tRNA(Lys) in two families.* Muscle Nerve, 1997. **20**(3): p. 271-8.

150. Rossmanith, W., et al., *The expanding mutational spectrum of MERRF substitution G8361A in the mitochondrial tRNALys gene.* Ann Neurol, 2003. **54**(6): p. 820-3.

151. Tiranti, V., et al., *A novel mutation in the mitochondrial tRNA(Val) gene associated with a complex neurological presentation.* Ann Neurol, 1998. **43**(1): p. 98-101.

152. Verma, A., et al., *A novel mitochondrial G8313A mutation associated with prominent initial gastrointestinal symptoms and progressive encephaloneuropathy.* Pediatr Res, 1997. **42**(4): p. 448-54.

153. Bidooki, S.K., et al., *Intracellular mitochondrial triplasmy in a patient with two heteroplasmic base changes.* Am J Hum Genet, 1997. **60**(6): p. 1430-8.

154. Santorelli, F.M., et al., *Novel mutation in the mitochondrial DNA tRNA glycine gene associated with sudden unexpected death.* Pediatr Neurol, 1996. **15**(2): p. 145-9.

155. Santorelli, F.M., et al., *A novel mitochondrial DNA point mutation associated with mitochondrial encephalocardiomyopathy.* Biochem Biophys Res Commun, 1995. **216**(3): p. 835-40.

156. Corona, P., et al., *Novel heteroplasmic mtDNA mutation in a family with heterogeneous clinical presentations.* Ann Neurol, 2002. **51**(1): p. 118-22.

157. Mancuso, M., et al., *A novel mitochondrial tRNAPhe mutation causes MERRF syndrome.* Neurology, 2004. **62**(11): p. 2119-21.

158. Hanna, M.G., et al., *MELAS: a new disease associated mitochondrial DNA mutation and evidence for further genetic heterogeneity.* J Neurol Neurosurg Psychiatry, 1998. **65**(4): p. 512-7.

159. de Coo, I.F., et al., *A mitochondrial tRNA(Val) gene mutation (G1642A) in a patient with mitochondrial myopathy, lactic acidosis, and stroke-like episodes.* Neurology, 1998. **50**(1): p. 293-5.

160. Shtilbans, A., et al., *A novel mutation in the mitochondrial DNA transfer ribonucleic acidAsp gene in a child with myoclonic epilepsy and psychomotor regression.* J Child Neurol, 1999. **14**(9): p. 610-3.

161. Manfredi, G., et al., *Identification of a mutation in the mitochondrial tRNA(Cys) gene associated with mitochondrial encephalopathy.* Hum Mutat, 1996. **7**(2): p. 158-63.

162. Bataillard, M., et al., *Atypical MELAS syndrome associated with a new mitochondrial tRNA glutamine point mutation.* Neurology, 2001. **56**(3): p. 405-7.

163. Melone, M.A., et al., *Revelation of a new mitochondrial DNA mutation (G12147A) in a MELAS/MERFF phenotype.* Arch Neurol, 2004. **61**(2): p. 269-72.

164. Nakamura, M., et al., *A novel point mutation in the mitochondrial tRNA(Ser(UCN)) gene detected in a family with MERRF/MELAS overlap syndrome.* Biochem Biophys Res Commun, 1995. **214**(1): p. 86-93.

165. Jaksch, M., et al., *Progressive myoclonus epilepsy and mitochondrial myopathy associated with mutations in the tRNA(Ser(UCN)) gene.* Ann Neurol, 1998. **44**(4): p. 635-40.

166. Seki, A., et al., *Mitochondrial encephalomyopathy with 15915 mutation: clinical report.* Pediatr Neurol, 1997. **17**(2): p. 161-4.

167. Hsieh, R.H., et al., *A novel mutation in the mitochondrial 16S rRNA gene in a patient with MELAS syndrome, diabetes mellitus, hyperthyroidism and cardiomyopathy.* J Biomed Sci, 2001. **8**(4): p. 328-35.

168. Tang, J., et al., *Mutational analysis of mitochondrial DNA of children with Rett syndrome.* Pediatr Neurol, 1997. **17**(4): p. 327-30.

169. Campos, Y., et al., *Bilateral striatal necrosis and MELAS associated with a new T3308C mutation in the mitochondrial ND1 gene.* Biochem Biophys Res Commun, 1997. **238**(2): p. 323-5.

170. Blakely, E.L., et al., *LHON/MELAS overlap syndrome associated with a mitochondrial MTND1 gene mutation.* Eur J Hum Genet, 2005. **13**(5): p. 623-7.

171. Kirby, D.M., et al., *Mutations of the mitochondrial ND1 gene as a cause of MELAS.* J Med Genet, 2004. **41**(10): p. 784-9.

172. Taylor, R.W., et al., *Progressive mitochondrial disease resulting from a novel missense mutation in the mitochondrial DNA ND3 gene.* Ann Neurol, 2001. **50**(1): p. 104-7.

173. Deschauer, M., et al., *Late-onset encephalopathy associated with a C11777A mutation of mitochondrial DNA.* Neurology, 2003. **60**(8): p. 1357-9.

174. Lertrit, P., et al., *A new disease-related mutation for mitochondrial encephalopathy lactic acidosis and strokelike episodes (MELAS) syndrome affects the ND4 subunit of the respiratory complex I.* Am J Hum Genet, 1992. **51**(3): p. 457-68.

175. Liolitsa, D., et al., *Is the mitochondrial complex I ND5 gene a hot-spot for MELAS causing mutations?* Ann Neurol, 2003. **53**(1): p. 128-32.

176. Crimi, M., et al., *A missense mutation in the mitochondrial ND5 gene associated with a Leigh-MELAS overlap syndrome.* Neurology, 2003. **60**(11): p. 1857-61.

177. Santorelli, F.M., et al., *Identification of a novel mutation in the mtDNA ND5 gene associated with MELAS.* Biochem Biophys Res Commun, 1997. **238**(2): p. 326-8.

178. Tiranti, V., et al., *A novel frameshift mutation of the mtDNA COIII gene leads to impaired assembly of cytochrome c oxidase in a patient affected by Leigh-like syndrome.* Hum Mol Genet, 2000. **9**(18): p. 2733-42.

179. Kirby, D.M., et al., *Leigh disease caused by the mitochondrial DNA G14459A mutation in unrelated families.* Ann Neurol, 2000. **48**(1): p. 102-4.

180. Ravn, K., et al., *An mtDNA mutation, 14453G-->A, in the NADH dehydrogenase subunit 6 associated with severe MELAS syndrome.* Eur J Hum Genet, 2001. **9**(10): p. 805-9.

181. Keightley, J.A., et al., *Mitochondrial encephalomyopathy and complex III deficiency associated with a stop-codon mutation in the cytochrome b gene.* Am J Hum Genet, 2000. **67**(6): p. 1400-10.

182. Valnot, I., et al., *A mitochondrial cytochrome b mutation but no mutations of nuclearly encoded subunits in ubiquinol cytochrome c reductase (complex III) deficiency.* Hum Genet, 1999. **104**(6): p. 460-6.

183. Varlamov, D.A., et al., *Metabolic consequences of a novel missense mutation of the mtDNA CO I gene.* Hum Mol Genet, 2002. **11**(16): p. 1797-805.

184. Bruno, C., et al., *A stop-codon mutation in the human mtDNA cytochrome c oxidase I gene disrupts the functional structure of complex IV.* Am J Hum Genet, 1999. **65**(3): p. 611-20.

185. Manfredi, G., et al., *A new mutation associated with MELAS is located in a mitochondrial DNA polypeptide-coding gene.* Neuromuscul Disord, 1995. **5**(5): p. 391-8.

186. Santorelli, F.M., et al., *A T-->C mutation at nt 8993 of mitochondrial DNA in a child with Leigh syndrome.* Neurology, 1994. **44**(5): p. 972-4.

187. Harding, A.E., et al., *Prenatal diagnosis of mitochondrial DNA8993 T----G disease.* Am J Hum Genet, 1992. **50**(3): p. 629-33.