BCL-2 GENOTYPES AND OUTCOMES AFTER TRAUMATIC BRAIN INJURY

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Background: Mortality and morbidity of patients following traumatic brain injury (TBI) remains extremely high. TBI sets into motion a cascade of apoptotic events that includes bcl-2, which inhibits apoptosis. Patients with higher levels of bcl-2 protein after TBI appear to have smaller areas of ischemia and better functional outcomes as measured by Glasgow Outcome Scores (GOS).

Purpose: The purpose of this study was to examine the relationship between BCL-2 genotypes in patients with severe TBI and global functional outcomes, cognitive-behavioral outcomes, mortality, and biological/clinical data.

Methods: This pilot study was an ancillary study that examined biological/clinical markers in TBI patients and global functional and cognitive-behavioral outcomes after severe TBI (n=230). Nuclear DNA was extracted from CSF or blood. Based on HapMap, the DNA was analyzed for the genotypes of 17 high priority tagging single nucleotide polymorphisms (tSNPs) with a minor allele frequency ≥0.3 via TaqMan® allele discrimination. Biological/clinical data (bcl-2 protein levels, neurometabolites (lactate, pyruvate, and lactate pyruvate (LP) ratio) and cerebral blood flow (CBF) were analyzed following the first five to six (protein only) days post injury. Mortality and global functional outcomes [GOS & Disability Rating Scale (DRS)], analyzed at 3, 6, 12, and 24 months. The cognitive behavioral outcomes [Neurobehavioral Rating Scale – Revised (NRS-R), Trails A, and Trails B] were analyzed at 3, 6, and 12 months post injury.

Statistical analysis for BCL-2's relationship with neuropsychological outcomes and biological/ clinical data overtime utilized was mixed modeling, (mortality utilized generalized mixed modeling).

Results: There were 3 tSNPs of particular interest: Rs1801018: homozygous variant (GG) significant associated with decreased mortality (p=0.0055; OR=5.01), higher GOS (p=0.0004), lower DRS (p=0.0002), lower Global CBF (p=0.0031), [presence of the variant allele (G)] lower LP ratios (p=0.024); all better outcomes. Rs17756073: presence of variant allele (G) was associated with higher NRS-R (p=0.0331) and longer Trails B times (p=0.0516); both poorer outcomes. Rs7236090: homozygous wild type (CC) was associated with lower bcl-2 protein concentrations when analyzed without outliers (p=0.0056); the literature associates this with poor outcomes.

Conclusion: BCL-2 genotypes had a significant relationship with global functional outcomes, cognitive behavioral outcomes, bcl-2 protein concentrations, neurometabolites, and CBF.

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PREFACE

What three things does having a child and writing a doctoral dissertation have in common? For one, both have the same gestational period as a human (or an elephant). Second, each major revision is like a contraction, both get you closer to the miracle at the end, but are never the less painful and can take your breath away. And the closer the "D" day comes the closer and harder the "contractions". The third thing in common is that they both take a village to come to fruition. I would like to thank the many "village people" that labored with me to give birth to my doctoral dissertation.

To my dissertation committee member: Yvette P. Conley, Sheila Alexander, Dianxu Ren, and Amy Wagner, without their knowledge, expertise, and support this study would not have been possible.

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To the families of the TBI patients and the TBI patients who altruistically allowed us to join in their painful journey as to shed light on genetics and outcomes after severe TBI in order to help others.

To my peers, friends, and colleagues at Duquesne University (especially, Eileen Zungolo and Linda Goodfellow), for their unwavering interest and encouragement to complete this study.

To my parents and sister, our personal story has motivated me to explore genetics after neurotrauma but it is your encouragement, love, and support through all of my academic and life endeavors that had made this study possible.

To my daughter Francesca (you have been an angel) and baby boy (Michael Ryan) who is on the way (you have been such a trooper); you have given mommy perspective and balance.

To my husband Michael, at times I have feared that I have made you into a dissertation widow; you have been my biggest supporter throughout this journey.

To the Sigma Theta Tau Eta chapter, the Leslie Hoffman Award, and the Conley lab for the financial support to implement this study.

In memory of my friends who have suffered a severe TBI: Scott, Jennifer, and Kerrie who are doing remarkably well and to Gretchen and Julie who have passed on.

1.0 INTRODUCTION

1.1 FACTS AND FIGURES

There are 1.4 million people in the United States (U.S.) who seek medical treatment each year for a new traumatic brain injury (TBI) (Langlois, Rutland-Brown, & Thomas, 2006). Of these 51,000 will die and 235,000 are hospitalized due to their injuries. An estimated 1.1 million are treated and released from the emergency room) (Langlois et al., 2006). There are countless others who sustain a TBI, but never seek medical attention. As a consequence of TBI, 5.3 million Americans live with long-term or life long disabilities (125,000 new cases added each year) (Thurman, Alverson, Dunn, Guerri, & Sniezek, 1999). The high risk groups are males between the ages of 15-24 and people 75 years of age or older (Langlois et al., 2006; National Institute of Neurological Disorders and Stroke [NINDS], 2008). African American's have the highest death rate from TBI (Langlois et al., 2006). The leading causes of TBI are related to falls (28%), motor vehicle- traffic crashes (20%), being stuck by/against events (19%), and assaults (11%) (Langlois et al., 2006). Blast related injuries are the leading cause of TBI's in active military personnel (Langlois et al., 2006).

One year post injury, 40% of people who were hospitalized for TBI report having at least one unmet need for continued services (Corrigan, Whiteneck, & Mellick, 2004). These needs are largely in the neuropsychological domain; memory and problem solving, managing stress and

emotions, controlling temper and employment/ job skills issues (Corrigan et al., 2004). Outpatient neuropsychological rehabilitation is most often not covered under health care insurance (Brain Injury Association of America [BIAA], 2007).

1.2 PRIMARY VERSUS SECONDARY INJURY

There are two types of injury that occur with TBI. First, the primary injury produces direct mechanical damage injury that results in contusion, blood clots, and fractures (Zhang, Chen, Jenkins, Kochanek, & Clark, 2005). Second, a cascade of injury results from the response to the primary injury that can produce secondary injury such as ischemia, edema, anoxia, oxidative stress, and cytotoxins that damage cell membranes and disrupts the integrity of the blood brain barrier and walls of the cerebral vessels (Lovasik, Kerr, & Alexander, 2001; Marion, 2001; Ng et al., 2000; Nicoll, Roberts, & Graham, 1995; Zhang et al., 2005). These cellular level injuries result in vasogenic edema and hyperemia which contribute to the increase of intracranial pressure (ICP), which is associated with poor outcomes (Lovasik, et al., 2001; Marion, 2001). Prevention is the only way to "treat" primary injuries (Bullock et al, 1996; Lovasik, et al., 2001; Marion, 2001) and impacting secondary injury is the best approach to improving outcomes. The ability to decrease such secondary injury is dependent on improving understanding of the complex, interrelated physiologic processes that produce secondary injury after TBI.

There are two common forms of secondary injury neuronal death following TBI: necrosis and apoptosis (Zhang et al., 2005). There are distinct morphological and biochemical and differences between these two forms of cell death. In short, necrosis is an unnatural death and does not require any energy expenditure. There is total lysis of the cell membrane which

results in a significant inflammatory response which is a hallmark of necrosis (Leppert, 2006). In contrast, apoptosis is programmed cell death. It is process characterized by an orderly genetically encoded suicide program that requires energy. It is associated with the shrinking and condensation of the nucleus along with bleb formation on the cell membranes surface without loss of membrane integrity. A hallmark of apoptosis is phagocytosis which secrets cytokines that inhibits inflammation (Leppert, 2006; Fadok et al., 1992).

1.3 BCL-2

Bcl-2 (B-cell lymphoma 2) is an oncogene protein that inhibits apoptosis. Overexpression of bcl-2 protein in the central nervous system can prevent apoptosis in the presence of neurons that respond to nerve growth factor. Bcl-2 protein has been shown to limit anti-apoptotic effects in neurons that respond to ciliary growth factor (Graham, Chen & Clark, 2000). Damage to the mitochondria from cellular events related to the physical injury results in the release of apoptosis promoters (bax and bad), survival promoters (bcl-x and bcl-2) or a ratio of both (Graham et al., 2000). Research indicates that patients with higher levels of bcl-2 protein, in there cerebrospinal fluid (CSF) or cerebral tissues, are likely to have better functional outcomes because less cell death occurs (Clark et al., 1999; Clark et al., 2000; Ng et al., 2000; Nathoo et al., 2004). Viable neuronal tissue should be preserved due to the cellular survival benefit of bcl-2 protein (Clark et al., 1999; Clark et al., 2000; Ng et al., 2000; Nathoo et al., 2004). The relationship between BCL-2 genotypes and biological/ clinical variables and outcomes after TBI have not been reported in the literature.

1.4 APOPTOSIS

TBI sets into motion a cascade of apoptotic events and the bcl-2 oncogene family has been shown to play a key role in programmed cell death, apoptosis- cellular suicide (Bredesen et al., 2000; Garcia, Martinou, Tsujimoto, & Martinou, 1992; Hockenbery, Nunez, Milliman, Schreiber, & Korsmeyer, 1990; Kane et al., 1993; Mah et al., 1993; Myers et al., 1995; Nunez et al., 1990). After TBI, apoptosis can occur with in the site of injury and in distant regions days to weeks after the trauma.

There are two classic pathways of apoptosis; cysteine-dependent aspartate-specific protease "caspase" dependent and "caspase" independent apoptosis. A role of thirds is speculated to describe the types of cell death after TBI; one third is related to caspase dependent apoptosis, one third is associated with caspase independent cell death, and the last thirds is attributed to necrosis (Zhang et al., 2005). The Bcl-2 family regulates apoptosis/cell death or survival by regulating the permeability of the mitochondrial outer membrane and permeability transition pore (mPTP) formation.

1.4.1 Caspase Dependent Pathways

There are two branches of caspase dependent pathways; extrinsic and intrinsic. These two branches describe whether the apoptosis initiation is initiated by intracellular or intracellular signals.

1.4.1.1 Extrinsic Pathway

Extracellular signals, which initiate the extrinsic pathway, may include hormones, growth factors, nitric oxide, or cytokines. They are extrinsic because they cross the plasma membrane or transduce to effect a response. These signals may positively or negatively induce apoptosis (Mohamad et al., 2005). TNF is a cytokine produced mainly by activated macrophages and is the major extrinsic mediator of apoptosis. Most cells in the human body have two receptors for TNF: TNF-R1 ad TNF-R2. Binding to the TNF-R1 receptor can indirectly lead to the activation of transcription factors that are involved with cell survival. Following TNF-R1 activation a balance between pro-apoptotic and anti-apoptotic members of the bcl-2 family is established (Mohamad et al., 2005).

1.4.1.2 Intrinsic Pathway

Intracellular apoptotic signaling is a response initiated by a cell in response to stress; glucocorticoids, heat, radiation, nutrient deprivation, viral infection, and hypoxia. The initiation of the intrinsic pathway of caspase dependent apoptosis is triggered by stress on the cellular organelle (i.e. mitochondria, ER). In a healthy cell, the outer membranes of the mitochondria express the protein bcl-2 on the surface. Bcl-2 is bound to a protein Apaf-1. Internal damage in the cell causes bcl-2 to release Apaf-1 which results in cytochrome c leaking from the mitochondria into the cytosol. The release of cytochrome c and Apaf-1 bind to molecules of caspase-9. The resulting complex of cytochrome c, Apaf-1, caspase -9, and ATP is called the apoptosome (Liu et al., 1996). As apoptosomes aggregate in the cytosol, caspase-9 cleaves and activates other caspases, especially caspase 3. The sequential activation of one caspase by another creates an expanding cascade of proteolytic activity which leads to digestion of structural

protein in the cytoplasm, degradation of chromosomal DNA, and phagocytosis of the cell (Fiskum et al., 2000; Kluck, Bossy-Wetzel, Green, & Newmeyer, 1997; Yang, J. et al., 1997).

1.4.2 Caspase Independent Pathway

Bcl-2 is at the heart of the caspase independent apoptosis cascade because of its integral role in inhibiting mitochondrial membrane permeability (Tanaka, 2005). When bcl-2 is inhibited, mitochondrial membrane pores are allowed to open. This event in turn allows for apoptosis via the direct release or the indirect activation of mitochondrial and nuclear proteins (i.e. with apoptosis inducing Factor [AIF], endonuclease G [Endo G], ploy(ADP-ribose) polymerase [PARP] and p53.) These activators of apoptosis are independent of caspase cascades.

1.5 PURPOSE

The purpose of this study was to examine the relationship between BCL-2 genotypes in patients with severe TBI and global functional outcomes, cognitive-behavioral outcomes, mortality, and clinical data. In addition, demographic factors, extent of injury, apolipoprotein E (APOE) genotypes, documentation of adverse events in the field, and participation in a hypothermia protocol were examined as potential mediating variables.

1.6 SPECIFIC AIMS AND RESEARCH QUESTIONS

The Specific Aims and the Research Questions (RQ) to be answered by this study were:

1. Specific Aim 1: Compare the relationship between BCL-2 genotype and global functional outcomes, cognitive-behavioral outcomes, and mortality attained by patients who have sustained a severe TBI.

RQ1. Is there a relationship between BCL-2 genotype and global functional outcomes (GOS and DRS,), cognitive-behavioral outcomes (NRS-R, Trails A, and Trails B), and mortality attained by patients who have sustained a severe TBI?

2. Specific Aims 2: Compare the relationship between BCL-2 genotype and biological/ clinical data.

RQ2. Is there a relationship between BCL-2 genotype and biological/ clinical data (bcl-2 protein, neurometabolites [lactate, pyruvate, and lactate pyruvate (LP) ratio], and CBF [right hemisphere, left hemisphere, and global]) in patients who have sustained a severe TBI?

1.7 DEFINITION OF TERMS

1.7.1 Independent Variables

1.7.1.1 BCL-2 Genotypes

BCL-2 genotype was determined from DNA extracted from CSF or blood using TaqMan technology (the generic terms of XX, YY, or XY [heterozygous]).

1.7.1.2 Dichotomized BCL-2 Genotypes

BCL-2 was dichotomized based on the frequency of the genotype in the database which was being analyzed (i.e. mortality, Trails A, bcl-2 protein, neurometabolite, etc.). Therefore, the homozygous genotype with the least frequency was combined with the heterozygotes and compared against the homozygous with the largest frequency in the particular database being analyzed.

1.7.2 Dependent Variables

1.7.2.1 Global Functional Outcomes

Global functional outcome were determined by the neuropsychological evaluation measures of Glasgow Outcomes Scale (GOS) and Disability Rating Scale (DRS) at 3 months, 6 months, 12 months, and 24 months after injury. Mortality rate was defined as subject's death occurring at 3, 6, 12, and 24 months after injury. The 3 month mortality captures death during hospitalization.

1.7.2.2 Cognitive-behavioral Outcomes

Cognitive-behavioral outcomes were determined by the neuropsychological evaluation measures of Neurobehavioral Rating Scale- Revised (NRS-R) and Trails Making Tests (Trails A and Trails B) scores at 3, 6, and 12 months post injury.

1.7.2.3 Biological/ Clinical Data

Bcl-2 protein was determined from analyzing bagged cerebrospinal fluid (CSF) collected during the first 6 days post injury via enzyme-linked immunosorbent assay (ELISA) kit.

Neurometabolites: Lactate, pyruvate, and lactate/ pyruvate ratio (LP ratio). During the first 5 days post injury, serial fresh CSF specimens were collected. These specimens were frozen and batch processed for the analysis of neurometabolites (lactate [µmol concentration], pyruvate [µmol concentration] utilizing high pressure liquid chromatography (HPLC). Lactate/ pyruvate ratio was calculated from the ratio of the lactate to pyruvate.

Cerebral blood flow (CBF): CBF was used to determine cerebral perfusion. CBF is the volume of blood in milliliters that passes through the cerebral vessels/100 gm brain tissue with in 1 minute. Right hemisphere, left hemisphere, and global CBF as determined by the enhanced xenon/computed tomography (Xe/CT) scan method.

1.7.3 Covariates

1.7.3.1 Age

Age was utilized as a continuous variable with a range of 16-75 years of age for the inclusion criteria.

1.7.3.2 Race

Race was dichotomized into Caucasians versus non-caucasians.

1.7.3.3 Gender

Gender was defined by standard anatomical terms of male versus female.

1.7.3.4 Glasgow Coma Scale (GCS)

Severe TBI was operationally defined as the admission GCS score of ≤8. GCS was dichotomized into two levels of severe coma 3-5 (more severe) versus 6-8.

1.7.3.5 Hypoxia

Hypoxia was defined as an oxygen saturation saO_2 of <90% for >30 minutes in the field or during the admission process.

1.7.3.6 Hypotension

Hypotension was defined as sustained systolic blood pressure <90 mmHg for >30 minutes in the field or during the admission process.

1.7.3.7 Hypothermia

Hypothermia was defined as the presence or absence of being enrolled in a randomized control clinical trial for a hypothermia intervention and receiving an intervention.

1.7.3.8 Seizure

Seizure was defined as a witnessed seizure in the field or during the admission process. This information was documented and tracked by research nurse collecting data based on field and emergency room documentation.

1.7.3.9 APOE

APOE genotype/haplotype (APOEε2, APOEε3, APOEε4) was determined by using Restriction Fragment Length Polymorphism (RFLP) technique, polymerase chain reaction (PCR)

and visualization under light. APOE was operationally defined as the presence or absence of APOEε4.

1.8 CONCEPTUAL FRAMEWORK

The conceptual framework designed for this study is represented in figure 1-1. It is driven by the Wilson Cleary Model of Health Related Quality of Life (Wilson & Cleary, 1995) (See Appendix A). This study focused on how the biological/ physiological variation of BCL-2 genotype influenced outcomes directly and symptom status as directly represented by Bcl-2 protein levels, neurometabolite concentrations (lactate, pyruvate, and LP ratio), CBF, and APOEε4, and how BCL-2 genotypes influenced these factors which then influenced global functional and cognitive-behavioral outcomes. The parent data set did not have general health perceptions or quality of life data available at the time which these analyses were conducted. Subsequent studies will address these issues.

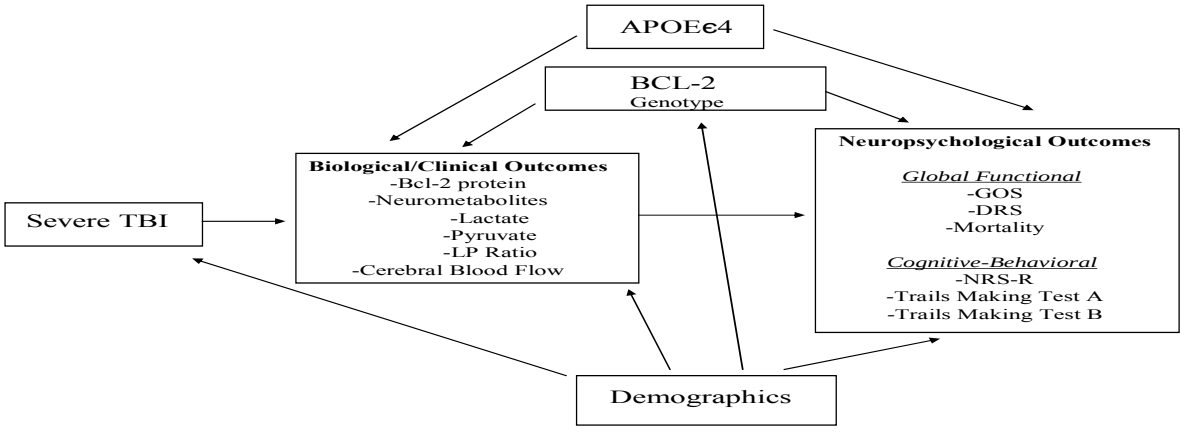


Figure 1-1: Conceptual Framework

2.0 BACKGROUND AND SIGNIFICANCE

2.1 TRAUMATIC BRAIN INJURY

2.1.1 Facts and Figures

There are 1.4 million people in the U.S. who seek medical treatment each year for a new TBI (Langlois et al., 2006). Of these 51,000 will die and 235,000 are hospitalized due to their injuries. An estimated 1.1 million are treated and released from the emergency room (Langlois et al., 2006). There are countless others who sustain a TBI, but never seek medical attention. As a consequence of TBI, 5.3 million Americans live with long-term or life long disabilities (125,000 new cases added each year) (Thurman et al., 1999). The high risk groups are males between the ages of 15-24 and people over 75 years of age (Langlois et al., 2006; NINDS, 2008). African American's have the highest death rate from TBI (Langlois et al., 2006). The leading causes of TBI are related to falls (28%), motor vehicle- traffic crashes (20%), being stuck by/against events (19%), and assaults (11%) (Langlois et al., 2006). Blast related injuries are the number one cause of TBI's in active military personnel (Langlois et al., 2006). Approximately half of all TBI's events involve alcohol (NINDS, 2008).

One year post injury, 40% of people who were hospitalized for TBI report having at least one unmet need for continued services (Corrigan et al., 2004). These needs are largely in the

neuropsychological domain; memory and problem solving, managing stress and emotions, controlling temper and employment/ job skills issues (Corrigan et al., 2004).

TBI's cost the U.S. $60 billion annually related to hospitalization and fatal TBI's. This figure underestimates the personal and life time costs of living with the sequelae after TBI (BIAA, 2006). Currently the state of the science in the acute care treatment of patients with severe TBI is supportive care with focus on maintaining low intracranial pressure as to avoid intracranial hypertension and maintaining adequate cerebral perfusion pressure (Bullock et al., 1996; Nolan, 2005; Vincent & Berré, 2005).

2.1.2 Primary Versus Secondary Injury

There are two types of injury that occur with TBI; primary and secondary injury. First, the primary injury produces direct mechanical damage injury that results in contusion, blood clots, and fractures (Zhang et al., 2005). Primary injury can be further classified by focal and diffuse injuries. Focal injuries are a result of direct injury contact; contusions, epidural hematomas, subdural hematomas, subarachnoid and intracranial hemorrhages. Diffuse injuries are injuries that are remote from the injury site, noncontact or injuries by rotational force; concussions and diffuse axonal injuries (Gennarelli et al., 1998; Meaney et al., 1995; Nolan, 2005). Prevention is the only way to "treat" primary injuries (Lovasik, et al., 2001; Marion, 2001; Nolan, 2005; Vincent & Berré, 2005). A cascade of injury results from the response to the primary injury that can produce secondary injury such as ischemia, edema, anoxia, oxidative stress, and cytotoxins that damage cell membranes and disrupts the integrity of the blood brain barrier and walls of the cerebral vessels (Lovasik et al., 2001; Marion, 2001; Ng et al., 2000; Nicoll et al., 1995; Zhang et al., 2005). These cellular level injuries result in vasogenic edema

and hyperemia which contribute to the increase of ICP, which is associated with poor outcomes (Lovasik, et al., 2001; Marion, 2001). TBI can result in neuronal loss in cortex, hippocampus (Kotapka, Graham, Adams, & Gennarelli, 1992), cerebellum and thalamus regions of the brain (Zhang et al., 2005; Raghupathi, 2004). The ability to decrease such injury is dependent on improving understanding of the complex, interrelated physiologic process that produce secondary injury after TBI.

Functional outcomes following severe TBI are likely to be dependent upon several factors including: primary and secondary cascades of injury factors such as apoptosis and BCL-2 activity, demographics, biological/clinical data.

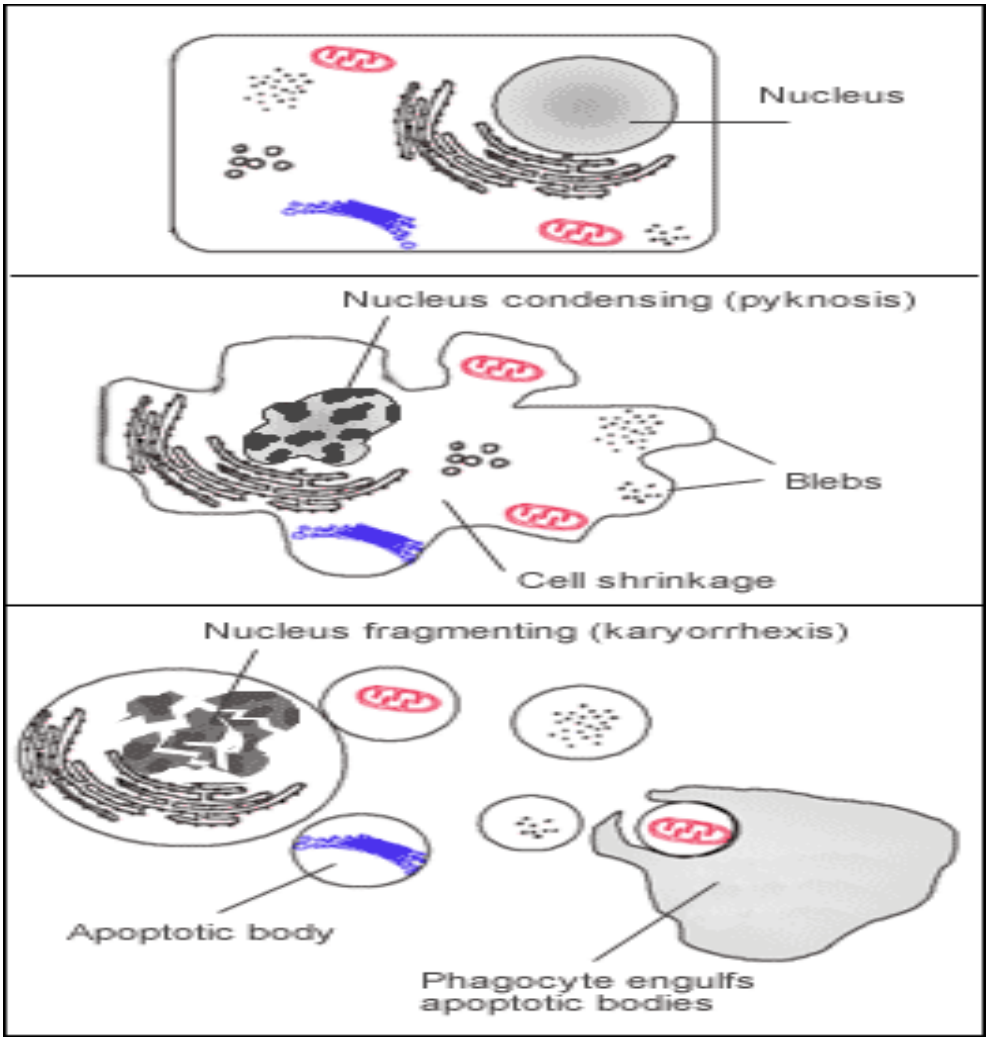
2.1.3 Secondary Injury: Necrosis versus Apoptosis

There are two common forms of neuronal death following TBI: necrosis and apoptosis (Zhang et al., 2005). There are distinct morphological and biochemical differences between these two forms of cell death.

Necrosis is defined as unnatural death of cells and living tissue. Necrosis can occur with mechanical injury as well as viruses, hypothermia, hypoxia, ischemia, and metabolic poisons. A series of morphological characteristic changes occur. The cell and the organelles (i.e. mitochondria) have irreversible swelling (disintegration) related to impaired ability to control plasma membrane permeability. Total lysis occurs with cell membrane breakdown allows cell contents leak out which is related to inflammation of surrounding tissues (Leppert, 2006; Proskuryakov, Konoplyannikov, & Gabai, 2003). On the biochemical level, in necrosis there is a loss of regulation of ion homeostasis. There is no energy requirement for necrosis; it is a passive process. There is random digestion of DNA and postlytic DNA fragmentation. Necrosis

affects groups of contiguous cells. A significant inflammatory response is a hallmark of necrosis (Leppert, 2006; Proskuryakov et al., 2003).

Apoptosis also know as programmed cell death, is an orderly genetically encoded suicide program. Cells that undergo apoptosis have morphological characteristic features that differ from necrosis. Apoptosis begins with cell cytoplasm shrinking and condensation of the nucleus. The mitochondria release cytochrome c, the cell membranes develop bubble like blebs on their surface without loss of membrane integrity. The chromatin (DNA and protein) in the nucleus degrade. The cell breaks into small, membrane wrapped, fragments also know as apoptotic bodies. The mitochondria membranes become leaky due to pore formation related to bcl-2 family proteins and allow pro-apoptotic factors to leak out feeding the cascade of events. Phospholipids and phosphatidylserines are translocated from hidden with in the plasma membrane to the exposed extracellular surface. Phagocytic cells, macrophages, and dendritic cells, bind to the phosphatidylserine and then engulf the cell fragments. Phagocytosis may also occur by neighboring cells. These phagocytic cells then secrete cytokines that inhibit inflammation; the absence of inflammation is a hallmark of apoptosis (Fadok et al., 1992; Leppert, 2006). (Refer to figure 2-1).



from http://en.wikipedia.org/wiki/apoptosis

Figure 2-1: Events of Cellular Apoptosis

Biochemically, apoptosis is a tightly regulated process involving activation and enzymatic steps. Apoptosis is energy dependent, requiring ATP. The fragmentation of the genomic DNA is an irreversible event that commits the cell to die and occurs before changes in plasma membrane permeability (pre-lytic DNA fragmentation). DNA fragmentation has been shown to result from activation of the endogenous Ca2+ and Mg2+ -dependent nuclear endonucleases. This enzyme cleaves DNA at sites located between nucleosomal units (linker DNA) generating non-random mono- and oligonudeosomal DNA fragments. Leaky

mitochondria release cyctochrome C and AIF into the cytoplasm. The caspase cascade is activated. Apoptosis affects individual cells and is induced by physiological stimuli (i.e. lack of growth factors and changes in hormonal environment). Apoptosis can be a normal regulatory process that is necessary is development, tissue turnover, atrophy induced by endocrine stimuli, negative selection in the immune system, and T-cell killing. Apoptosis also accounts for cell deaths related to exposes from cytotoxic compounds hypoxia and viral infections (Bredesn, 2000; Elddah & Faden, 2000; Kroemer, 2003; Kromer & Reed, 2000).

Apoptosis occurs when there is a withdrawal of a positive signal for cell survival (nerve growth factor and Interleukin-2) and the receipt of negative signals (increased levels of oxidants within the cell, damage to the DNA by the oxidants, UV light, x-rays, chemotherapeutic drugs, binding of death activators (TNF-a, TNF-b, FasL) to cell surface receptors (TNF and Fas [aka CD95]) (Bredesn, 2000; Elddah & Faden, 2000; Kroemer, 2003; Kromer & Reed, 2000). In TBI apoptosis can occurs with in the site of injury and in distant regions days and weeks after trauma.

2.2 BCL-2 IN APOPTOSIS

2.2.1 Overview of Apoptosis in TBI

The bcl-2 oncogene family has a key role in programmed cell death, apoptosis. An overview of the apoptosis cascade illustrates the complexity of the apoptosis cascade with direct attention on bcl-2 (Lu, Ashwell et al, 2000). The caspase dependent and independent cascades will be discussed at length in sections (Section 2.3).

After the primary TBI assault three cascades of events transpire to define secondary injury. First, primary injury leads to edema, cerebral ischemia and tissue anoxia. Second, the blood brain barrier and the cell membranes are injured by the initial injury. The third branch of the cascade results in excitotoxin release from the hyperactive and derogate cells (Nicoll et al, 1995; Lu, Ashwell et al, 2000; Ng et al, 2000).

Primary injury leads to edema, cerebral ischemia and tissue anoxia. This decreases cell energy supply, thus decreasing ion pumping and increasing sodium and calcium in the cells which causes mitochondrial damage and activates the bcl-2 family of oncogenes. As the cell energy supply decreases, there is a decrease in antioxidant activity which results in the release of free radicals. The consequence is damage to cellular macromolecules and activation of caspase and calpain that ultimately leads to cell death. Mitochondrial damage further increases tissue edema, ischemia and anoxia and further promotes this cascade. Mitochondrial damage will either activate the apoptosis promoters, bax and bad or survival promoters, bcl-2 and bcl-xL. If the apoptosis promoters are activated, cytochrome c is released which activates caspase and calpain and subsequent cell death (Lu, Ashwell et al, 2000).

The second cascade involves the blood brain barrier and the cell membranes are being injured by the initial injury. The disruption of tissue integrity results in an increase in microglial activation, proinflammatory cytokines and caspase/ calpain activation and then cell death. Disruption in the blood brain barrier and cell membranes also results in the release of free radicals which damage macromolecules activate caspase and calpain and lead to cell death. Increase in free radicals also further breaks down the blood brain barrier and cell membranes which perpetuate the cascade of events. At the same time, injuries to the blood brain barrier and cell membrane result in edema, tissue ischemia and anoxia which feed the first cascade of events,

including the activation of the bcl-2 family. This second cascade is a key stone because it also feeds the third cascade (Lu, Ashwell et al, 2000).

In the third cascade, excitotoxins are released from the hyperactive and derogated cells. This leads to an increase in extracellular glutamate and increase in sodium and calcium in the cells. Nitric oxide synthesis activation results in free radical release, phospolipase, endonuclease and protein kinase activation which leads to further damage to the cellular macromolecules which activate caspase and calpain and hence cell death. The increase of intracellular calcium and sodium can directly activate caspases and calpains, and initiate subsequent cell death (Lu, Ashwell et al, 2000).

2.2.2 Bcl-2 Structure

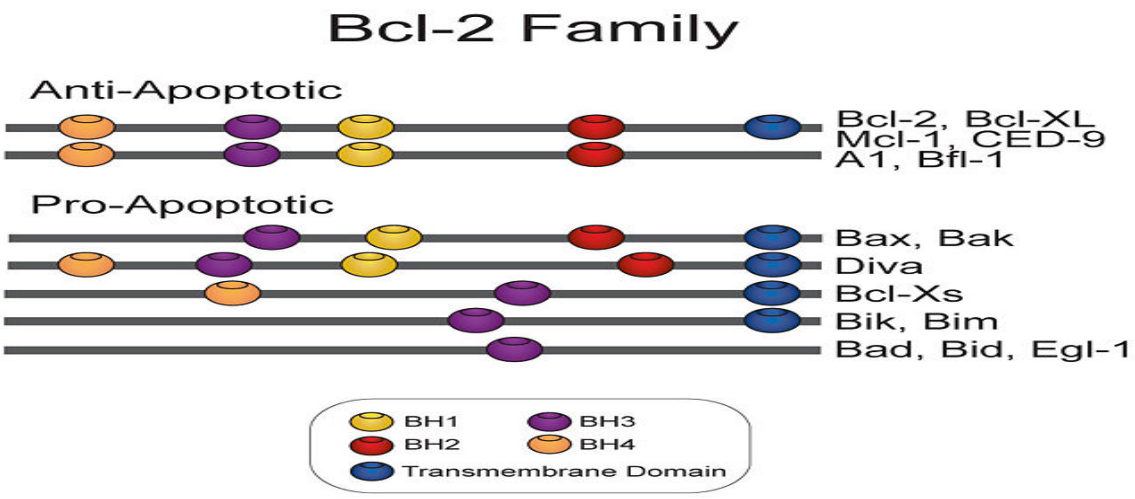
BCL-2 (B-cell lymphoma 2) specifically, is a protoncogene that inhibits apoptosis; it is a survival promoter. It is located with in the mitochondrial membrane, endoplasmic reticulum, and nuclear envelope (Lithgow, van Driel, Bertram, & Strasser, 1994). The normal chromosomal position for BCL-2 is on18q21 (Tsujimoto, 1984). BCL-2 is encoded by a 230kb gene that results in a 22-26 kDa protein. Bcl-2 protein has approximately 239 amino acids (196, 034 bases) **(**Genecards, 2007). Bcl-2 is currently known to have three exons (GenomeUSCS, July 2008) with 646 known SNP's and 36 tagging SNP's (HapMap, April 2007). The overexpression of Bcl-2 is associated with G1 arrest in the cell cycle (Chen, Gong, & Almasan, 2000). The Bcl-2 family has a general structure that consists of a hydrophobic helix surrounded by seven amphipathic helices (Figure 2-2).

from http://en.wikipedia.org/wiki/Bcl2

Figure 2-2: BCL-2 Crystal structure

2.2.3 Bcl-2 Family Members

There are two camps in this Bcl-2 family of protoncogenes; survival promoters and death promoters. The membership to each camp is based on the presence or absence of four conserved BCL-2 homology (BH) domains designated as BH1, BH2, BH3, and BH4. Each of the BH domains corresponds to helical segments and is crucial for functioning. (Refer to Figure 2-3).



from http://en.wikipedia.org/wiki/BCL-2

Figure 2-3: Bcl-2 Family

The BH's are essential for homocomplex and heterocomplex formation. All of the mammalian BCL-2 family survival promoter members (bcl-2, bcl-xl, bcl-w, and mcl-1) display sequence conservation in all four BH domains. BH-4 is essential for the coding of pro-survival proteins. BH4 blocks the VDAC channel, which in turn blocks the release of cytochrome c (Shimizu, Ide, Yanagida, & Tsujimoto, 2000). Bcl-2 family members who are cell death promoter are subdivided into Bax-like and BH3 only groups. All have the absence of BH-4 or

have the sole presence of BH-3 homolog domains. The bax-like group (bax, bak, Bok) has a structure similar to bcl-2 and binds to bcl-2 (Adams & Cory, 2007). The BH-3 only group (bid, bad, bim, or bik) have sequences that are unrelated to bcl-2 or each other. Bcl-xs is a death promoter possessing both BH-3 and -4, because of the absence of BH 1and 2 negates the survival ability associated with BH-4 (Graham et al., 2000). Complexes with BH3 domains bax, truncated bid (tbid), and bad facilitate the release of cytochrome c via the pore forming capabilities of BH3. Pro-survival proteins (bcl-2, bcl-xl, and mcl-1) are capable of preventing the release of mitochondrial proteins (i.e. cytochrome *c*, endonuclease G, and AIF) through the pores (Zhang et al., 2005). For example, in the absence of survival factors, BH3 binds and inactivates bcl-2 and /or bcl-xl in the outer mitochondrial membrane. Bad is then dephosphorylated, thus promotes apoptosis. In the presence of cell survival factors a series of kinases are activated. Bad is then phosphorylated, which dissociates itself from Bcl-2 and/ or Bcl-xl allowing for survival (Mohamad et al., 2005; Zha, Aime-Sempe, Sato, & Reed, 1996). Heterodimerization is not required for pro-survival function but it is essential for the pro-death function of the BH3 only group of proteins (Chittenden et al., 1995).

There transmembrane (Tm) domain is involved in anchoring both survival and death promoter proteins of the Bcl-2 family to the mitochondria (Graham et al., 2000). All members of the Bcl-2 family have a Tm domain excluding bid.

2.2.4 The Interaction of Anti- and Pro-Apoptosis Family Members

The process by which anti- and pro- apoptosis family members interact has yet to be fully elucidated. The literature supports that BH-3 only pro-apoptotic proteins can not directly activate apoptosis and that they act upstream of bax-like family members to ultimately activate them (bax

and bak) and induce apoptosis. Adams and Cory (2007) review the two common theories apoptotic activation: 1) direct versus indirect activation and 2) regulation.

Direct activation is a process by which BH-3 only "activators" (i.e. bim and tbid) bind to bax-like family members (bax and bak) thus triggering apoptosis. Other BH-3 only family members (i.e. bad) are "sensitizers" and displace bim and tbid from pro-survival proteins and then bim and tbid activate bax and bak. (See figure 2-4a).

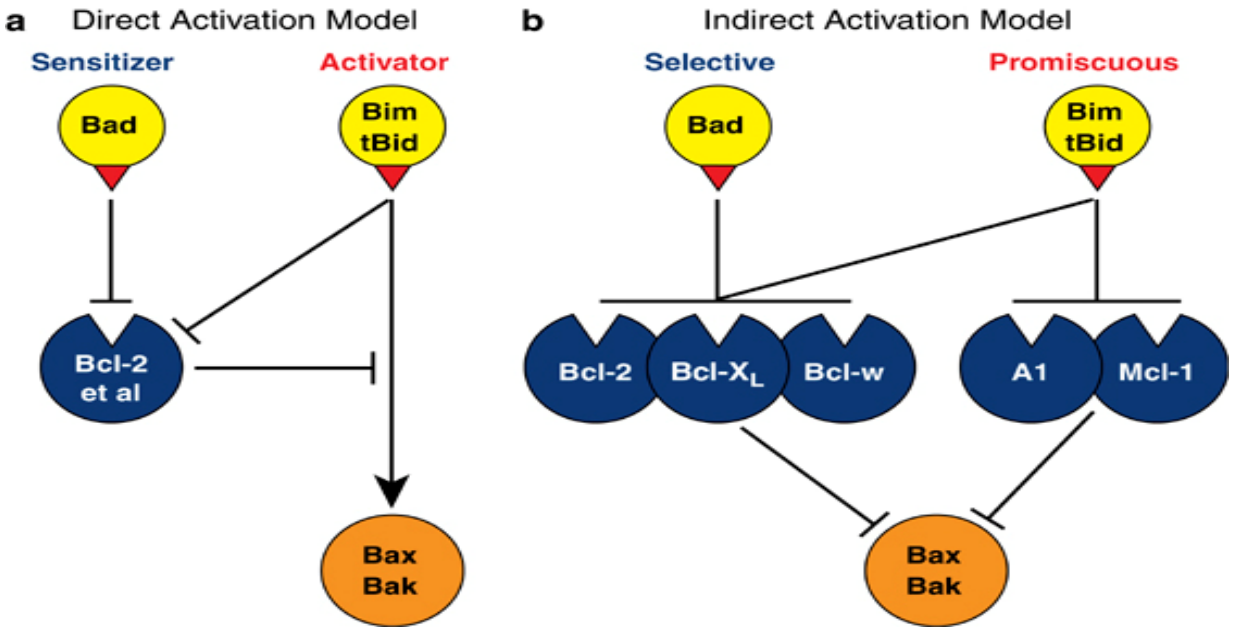


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Figure 2-4: Activation of Bcl-2 by Pro-Apoptotic Family Members

Indirect activation of apoptosis proposes that BH-3-only proteins guard specific ("selective") pro-survival proteins and that bad "guards" bcl-2, bcl-xl, and bcl-w. Bim and tbid are potent "promiscuous" pro-apoptotic proteins and can bind to a greater range of anti-apoptotic proteins (bcl-2, bcl-xl, bcl-w, A1, and mcl-1) and therefore are able to either engage or neutralize the pro-survival proteins and prevent them from counteracting bax or bak. (See figure 2-4b).

The regulation of bax-like family members (bak and bax) which are proported to lead to apoptosis are mediated by BH-3 only proteins. Bak regulation in a healthy cell involves bcl-xl and mcl-1 anti-apoptosis proteins. When bak is "primed" or bound to a BH-3 only family member it is then also able to bind to the anti-apoptotic family member (i.e. Bcl-x_L) and consequently is able to inhibit survival. Apoptosis occurs when BH-3 only protein remains bound to the anti-apoptosis family member, liberates bak. Free primed bak merge in an oligomer formation. This in turn elicits the permeabilization of the outer mitochondrial membrane and allows the release of cytochrome c. (See figure 2-5a).

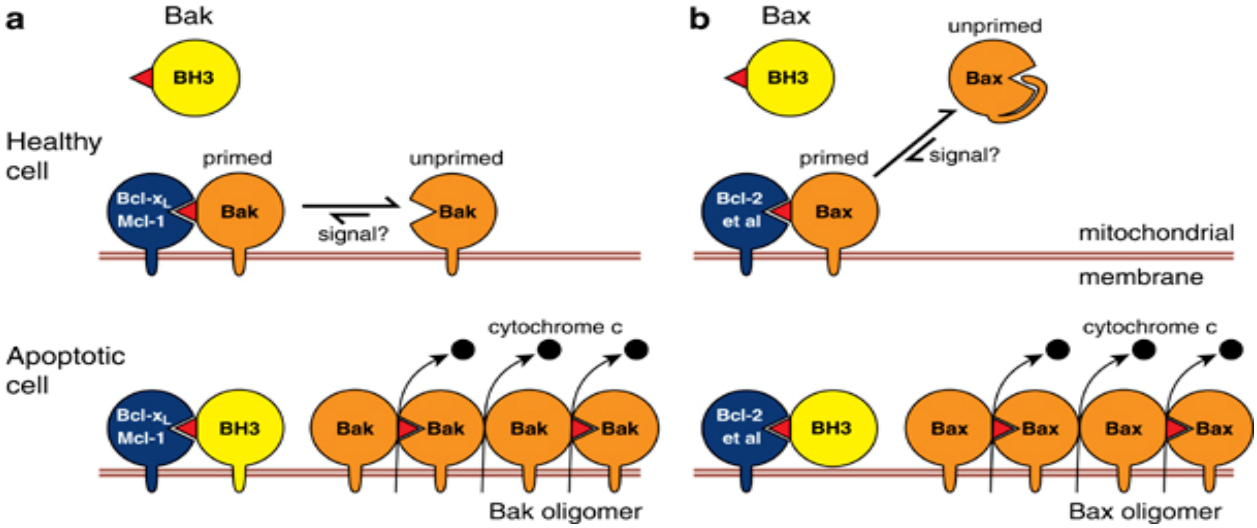

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Figure 2-5: Regulation of Bax-like Pro-Apoptotic Family Members

Bax regulation in a healthy cell involves bcl-2, bcl-w, A1 and bcl-b anti-apoptosis proteins. Like, bak, when bax is "primed" or bound to a BH-3 only family member it is also able to bind to the anit-apoptotic family member (i.e. Bcl-2). Apoptosis occurs when BH-3 only protein remains bound to the anti-apoptosis family member, liberates bax. Free primed bax merge in an oligomer formation. This in turn elicits the permeabilization of the outer mitochondrial membrane and allows the release of cytochrome c. In addition, unprimed bax is in

the cytosol and the anti-apoptotic family members are able to inhibit bax activation. (See figure 2-5b).

2.2.5 Isoforms

There are two isoforms known for the bcl-2 protein, with alternative splicing; bcl-2α and bcl-2ß (Akgul, Moulding, & Edwards, 2004). Bcl-2α is reported to have 239 amino acids, is 26kDa in size, have BH 1, 2, 3, 4, and TM homolog domains and are active in the mitochondria (Akgul et al., 2004). Bcl-2ß has 205 amino acids, is 22 kDa in size, have BH 1,2, 3, and 4 homolog domains and are active in the cytoplasm (Akgul et al., 2004).

The human Bcl-2 protein consists of BH domains, the predicted α-helical segments, and a transmembrane (TM) anchor. The first α-helix is associated with the binding of Raf-1 and calcineurin. A long flexible loop between the first and second α-helices is required for Bcl-2 phosphorylation and may represent a negative-regulatory domain. The second α-helix is associated with BH3 domain and plays the role is binding during dimerization (ligand domain) of Bcl-2 family proteins. The fifth and sixth α-helices are predicted to participate in channel formation by penetrating lipid bilayers (Reed, 1997).

Bcl-2 over-expression promotes survival when apoptosis is induced by: deprivation of nerve growth factor in sympathetic neurons, serum and growth factor withdrawal, calcium ionophore A23187, glucose withdrawal, and membrane perioxidation free radical (Garcia et al., 1992, Kane, 1993; Mah, 1993, Zhong et al., 1993). Bcl-2 was not found to exhibit survival when apoptosis is induced by cytotoxic T-cells (Vaux, Aguila, & Weissman, 1992), TNF

(Vanhaesebroeck et al., 1993), B-cell lines (Cuende et al., 1993), and neurons that are dependent upon ciliary neurotrophic factor (Allsopp, Wyatt, Paterson, & Davies, 1993).

2.2.6 Mitochondrial Role in Apoptosis

The mitochondria play a pivotal role in apoptosis. (See figure 2-6). The mitochondria is a cellular organelle that contains two membranes; inner and outer. The inner membrane surrounds the mitochondrial matrix. It is highly folded to form cristae, molecular complexes of the electron transport chain. This electron transport chain generates the hydrogen ion gradient that is essential to make ATP. Cellular ATP is produced by oxidative phosphorylation (Harris & Thompson, 2000). Homeostasis which maintains intracellular ATP/ADP ratio is a result of the free exchange of substrates and ATP/ADP between the mitochondria and the cytosol (Harris & Thompson, 2000). The outer mitochondrial membrane surrounds the inner mitochondrial membrane. This intermembrane space holds proteins specific to apoptosis promoting proteins (cytochrome c, AIF, pro-caspases). Transport across the outer membrane is less tightly controlled than transport across the inner mitochondrial membrane (Harris & Thompson, 2000). The voltage dependent anion channel (VDAC) is the most common protein in the outer membrane and is permeable to molecules less than 5000 daltons making the outer membrane highly permeable (Mannella, 1992). The outer membrane is impermeable to holo-cytochrome c. The ability to sequester cytochrome c/ holo-cytochrome c is essential for cell survival (Yang, 1997; Harris & Thompson, 2000). The release of cytochome c leads to caspases activation in the apoptosis cascade (Cai, Yang, & Jones, 1998; Skulachev, 1998).

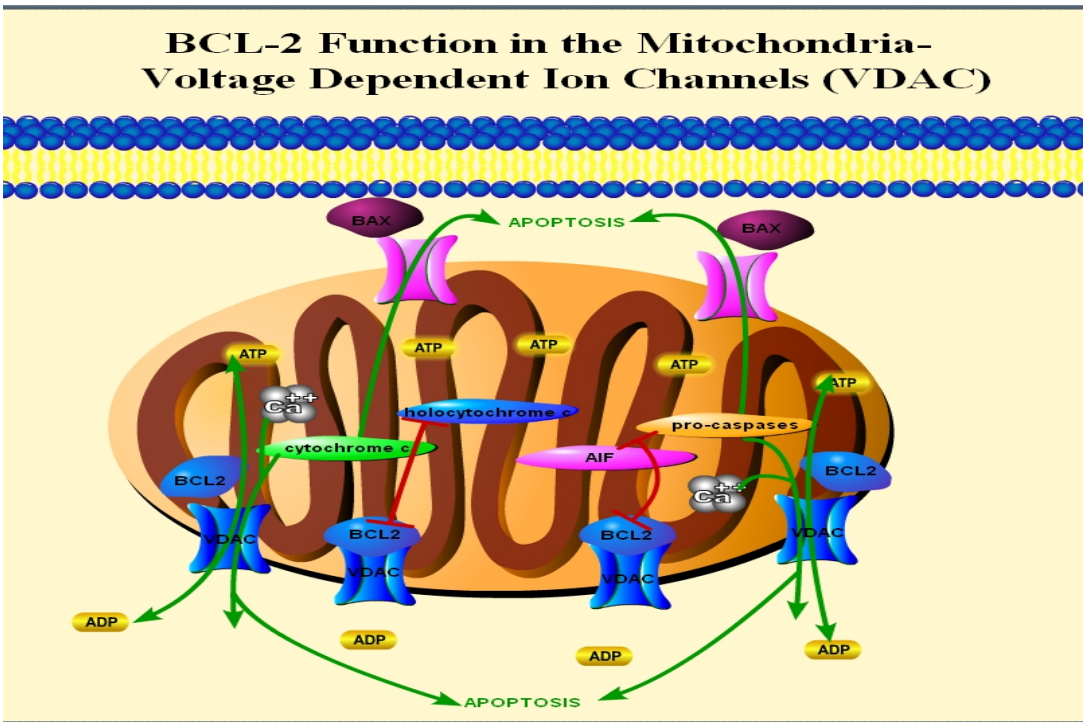

Figure 2-6: Bcl-2 Function in the Mitochondria

VDAC and Bcl-2 family proteins reside in the outer membrane of the mitochondria. Bcl-2 family of proteins modulates ATP/ADP via the VDAC. VDAC can exist in open or closed formation (Vander Heiden et al., 2000). When VDAC is closed ATP/ADP and other metabolic anions can not pass from the cytosol through the outer membrane. In the open formation, passage is granted, but the BCL-2 family of proteins are the gate keepers that maintain ATP/ADP exchange. Therefore hyperpolarization of the mitochondria can be impeded by bcl-2, preventing swelling and rupture that leads to the release of cytochrome c and ultimately prevents cell death (Rostovtseva & Colombini, 1996; Vander Heiden et al., 2000; Harris & Thompson, 2000).

Changes in ADP/ATP ratio have been used to differentiate the different modes of cell death and viability. Increased levels of ATP and decreased levels of ADP are associated with proliferating cells. In cell death there is a continuum where the type of cell death that ensues is

related to the severity of the decrease in ATP and increase in ADP. Apoptotic cells have decreased levels of ATP and increased levels of ADP. Where as necrosis has a pronounced decrease in levels of ATP and increase in levels of ADP (Leppert, 2006).

2.2.7 Mitochondrial Membrane Potential Regulation by Bcl-2

Mitochondria regulate cell death mediated by Bcl-2 family of proteins. Bax and Bak are associated with mitochondrial membrane permeabilization (MMP). Alterations in MMP are early events in the cell death process preceding other non-mitochondrial signs of apoptosis (Kroemer, 2003). During apoptosis several mitochondrial events occur. There is a loss of mitochondrial membrane potential ($\Delta\Psi m$)[Delta Psim] (electrochemical gradient) across the inner membrane, result in uncoupling of oxidative phosphorylation, generation of superoxide free radicals, and dumping of matrix associated Ca^{2+} into the cytosol (Jurgensmeier et al., 1998; Kim, 2005). During apoptosis the $\Delta\Psi m$ across the mitochondrial membrane collapses; this collapse commits a cell to die (Kroemer, 2003).

The induction of cellular apoptosis is dependent on mitochondrial permeability transition pores (mPTP) (Kroemer & Reed, 2000). The collapse maybe associated to the formation of pores in the mitochondria by dimerized Bcl-2 family cell death promoters; Bax or activated Bid, Bak, or Bad proteins (Jurgensmeier et al., 1998; Kroemer & Reed, 2000; Leppert, 2006). The overexpression of Bcl-2 in cells has been reported to prevent all of the following: the loss of $\Delta\Psi m$, release of cytochrome *c*, and activation of caspases which are ultimately responsible for apoptosis (Jurgensmeier et al., 1998).

Mitochondria play a significant role in cerebral energy metabolism and cellular Ca^{2+} homeostasis. Mitochondrial alterations in respiration and respiratory control are apparent 1 hour

to 14 days after controlled cortical impact injury (Fisksum, 2000). When neurons are exposed to excitotoxic levels of excitatory neurotransmitters, it results in the accumulation of Ca^{2+} in the mitochondria, leading to mitochondrial dysfunction and delayed cell death (Fiskum, 2000). Compromised ability for respiration and phosporylation affects the mitochondria's ability to sequester Ca^{2+}, which can contribute to cell death related to lowering the $\Delta\Psi m$ and contributes to osmotic swelling and lysis (Fiskum, 2000). The accumulation of Ca^{2+} can also result in the increase of the inner MMP the promotion of pro-apoptotic proteins (i.e. cytochrome c) during the exposure to toxic levels of excitatory neurotransmitters. Excessive mitochondrial Ca^{2+} related to excitotoxic cell death (Fiskum, 2000). The increase in Ca^{2+} is related to bax/bak activity in the ER of the apoptosis cascade. Bcl-2 on the ER can interfere with calcium –mediated death signals (Thomenius & Distelhorst, 2003).

2.2.8 Bcl-2 Family's Mechanism of Anti- and Pro- Apoptosis

The exact mechanism(s) by which the Bcl-2 family of proteins either promote cell death or promote cell survival has yet to be definitively elucidated. Bhar and colleagues (2000) reviews the four common theories to explain how cytochrome c may escape from the intermembrane space in the mitochondria through the outer membrane of the mitochondria into the cytsosol.

1. Ca^{2+} concentration, pH, voltage or redox state influence whether the PTP are open or closed.

Mitochondrial depolarization in apoptosis is associated with the permeability transition (PT) goes through the permeability transition pore (PTP) opening of the mitochondria matrix. As a result of these open pores, there is osmotic swelling in the inner membrane of the mitochondria which in turn rupture the outer membrane of the mitochondria and release cytochrome c and subsequent cell death (Susin et al., 1998). The pore structure itself is theorized to have two

components adenylate translocator (ANT) that is necessary for ATP/ADP exchange and VDAC which is regulated by the Bcl-2 family; Bcl-xL (pro-survival) and Bax (pro-death).

2. Cytochrome c release via Bax expression in VDAC

3. Bax heterodimers and other pro-death Bcl-2 family members form channels in the outer membrane of the mitochondria that allow cytochrome c to release. The formation of these channels is believed to be a result of the pro-survival Bcl-2 family members inhibiting the polymerization of Bax, lending itself to heterodimer formation and subsequent pore channels.

4. If PTP is closed during apoptosis then ATP/ADP exchange blocked and accumulates in the mitochondria. The lack of ADP renders phosporylation ineffective. The inner membrane swells (distends), the outer membrane of the mitochondria ruptures and cytochrome c is then released.

2.3 BCL-2 FAMILY AND APOPTOSIS CASCADES

The Bcl-2 family of protoncogenes plays an integral role in the regulation of apoptosis (Bredesen, 2000; Garcia et al., 1992; Hockenbery et al., 1990; Kane et al., 1993; Mah et al., 1993; Myers et al., 1995; Nunez et al., 1990). There are two classic pathways of apoptosis; cysteine-dependent aspartate-specific protease “caspase” dependent and “caspase” independent apoptosis. The Bcl-2 family function to regulate the permeability of the mitochondrial outer membrane and permeability transition pore (mPTP) formation. This is believed to be related to the fact that Bcl-2 has the tertiary structure similar to that of a bacterial pore forming protein (Muchmore et al.,1996).

The bcl-2 protein appears to function in a feedback loop system with caspases. It inhibits caspase activity either by preventing the release of cytochrome c from the mitochondria and/or

by binding to the AIF. Bcl-2 is the regulator of extrinsic and intrinsic caspase dependent pathways of apoptosis as well as caspase independent apoptosis (Muchmore et al., 1996).

2.3.1 Caspase Dependent and Independent Apoptosis Pathways

2.3.1.1 Caspases

Cysteine aspartic acid-specific proteases "caspases" typically have one of two function; activate pro-inflammatory cytokines or promote apoptosis. Caspases engage in proteolysis which is an irreversible form of posttranslational modification (Thornberry & Lazebnik, 1998). There are 14 members in the caspase family. Seven members are involved in apoptosis; apoptosis activators -8, -9 and apoptosis executioners -2, -3, -6, - 7, -10, -12 (Eldaddah & Faden, 2000; Salvesen & Dixit, 1997; Thornberry & Lazebnik, 1998). In the brain certain caspase activity is dependent upon the region of the brain that is undergoing apoptosis; caspase -8 and -9 activation precedes caspase -3 execution in the cortex (Raghupathi, 2004). While only caspase -9 activation is attributable to activating caspase -3 execution in the hippocampus and thalamus (Raghupathi, 2004). Caspase 3, ultimately cleaved by these activator caspases and apoptosis, is then irreversible (Leppert, 2006; Zhang et al., 2005). Apoptosis is typically classified based upon caspase activity; the extrinsic and intrinsic pathway and caspase independent pathways. The literature suggests that there can be crosstalk between the extrinsic and intrinsic caspase dependent pathways via the cleavage of Bid by caspase -8 with subsequent translocation to the mitochondria leading to the release of cytochrome c (Li et al., 1998; Luo et al., 1998; Zhang et al., 2005).

2.3.1.2 Extrinsic Pathway of Apoptosis -Caspase Dependent

Extracelluar signals, which initiate the extrinsic pathway, may include hormones, growth factors, nitric oxide, or cytokines. They are extrinsic because they cross the plasma membrane or transduce to affect a response. These signals may promote or inhibit apoptosis. (Refer to figure 2-7).

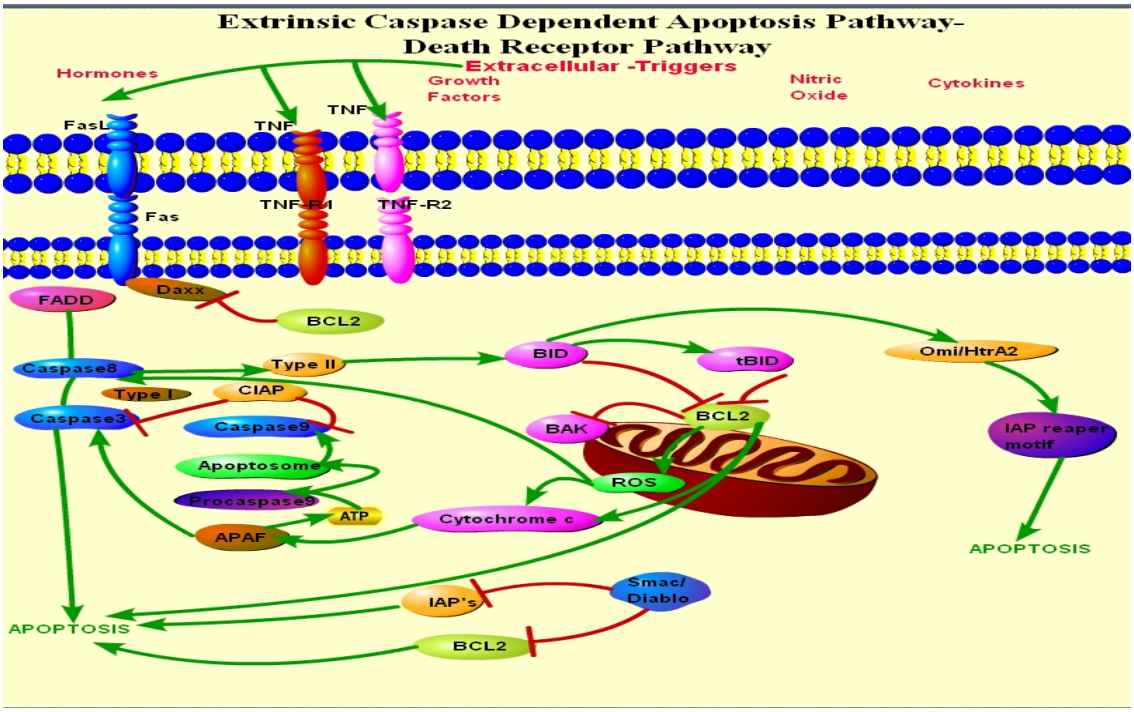

Figure 2-7: Extrinsic Caspase Dependent Mitochondrial Apoptosis Pathway

TNF is a cytokine produced mainly by activated macrophages and is the major extrinsic mediator of apoptosis. Most cells in the human body have two receptors for TNF: TNF-R1 and TNF-R2. In apoptosis TNF binds to TNF-R1 to initiate the pathway that leads to caspase activation via intermediate membrane proteins (TNF receptor-associated death domain [TRADD] and Fas-associated death domain protein [FADD]). Binding to the TNF1 receptor can also indirectly lead to the activation of transcription factors involved in cell survival. Following TNF-R1 activation in mammalian cells, a balance between pro-apoptotic and anti-apoptotic members of the Bcl-2 family is established. Pro-apoptotic homodimers that form in the outer-

membrane of the mitochondrion are required to make the mitochondrial membrane permeable for the release of caspase activators such as cytochrome c and SMAC. When the death signal is received, products of the activation cascade displace voltage-dependent anion channel isoform (VDAC2) and BAK is able to be activated (Itoh et al., 1991; Muzio et al., 1996; Salvesen & Dixit, 1997). TNF is related to the opening of mPTP, and in doing so, the loss of $\Delta\Psi m$ induces ROS and therefore contributing, via PTP opening in the mitochondria matrix, subsequent osmotic swelling in the inner membrane of the mitochondria which in turn rupture the outer membrane of the mitochondria and release cytochrome c and caspase-8 activation (Bahr, 2000; Breckenridge & Xue, 2004; Kim, 2005). This in turn leads to the cleavage of Bid to truncated Bid (tBid) which translocates from the cytosol to the outer mitochondrial membrane allowing the pores to open and cytochrome c to release (Li, Zhu, Xu, & Yuan, 1998; Luo, Budihardjo, Zou, Slaughter, & Wang, 1998). (Refer to figure 2-7).

Fas is a member of the TNF superfamily. The Fas receptor binds the Fas ligand (FasL), a transmembrane protein. The interaction between Fas and FasL results in the formation of the death-inducing signaling complex (DISC), which contains the FADD, caspase-8, and caspase-10. There are two types of cells, type 1 and type 2. Type 1 processed caspase-8 and directly activates other members of the caspase family, and triggers the execution of apoptosis. Type II cells the Fas-DISC starts a feedback loop that spirals into increasing release of pro-apoptotic factors from mitochondria and the amplified activation of caspase-8. Fas has two known apoptotic pathways, Daxx is a Fas binding protein that is able to be blocked by Bcl-2. The other Fas pathway is mediated downstream by FADD and is insensitive to Bcl-2 and leads to apoptosis (Yang, Khosravi-Far, Chang, & Baltimore, 1997). Initiator caspases (caspase-8) can induce MMP by cleaving pro-death Bid into truncated Bid (tBid). It is tBid that amplifies the weak signal of

initiator caspases by promoting the release of cytochrome c, Smac/Diablo and Htra2/Omi to activate the apoptosome and reverse IAP inhibition of caspases (Breckenridge & Xue, 2004; Muzio et al., 1996; Yang, et al., 1997). (Refer to figure 2-7)

Bcl-2 family of proteins, Bax, Bak, and Bcl-2 are found in the endoplasmic reticulum as well as the mitochondria. Therefore these proteins are capable of affecting ER Ca2+ homeostasis and Ca2+ uptake by the mitochondria and potentially affecting mPTP (Breckenridge & Xue, 2004). The over-expression of Bcl-2/Bcl-xL can inhibit the translocation of Bax/Bak and therefore preventing oligomerization of the pro-apoptotic proteins. Apoptosis is inhibited by sequestering small pro-apoptotic molecules in the mitochondria (Breckenridge & Xue, 2004; Kim, 2005; Wang, et al., 2004). There are several kinds of small pro-apoptotic molecules which are released from the mitochondria they include; cytochrome c, SMAC/ DIABLO and Omi/HtrA2 (Kim, 2005). (Refer to figure 2-7).

Cytochrome c is an apoprotein. Enzyme cytochrome c heme-lyase adds a heme group to the cytochrome c and converts it to a globular protein known as holocytochrome c. It is holocytochrome c that is the catalyst to activate caspase (Vander Heiden & Thompson, 1999). Once cytochrome c is released it binds with Apaf-1 and ATP, which then bind to pro-caspase-9 to create a protein complex known as an apoptosome. The apoptosome cleaves the pro-caspase to its active form of caspase-9, which in turn activates the effector caspase-3 (Kim, 2005). (Refer to figure 2-7).

Second mitochondrial activator of caspase (Smac /DIABLO) is a caspase coactivator. Smac /DIABLO potentates apoptosis triggered by UV and irradiation, cytotoxic drugs and DNA damage and ligation of the Fas/CD95 death receptor. Smac/DIABLO escapes from mitochondria via a caspase-catalysed event that occurs downstream of cytochrome c release (Adrain, Creagh,

& Martin, 2001). Smac/ DIABLO binds to inhibitor of apoptosis proteins (IAPs) and inhibits them, preventing the IAPs from arresting the apoptotic process and therefore allowing apoptosis to proceed. Smac/ DIABLO also inhibits bcl-2-over-expressing cells (Kim, 2005). (Refer to figure 2-7).

Omi/HtrA2 is a proapoptotic mitochondrial serine protease involved in caspase-dependent as well as caspase-independent cell death. Omi/HtrA2 is able to cleave itself generating an IAP interacting reaper motif (Kim, 2005). Omi/HtrA2 is also important in the process of apoptosis independently of the presence of the reaper motif. The release of Omi/HtrA2 is promoted by the 'BH3-only' Bcl-2 family member; Bid (Martins, 2002). (Refer to figure 2-7).

As mentioned above there are “Inhibitor of apoptosis proteins” (IAP’s). IAP’s have at least five members to this family; c-IAP1, c-IAP2, XIAP, NIAP, and surviving. All IAP’s except for NIAP inhibit apoptosis by binding to the active form of terminal/ executioner caspapses-3, -7 and -9 which cause actual degradation of enzymes. They do not interact with caspase-8 (Deveraux et al., 1998). (Refer to figure 2-7).

2.3.1.3 Intrinsic Pathway of Apoptosis Caspase Dependent

Intracellular apoptotic signaling is a response initiated by a cell in response to stress; glucocorticoids, heat, radiation, nutrient deprivation, viral infection, and hypoxia. The initiation of the intrinsic pathway of caspase dependent apoptosis is triggered by stress on the cellular organelle (i.e. mitochondria and ER). In a healthy cell, the outer membranes of the mitochondria express the protein Bcl-2 on their surface. Bcl-2 is bound to a molecule of the protein Apaf-1. Internal damage in the cell causes Bcl-2 to release Apaf-1 which results is cytochrome c leaking out of the mitochondria. (Refer to figure 2-8). The release of cytochrome c and Apaf-1 bind to

molecules of caspase-9. The resulting complex of cytochrome c, Apaf-1, caspase -9 and ATP is called the apoptosome (Liu et al., 1996). As apoptosomes aggregate in the cytosol, caspase-9 cleaves and activates other caspases, esp. caspase 3. The sequential activation of one caspase by another creates an expanding cascade of proteolytic activity which leads to digestion of structural protein in the cytoplasm and degradation of chromosomal DNA and phagocytosis of the cell (Fiskum, 2000; Kluck et al., 1997; Yang, J. et al., 1997). (Refer to figure 2-8).

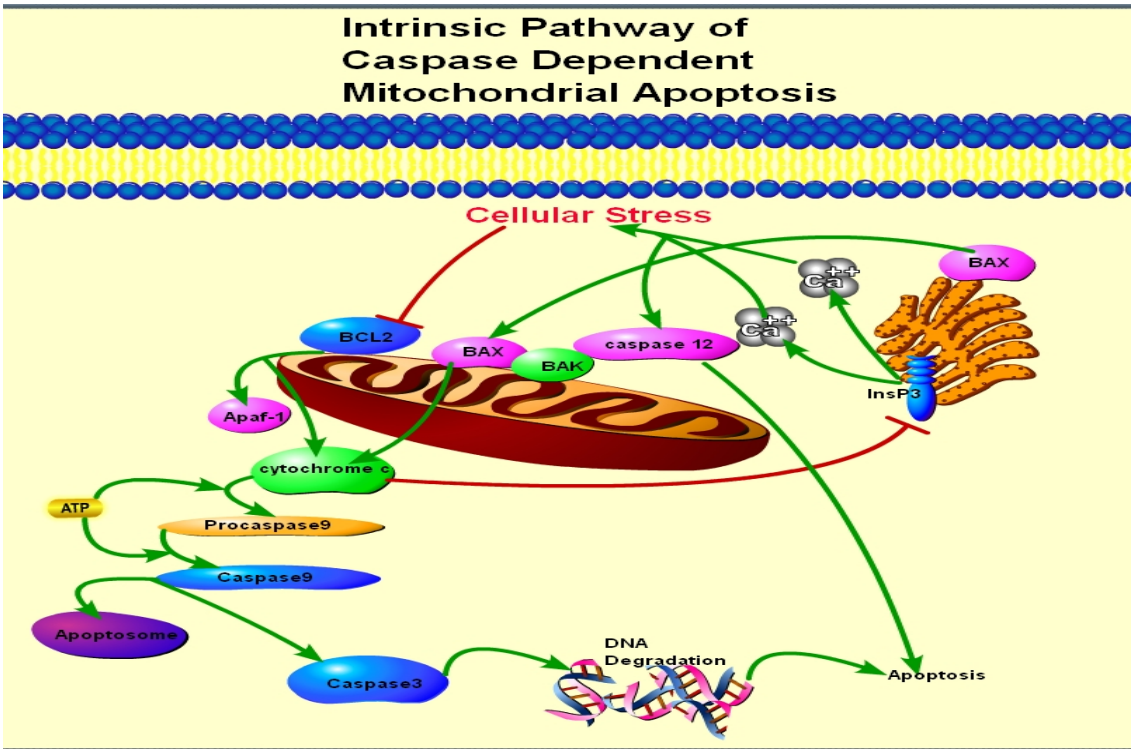

Figure 2-8: Intrinsic Pathway of Caspase Dependent Mitochondrial Apoptosis

Cytochrome C released from the mitochondria can translocate to the ER to block inositil-(1,4,5)-triphosphate (InsP3) receptor. This amplifies Ca2+ signaling and further release of cytochrome c from the mitochondria. ER stress can interfere with the ER Ca2+ homeostasis and accumulation of excess protein which activates ER localized caspase 12 contributing to apoptosis (Boehning et al., 2003; Larner, Hayes, McKinsey, Pike, & Wang, 2004; Zhang et al., 2005). Bax is translocated from the ER to the mitochondria during the process of apoptosis. The

translocation results in the oligomerization of Bax/Bak which activates Bax/Bak and the subsequent insertion into the outer membrane of the mitochondria forming mPTP's. Ultimately the release of cytochrome c from the mitochondria activates caspase-9, which in turn activates the executioner caspase-3 and culminates in apoptosis (Baliga & Kumar, 2003; DeGiorgi, et al., 2002; Kim, 2005). (Refer to figure 2-8).

2.3.2 Caspase Independent Apoptosis

Bcl-2 is at the heart of the caspase independent apoptosis cascade because of its integral role in inhibiting mitochondrial membrane permeability (Tanaka et al., 2005). The inhibition of bcl-2 allows the pores to open and allows the direct release or indirect activation of mitochondrial and nuclear proteins that are able to induce apoptosis without activating the caspase cascade some specific examples include; AIF, EndoG, PARP, and p53. (See figure 2-9).

Apoptosis Inducing Factor (AIF) is an evolutionary conserved flavoprotien in the mitochondria which is released after mitochondrial insult related to mitochondrial membrane depolarization (Zhang et al., 2005). AIF is translocated to the nuclei and induces the hallmark event of DNA fragmentation. AIF caspase independent apoptosis does occur in TBI, oxidative stress, and brain ischemia in vivo (Zhang et al., 2005). (Refer to figure 2-9).

Endonuclease G (EndoG) is a mitochondrion-specific nuclease. It translocates to the nucleus during apoptosis. Once released from mitochondria, endoG cleaves chromatin DNA into nucleosomal fragments independently of caspases; therefore a component of the caspase-independent apoptotic pathway (Li, Luo, & Wang, 2001). (Refer to figure 2-9).

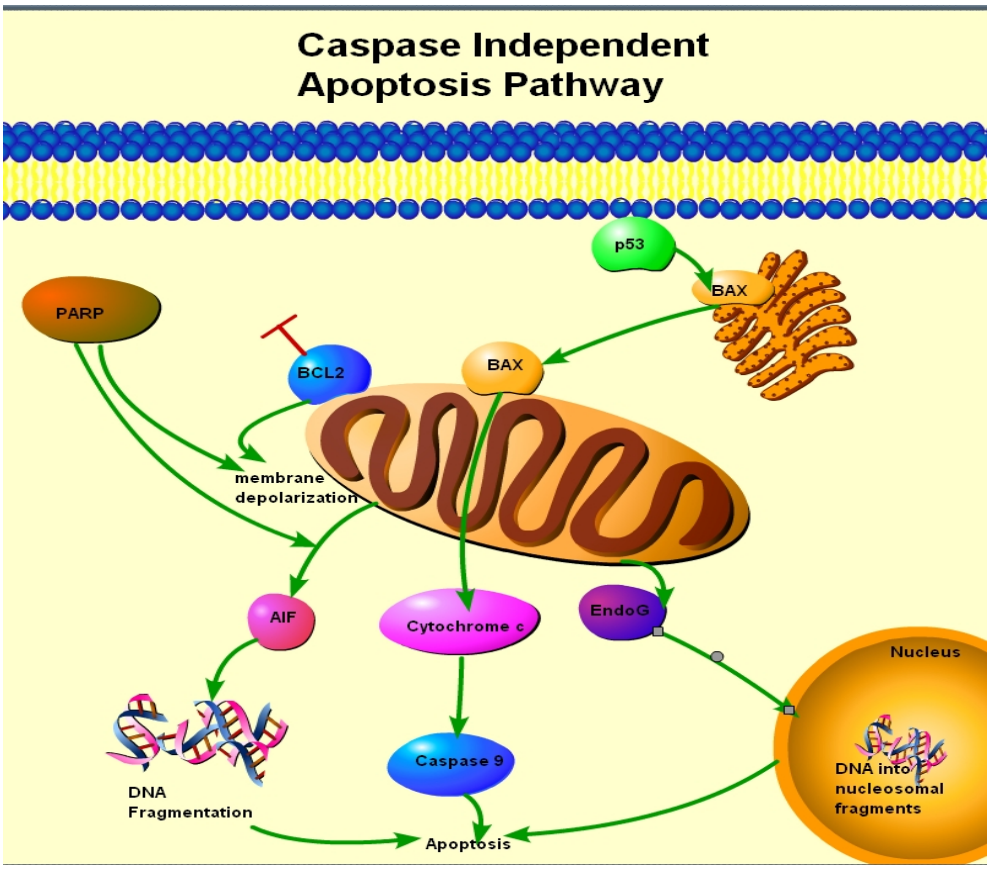

Figure 2-9: Caspase Independent Apoptosis Pathway

Poly(ADP-ribose) polymerase (PARP) (PARP1) plays a role in both necrotic cell death and apoptosis. In severe conditions of depletion of NAD+ via PARP, activation exacerbates energy failure in the cell, hence membrane leakage and classic necrosis. When the cell is in incomplete energy failure, representative of TBI models of injury, PARP contributes to mitochondrial membrane depolarization, AIF release, and apoptosis (Zhang et al., 2005). PARP1 activation is required for the release and translocation of AIF from the mitochondria to the nucleus. Conversely, AIF is necessary for PARP1 dependent cell death (Yu et al., 2002; Yu et al., 2006). This results in caspase independent apoptosis. (Refer to figure 2-9).

Pro-apoptotic gene product, p53 is a transcription factor that leads to the induction and up regulation of bax with mitochondrial translocation hence increasing the bax/bcl-2 ratio (Lu,

Moochhala, Kaur, & Ling, 2000; Miyashita & Reed, 1995). The protein, p53 indirectly triggers the intrinsic caspase dependent cascade due to the increase of Bax resulting in the direct activation of the intrinsic pathway by release of cytochrome c from the mitochondria and subsequent caspase -9 activation (Bredesen, 2000). The effects of p53 on Bcl-2 expression may be attributed to the cis-acting p53 negative response element located at the 5' untranslated region of the BCL-2 gene (Miyashita, Harigai, Hanada, & Reed, 1994).

2.4 BCL-2 AND TBI

In the previous section, a detailed explanation was given to the importance of Bcl-2 in apoptosis. Apoptosis is an intricate event requiring the orchestration of numerous cellular players. This section will review the evidence of the role of bcl-2 as it pertains to TBI.

Zhang (et al., 2005) speculate a rule of thirds in the types of cell death after TBI; one third of cell death is related to caspase dependent apoptosis, one third is associated with caspase independent cell death, and the last third is attributed to necrosis. In TBI, apoptosis may be triggered by edema (i.e. increased ICP and inflammation) and hemorrhage (Zhang et al., 2005). Cells that are vulnerable to apoptosis after TBI include neurons, oligodendrocytes, astrocytes (glutamate uptake, lactate, and antioxidant production) and microglia (Zhang et al., 2005). Bcl-2 can block the axonal injury and loss of axonally transported trophic factors associated with TBI (Garcia et al., 1992; Graham et al., 2000). Bcl-2 can also regenerate severed axons (Chen, Schneider, Martinou, & Tonegawa, 1997). Apoptosis is apparent 1 hr- 14 days after injury with bcl-2 protein levels peaks 12-72 hours after injury in the animal model (Xiong, Lin, Chen,

Peterson, & Lee, 2001). One study suggests that Bcl-2 expression peaks on day 4 after brain injury (Lee, J. et al., 2004).

2.4.1 Review of the Literature in Animals

Tables 2-1 to 2-6, reviews all of the Bcl-2 literature with direct relevance to TBI in the animal literature from 1960-2008 in the OVID and CINAHL databases, with 23 relevant articles spanning 1997-2008.

The literature commences with an investigation if bcl-2 mRNA increases in the rat brain post experimental model TBI. Clark and colleagues (1997) report that bcl-2 mRNA increases in the ipsilateral cortex (6, 24, and 72 hours), dentate gyrus (6 and 24 hours), hippocampus cornu ammonis area 1[CA1] (24 hours), hippocampus cornu ammonis area 3 [CA3] (6 and 24 hours) post TBI. Bcl-2 protein increases in the cortex 8 and 72 hours post injury ($P<0.05$) compared to controls (Clark et al., 1997). These studies established that bcl-2 mRNA and protein increase after TBI in experimental adult rat animal models.

Further studies examined the relationship of injury to the immature brain as apoptosis is essential to the developmental process and brain injury may further highten the apoptotic process. The 3 and 7 day old immature rat brain undergoes significant apoptosis in the 1 hour after injury (Bittigua et al., 1999; Morrison, Eberwine, Meaney, & McIntosh, 2000). In an in vitro model of 4 day old rat brain, bcl-2 mRNA expression significantly decreased 24 hours after injury compared to controls ($P<0.005$) and at 6 hours ($P<0.01$) and return to baseline 48 hours injury (Morrison et al., 2000). In the in vivo rat model bcl-2 depression is evident at 2 hours and persists for 48 hours and with a slow and incomplete recovery at 120 hours post injury

(Felderhoff-Mueser et al., 2002). Apoptosis and/ or the decrease in bcl-2 activity has been noted earlier after injury in the immature brain versus the adult brain and may indicate why children may have different resposes to similar injury.

The human BCL-2 transgenic (TG) versus wild type (WT) murine models were utilized in three of the studies reviewed (Raghupathi et al., 1998; Nakamura et al., 1999; Tehranian et al., 2006). The TG models all support the evidence that bcl-2 over-expression is associated with significant reduction in the percent of lesion after TBI ($P<0.01$ to $P<0.05$). One study reports TG mice had a reduced deficits on the incline plane test ($P<0.05$) versus WT mice, however this study also reports no significant differences in other neurological assessments (Raghupathi et al., 1998). Additional studies also support the evidence that bcl-2 over-expression does not significantly improve murine neurological performance on neurological exam and Morris Water Maze (MWM) tests 24 hours to 1 week after injury (Nakamura et al., 1999; Tehranian et al., 2006). The results of Nakumura et al. (1999) study are confounded with their report that pre-injury TG mice have significant learning impairment versus WT ($P<0.05$). In evaluating the potential behavioral and functional outcomes of bcl-2 over-expression after TBI, the limitations of the TG model need to be considered. Murines have only small behavioral deficits associated with injury to the cortex (Bederson et al., 1986). TG murines have life long over-expression of bcl-2 having larger brains than WT littermates (Tehranian et al., 2006) and therefore developmental apoptosis is interrupted which may explain the learning deficits in the pre-injured TG mice (Nakamura et al., 1999). The over-expression of bcl-2 in humans is not life long. The incongruence between decreased lesion volume and behavioral outcomes after TBI as seen in the murine population after TBI may not exist in humans. Pediatric humans with increased expression of bcl-2 after TBI are reported to have significantly better functional outcomes and

decreased mortality than in subjects with negative (low) bcl-2 expression after TBI (Clark et al., 2000; Ng et al., 2000; Nathoo et al., 2004).

BCL-2 fusion protein- recombinant adenovirus gene therapy intervention after TBI in rats has shown to significantly reduce apoptosis 3 days post injury ($P<0.01$) (Yang X., Zheng, Liu, & Feng, 2006). This is in congruence with the TG models for bcl-2 over-expression which indicate decreased lesion size. The gene therapy model indicated that one week after injury the tilt board functional outcome measure for the rats were no different than the sham control however, 2-4 weeks after injury, experimental gene therapy group had significantly better tilt board outcomes ($P<0.005$) (Yang, X. et al., 2006). This maybe a factor of a broader timeframe to ascertain differences in outcomes or absence of life long over-expression bcl-2, a combination of both, or possibly another factor.

There are additional intervention studies that resulted in altered bcl-2 expression that offer areas of therapeutic means by which to manipulate bcl-2 expression and the potential benefits from the altered states.

An in vitro study used a pharmaceutical bcl-2 protein derivative, Bcl-220-34, to perfuse hippocampal slices after a fluid percussion model of injury. Bcl-220-34 in the hippocampus was protective against hypoxia, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA], N-methyl-D-aspartic acid [NMDA], and nitric oxide [NO] (Panizzon, Shin, Frautschy, & Wallis, 1998). Another pharmaceutical study utilized N-acetylcysteine (NAC), an antioxidant, which can inhibit gene expression induced by ROS. It was able to reduce bcl-2 short bands and bax in injured rats after TBI (Xiong , Lin, Chen, Peterson, & Lee, 2001). Mifepristone (glucocorticoid receptor antagonist) when used as a pretreatment can protect neuronal loss in the CA1 hippocampal region 24 hours following TBI. It is also associated with decreased basal bcl-2

mRNA in CA1 and dentate gyrus. Mifepristone did not affect basal bax or p53 levels therefore it is not believed to be involved with transcriptional regulation of bcl-2, bax, or p53 apoptosis related genes (McCullers, Sullivan, Scheff, & Herman, 2002). Recombinant human erythropoietin (rhEPO) was found to increase the expression of bcl-2 mRNA and protein and decreased DNA fragmentation in rats with TBI when compared to vehicle treated rats (Liao, Zhi, Shi, & He, 2008). While there are several pharmaceutical interventions none of the results have been duplicated. More research needs to be conducted in this arena with neurobehavioral outcomes added to the study designs.

An alternate intervention to alter bcl-3 states after TBI in the murine models is hyperbaric oxygenation (HBO). Bcl-2 expression was shown to be lower in hypoxic states and that bcl-2 expression can increase significantly with HBO (Liu, Kim, Yang, Jemmerson, & Wang , 2006 Vlodavsky, Palzur, Feinsod, & Soustiel, 2005). The mechanism behind this maybe related to HBO ability to lower cytochrome c and bax expression at 3, 6, 12 and 24 hours post TBI ($P<0.05$) (Liu et al., 2006). Additional research needs to be done in this area as well.

Additional studies examined the relationship of bcl-2 with other pro- and anti-apoptosis markers that ascertain the timing and brain location of bcl-2 as a player in the larger apoptosis cascades. These studies are important to designing the timing of an intervention for the specific location of injury as they pertain to possible alterations in bcl-2 concentrations. Following TBI, bcl-2 and bax bands intensities reduce 2 hrs after injury ($P<0.05$) more importantly the bax:bcl-2 ratio increases at 2 hrs and remains elevated at 7 days s/p injury ($P<0.05$) (Raghupathi et al., 2003). Bcl-2 levels tend to down regulate in early TBI (i.e. 30 minutes to 6 hours) (Dong, Singh, Dendle, & Prasad, 2001; Lu, J., Moochhala, Kaur, & Ling, 2000; Raghupathi et al., 2002; Strauss, Narayan, & Raghupathi, 2004). However, bcl-2 expression is significantly higher 24

hours after injury post injury of TBI of any severity (Hellmich et al., 2005). These studies provide some evidence that early post injury there is a reduction in the anti-apoptotic concentration of bcl-2 and an increase in bax:bcl-2 ratio. It has yet to be determined if this temporal pattern influences neurobehavioral outcomes.

The location of the injury may play a role in bcl-2 activity. In diffuse traumas, there is an increase bax expression, induction of increase in active caspase -3 expression with a simultaneous increase in bcl-2 expression for the first 5 days after injury (Cernak, Chapman, Hamlin, & Vink, 2002). One study found that bcl-2 mRNA is up regulated ipsilaterally 10 days after injury suggesting that the therapeutic window is quiet large (Wennersten, Holmin, & Mathiesen, 2003). Yet, cytochrome c significantly increases and bcl-2 significantly decreases 30 minutes to 72 hours post TBI in the injured cortex and ipsilateral hippocampus (Dong et al., 2001). One study examined the long term activity of anti-(bcl-2 and heat shock protein 70) and pro-(casapes-3 and-9) apoptotic genes at 3, 6 and 24 months post TBI. Both anti- and pro-apoptotic genes were down regulated in the hippocampus; long term neuronal survival/loss may not be mediated by apoptosis related genes (Shimanura et al., 2005). Due to the evidence that the therapeutic window for bcl-2 activity may be as large as 10 days, but cytochrome c activity peaks are 72 hours post injury, there may be additional members of the apoptosis cascade that may be influencing the temporal/ location pattern exhibited in the bcl-2 concentrations after acute experimental TBI in animal models.

Table 2-1: Bcl-2 and Traumatic Brain Injury in Animals: Literature Review

Bcl-2 and Traumatic Brain Injury in Animals: Literature Review 1960-2008						
Population Subjects (n)	**Mechanism of Injury**	**Tissue Analyzed**	**Treatment**	**Method of Analysis**	**Summary of Results**	**Reference**
Adult Male Sprague-Dawley rats n=65	CCI	Peritrauma Hippocampus Dentate gyrus		-*In situ* hybridization -Western Blot -Immunohistochemistry -TUNEL	Increase in bcl-2 mRNA at 6 and 24 hrs after injury (ipsilateral cortex, dentate gyrus, CA1 (24 hrs only) and CA3. At 72 hrs ipsilateral cortex only. Bcl-2 protein increased in cortex at 8 and 72 hrs ($P<0.05$ vs control) <2%of TUNEL-positive cells display characteristics of apoptosis or necrosis which express bcl-2.	Clark et al., 1997
Male Sprague-Dawley rats	Fluid percussion trauma	Hippocampal CA1 pyramidal neurons	Bcl-2^{20-34} peptide medication	-Electron micrographs -Electrophysical function (Shaffer collateral stimulation)	-Bcl-2^{20-34} provided protection with CA1 antidromic PS recovery of 92% ±1 ($P<0.05$) -Bcl-2^{20-34} protective against hypoxia, AMPA, NMDA & NO	Panizzon et al., 1998
BCL-2 TG mice n=21 WT littermates n=18	Lateral CCI	Cortical hippocampal		-Functional Outcomes -Histology	-7 days s/p CCI TG mice reduced cortical lesion ($P<0.01$) -TG mice had reduced deficit in cline plan test ($P<0.05$) vs WT No significant difference TG vs WT on other neuroscores.	Raghupathi et al., 1998
Wistar rat pups Age 3, 7, 10, 14 and 30 days.	Weight Drop (Modified based on age)	Frontal, parietal, cingulated, retrosplenial, cortex, caudate nucleaus, dentate gyrus, mediodorsal, laterodorsal, ventral, thalamus, subiculum		TUNEL	Immature brain (3 & 7 days) undergoes significant apoptosis in the first hour after injury significant in all tissue analyzed from ($P<0.05$ to $P<0.001$) [except in the dentate gyrus on day 3] bcl-2 levels are down regulated 1 hr after trauma.	Bittigua et al., 1999

Table 2-1 continued

Bcl-2 and Traumatic Brain Injury in Animals: Literature Review 1960-2008						
Population Subjects (n)	**Mechanism of Injury**	**Tissue Analyzed**	**Treatment**	**Method of Analysis**	**Summary of Results**	**Reference**
8 week old BCL-2 TG mice n=13 WT mice n=9	CCI moderate TBI	Cortical Hippocampus Dentate gyrus Thalamus		-Histopathological Immunohistochemistry -Cognitive MWM	Percent tissue loss TG smaller vs WT hemisphere ($P<0.01$) Hippocamal ($P<0.001$) Dentate Gyrus ($P<0.01$). TG had increased loss in dorsal thalamus ($P<0.05$) ventral thalamus ($P<0.01$) MWM 1 week s/p injury TG vs WT no significant difference.(Pre-injury TG learning impaired $P<0.05$ vs WT)	Nakamura et al., 1999
Male Wistar rats n=24	Weight drop	Cerebral cortex		Double immunolabelling TUNEL	-2 hours after injury Fos immunoreactivity, -At 4 hours bax elevated and bcl-2 down regulated, increase p53 -colocalization of Bax immunoreactivity with bcl-2 and p53	Lu et al., 2000
4 day old Sprague-Dawley rat pups n=19 from 3 different litters were used to make the organotypic brain cultures	*In vitro* mechanical injury (deformation of the silicone membrane) to organotypic cultures of rat brain Mild to moderate injury	unknown		Reverse Northern Hybridization	Bcl-2 mRMA expression decreased 24 hrs after injury compared to controls ($P<0.005$) 6 hr ($P<0.01$), and returned to baseline 48 hours after injury. Bax mRNA were not altered after injury	Morrison et al., 2000

Table 2-1 continued

Bcl-2 and Traumatic Brain Injury in Animals: Literature Review 1960-2008						
Population Subjects (n)	**Mechanism of Injury**	**Tissue Analyzed**	**Treatment**	**Method of Analysis**	**Summary of Results**	**Reference**
Rats	Lateral Fluid Percussion	Cortex (Injured left and contralateral right) Hippocampus (ipsilateral and contralateral)		RT-PCR (Bcl-2) Western Blots (cytochrome c)	Bcl-2 significantly decreased 2 hr s/p injury to 48 hrs in the injured cortex and ipsilateral hippocampus. Cytochrome c was significantly increased in these areas. Cytochrome c increases in the injured cortex at 30 min, 48 and 72 hrs; and in the ipsilateral hippocampus at 2 to 72 hrs.	Dong et al., 2001
Male Sprague-Dawley Rats	CCI	Forebrain	N-acetylcysteine (NAC)	Western Blots	Bcl-2 not detected in uninjured rat brain. 1, 4 and 12 hrs s/p injury shortened Bcl-2 bands were detected in injured hemisphere. Bax was evident in both the uninjured and injured rats. Bcl-2 short band and bax were reduced in NAC tx injured rats	Xiong et al., 2001
7 day old rat pups	weight drop model	Right parietal cortex Fresh tissue from cingulate and parietal cortex, striatum and thalamus		TUNEL Immunohistochemistry silver staining RT-PCR Western Blot fluorescence assays	Trauma triggers a down regulation of bcl-2 and bcl-xL evident 2 hrs post injury and persisting 48 hors with a slow and incomplete recovers by 120 hrs post injury ($P<0.001$)	Felderhoff-Mueser et al., 2002
Adult male Sprague-Dawley Rats	Mild fluid percussion injury	Cortex		TUNEL Morphological changes	Decreased bcl-2 cellular immunoreactivity in the cortex at 2 hrs s/p injury. No change in bax levels. Loss of bcl-2 at 6 hrs in the hippocampus CA3 followed by overt neuronal loss at 24 hrs s/p injury. No increase of apoptosis (bax) in the hippocampus. Evidence of bax:bcl-2 ratio in the cortex and hippocampus.	Raghupathi et al., 2002

Table 2-1 continued

Bcl-2 and Traumatic Brain Injury in Animals: Literature Review 1960-2008						
Population Subjects (n)	**Mechanism of Injury**	**Tissue Analyzed**	**Treatment**	**Method of Analysis**	**Summary of Results**	**Reference**
Male Sprague-Dawley Rats	2m impact-acceleration model of diffuse axonal injury	cortex		TUNEL Western Blot	Increased active caspase-3 expression was correlated with increased bcl-2 levels (P<0.001) for the first 5 days after injury. Bcl-2 overexpression significant days 3-5 (P<0.05) in the cortex. Maximal DNA fragmentation at 3 days after injury	Cernak et al., 2002
Young adult male Sprague-Dawley rats n=60	Unilateral CCI	Hippocampus (CA1, CA3 and dentate gyrus)	Spironolactone Mifepristone	Optical fractionator *In situ* hybridization	Spironolactone and Mifepristone decreased basal Bcl-2 mRNA in CA1 and dentate gyrus. They did not affect basal bax or p53 levels. Injury decreased bcl-2 mRNA in the dentate gyrus but did not affect bax or p53 levels.	McCullers et al., 2002
Adult male Sprague-Dawley rats	Lateral fluid percussion Moderate severity	Cortex Hippocampus		Immunohistochemistry Western Blot In situ hybridization	Bcl-2 and bax bands intensities reduce 2 hrs after injury (P<0.05). bax:bcl-2 Ratio increases at 2 hrs and remains elevated at 7 days s/p injury (P<0.05).	Raghupathi et al., 2003
Adult Male Sprague-Dawley rats	Unilateral CCI	Hippocampus		TUNEL Immunohistochemistry In situ hybridization	Bcl-2 mRNA was up regulated ipsilaterally 10 days after injury compared to controls (P<0.05) Bax mRNA peaks on day 6 (P<0.05)	Wennersten et al., 2003
Adult Male Sprague-Dawley rats	TBI Model I Lateral Fluid percussion TBI Model II Lateral CCI	Cortex Hippocampus		RNase Protection Assays (RPA)	Bcl-2 decreases 75-80% in the first 6 hrs after injury and return to normal levels by day 1 and decrease again until day 3.	Strauss et al., 2004
Adult Male Sprague-Dawley rats	Fluid Percussion Mild -Severe	Hippocampus (CA1, CA3, dentate gyrus)		RNase Protection Assays (RPA)	Bcl-2 expression at 24 hr was higher than at 4 hr regardless of TBI severity (P=0.046).	Hellmich et al., 2005

Table 2-1 continued

Bcl-2 and Traumatic Brain Injury in Animals: Literature Review 1960-2008						
Population Subjects (n)	**Mechanism of Injury**	**Tissue Analyzed**	**Treatment**	**Method of Analysis**	**Summary of Results**	**Reference**
3-4 month old Male Sprague-Dawley rats		Hippocampus (CA1, CA3, dentate gyrus) (24 hr and 3, 6,& 12 months)		RNase Protection Assays (RPA)	MRNA expression of Bcl-2 and Heat Shock protein 70 was down regulated in neurons at 3, 6 (BCL-2 only) and 12 months after TBI ($P<0.05$). Caspase -3 and -9 decreased in the neurons at 3, 6 (-9 only) and 12 months after injury ($P<0.05$). Bcl-2, Heat Shock protein 70, Casapse -3 and -9 decrease in glial cells at 3 and 12 months ($P<0.05$).	Shimamura et al., 2005
Sprague-Dawley rats	Focal cerebral contusion Cortical dynamic deformation	Traumatic penumbra area	Hyperbaric oxygen (HBO)	Immunohistochemical staining	Bcl-2 expression was lower in hypoxic rats vs non hypoxic. Bcl-2 expression increased with HBO in hypoxic and non-hypoxic groups ($P< 0.05$)	Vlodavsky et al., 2005
Adult male Sprague-Dawley rats n=40 Treatment n=40 controls	Weight drop model	Right parietal cortex	Hyperbaric Oxygenation (HBO)	Immunohistochemistry Electronmicroscope	TBI rats with HBO treatment had: Lower cytochrome C and BAX expression at 3, 6,12 and 24 hrs post injury ($P<0.05$) Higher BCL-2 expression at 3,6,12,24,and 72 hr post injury ($P<0.01$)	Liu et al., 2006
BCL-2 TG Mice WT Mice	CCI	Hippocampus		Western Blot TUNEL MWM	BCL-2 TG mice had smaller contusion volumes and increased number of surviving neuron in CA2 24 hr after injury ($P<0.05$) No difference in motor function and MWM.	Tehranian et al., 2006

Table 2-1 continued

Bcl-2 and Traumatic Brain Injury in Animals: Literature Review 1960-2008						
Population Subjects (n)	**Mechanism of Injury**	**Tissue Analyzed**	**Treatment**	**Method of Analysis**	**Summary of Results**	**Reference**
Wistar Rats n=45 BCL-2 gene therapy n=45 controls	Weight drop model	Right parietal lobe	BCL-2 fusion protein-recombinant adenovirus	Immunohistochemistry Western Blotting Fluorescence microscopy TUNEL Behavioral- tilt board	Experimental group had less apoptosis 3 days post injury ($P<0.01$). 1 week after injury no difference in tilt board score; 2-4 weeks after injury experimental group had better outcomes ($P<0.005$)	Yang et al., 2006
Female Wistar Rats n=60 rhEPO-treated n=60 vehicle-treated n=10 sham operated	Feeney free falling model	Right somatosensory cortex	rhEPO-treated rhEPO 5000 IU/kg administered intraperitoneally once a day for 7 days post injury. Vehicle- treated	RT-PCR Western blotting immunofluorescence TUNEL	rhEPO –treated TBI group bcl-2 mRNA and protein greater than vehicle-treated Bcl-2 mRNA peaked at 24 hours rhEPO –treated less DNA fragmentation than vehicle-treated rhEPO protects neurons by enhancing bcl-2 expression	Liao et al., 2008

2.4.2 Review of the Literature in Humans

The literature on bcl-2 and TBI in humans is limited. There were seven relevant articles that addressed these specific issues in the literature from Ovid search from 1960- 2008. The seven articles were published between May 1999 and June 2008 and utilize small sample sizes. **None of the articles address BCL-2 genotypes.** Tables 2-7 and 2-8 summarizes the findings of the human studies.

From the studies that have been conducted in humans the empirical evidence establishes that bcl-2 levels increase in humans brain and/or CSF after TBI compared to patients without TBI or neurological injury (Clark et al., 1999; Clark et al., 2000; Yang & Xue, 2004).

TBI subjects who up regulated bcl-2 had an incidence of a lower mortality rate and better GOS scores (Clark et al., 2000; Ng et al., 2000; Nathoo et al., 2004). Minambres and colleagues (2008), report that *in vivo* samples had significantly higher bcl-2 concentration compared to post mortem autopsy ($p=0.027$). However, it is unclear at what time point the post mortem specimens were harvested and the inconsistent procedure in fixating the tissues in formalin (surgical tissues 24 hours and the autopsy brain for 15 days.) This raises questions if the significant down-regulation of bcl-2 in the post mortem brain tissue was due to a medically relevant conclusion that patients with lower concentration of bcl-2 have increased mortality or if bcl-2 levels naturally down-regulate post mortem or if the procedure by which the tissues were harvested and fixed affected bcl-2 concentrations.

The time point at which bcl-2 concentrations peak in humans is not clear in the literature. One study reported that bcl-2 concentrations increases 8 hours after injury and peak on days 2-3 and remains higher for 5-7 days (Yang & Xue, 2004) while another study reports two peak

points among the patients, day 3 and 5 (Uzan et al., 2006) the peak window may be patient dependent.

Among the seven studies, three examined ICP as a clinical outcome. One study reports that bcl-2 positive patients had lowered ICP trending towards significance (p=0.057), however then sample size was small (n=11)(Ng et al., 2000). Two additional studies found there to be no effect of bcl-2 concentrations on ICP (n=29, p= 0.8, Nathoo et al., 2004; n=14, p= 0.9, Uzan et al., 2006).

Based on the studies reviewed, there is a growing body of evidence that bcl-2 concentrations are increased after TBI, and the bcl-2 levels are related to global functional outcomes (survival versus mortality) after TBI. More research is needed to establish a relationship with clinical, biological, global functional outcomes, and cognitive- behavioral outcomes after TBI. None of the studies examined BCL-2 genotypes in the TBI population. This study was designed to address this gap in the literature.

Table 2-2: Bcl-2 and Traumatic Brain Injury in Humans: Literature Review

Bcl-2 and Traumatic Brain Injury in Humans: Literature Review 1960-2008						
Population Subjects (n)	**Severity of Injury**	**Tissue Analyzed**	**Measurements**	**Outcome Measure**	**Summary of Results**	**Reference**
Adults n=8 TBI n=6 controls	All TBI w/ surgical decompression for increased ICP Initial GCS 3-15	Contused cerebral cortex (TBI) Temporal Lobe (controls)	Western Blot Immunohistochemistry TUNEL	GOS at 3 months median 3 . GOS based on level of expression not available.	TBI subjects had bcl-2 levels higher than the controls (P=0.020) There was no significant increase in bcl-xL or bax expression between TBI and controls.	Clark, et al., 1999
Adult n=11	GCS≤8	Contused frontal, temporal & parietal tissues	Immunohistochemistry TUNEL	ICP mortality	Bcl-2 positive subject had survival after 6 months (P=0.01) Bcl-2 positive subject had lower ICP (P=0.057)	Ng, et al., 2000
Pediatrics n=23 TBI n=19 controls	GCS≤8	CSF Temporal Lobe	ELISA on CSF TUNEL on Temporal Lobe	GCS GOS-(most recent follow-up visit)	Bcl-2 levels increased TBI subjects vs control, P=0.01 Increased bcl-2 in TBI patients that survived vs died P=0.02	Clark, et al., 2000
Adults n=29 control=3	Admission GCS 5-14 Mean 11.2±2.46	ER Craniotomy & Biopsy of perischemic zone (PIZ) cerebral contusion	Immunohistochemistry Immunostaining	GOS ICP	Bcl-2 negative status independent predictor of poor outcomes P<0.04, odds ratio 5.5 Bcl-2 Positive patients better GOS at 18 months or longer (3.8±1.6) P=0.03. Bcl-2 positive and negative patients no significant difference in ICP (p=0.8). PIZ was significantly higher for Bax (P=0.005) and caspase-3 (P=0.005).	Nathoo, et al., 2004

Table 2-2 continued

Bcl-2 and Traumatic Brain Injury in Humans Literature Review 1960-2008						
Population Subjects (n)	**Severity of Injury**	**Tissue Analyzed**	**Measurements**	**Outcome Measure**	**Summary of Results**	**References**
Adults n=40 TBI n=5 controls	Average GCS=7.22±3.19	Internal Decompression of the cerebral cortex	Immunohistochemistry TUNEL	Outcome data not available	Controls had minimal bcl-2 (mRNA and protein) TBI had bcl-2 (mRNA and protein) increased 8 hr after trauma, peaked 2-3 days later, remained higher than controls days 5-7. Bax (mRNA /protein) was high in the controls. Bax became significantly higher in TBI at 20-28 hrs. & 2-3 days post injury.	Yang, et al., 2004
Pediatric (n=3) & Adults (n=11) n= 14 TBI n= 14 controls	GCS≤8 in the TBI group	CSF	sFAS & Bcl-2 via ELISA Caspase-3 via colorimetric activity assay kit	GOS at discharge 50% mortality rate	Caspase-3 correlates to ICP(p=0.01) and CCP (p=0.04) Bcl-2 concentrations peaks days 3 and 5 no correlation with ICP, CPP, or CT findings (p>0.05) No correlation between bcl-2 and sFAS or capsase-3.	Uzan et al., 2006
Adults n=40 *Ex vivo* study n= 16 TBI n= 5 controls	GCS≤8	Pericontusional zone (PCZ)	Immunohistochemistry TUNEL	GOS at 6 months 47.5% mortality rate at 6 months	In viva samples compared to post mortem samples had significantly higher expression of bcl-2 (p=0.027) and bcl-xL (p=0.014) In vitro studies apoptotic rate pf PC12 cells independent factor in mortality at 6 months (p=0.014).	Minambres et al., 2008

2.5 OUTCOMES

2.5.1 Neuropsychological

The gold standard in evaluating outcomes after TBI has been the global functional outcomes measurement of Glasgow Outcome Score (GOS). GOS is a feasible tool to quickly gage a patient's ability to perform ADL's and the amount of assistance required (Jennett & Bond, 1975; King, Calier & Marion, 2005). There are a host of functional outcome measures to assess ADL functioning and participation in care (i.e. GOS, DRS, Functional Independence Measurement [FIM], and Community Integration Questionnaire [CIQ]). For research purposes, batteries of physical functional outcome test are administered routinely. Less consistent is the administration of cognitive-behavioral outcome measurements. Part of this is the reality that TBI patients injuries can fall on a continuum of mild to severe and that the measurements of outcomes after TBI therefore have the potential of ranging from no impairment to death. Global functional outcome measurements are necessary to capture the outcomes for those with severe impairments or death. More sensitive measurements of outcomes, cognitive-behavioral, are necessary for those who are capable of participating in neuropsychological testing/ rehabilitation to truly assess recovery and impairment.

Timing of initiating the first neuropsychological testing is debatable. Severe TBI patients are often unable to participate in neuropsychological testing during the first 3 months of injury because of persistent psychosyndromes that disturb orientation and concentration (Lippert-Gruner, Kuchta, Hellmich, & Klug, 2006). However, 62% of patients with moderate to severe

TBI, regardless of post traumatic amnesia status, were able to complete a neuropsychological outcomes brief battery of tests from 2-6 weeks after injury administered in an inpatient rehabilitation setting (Kalmar et al., 2008). In this population, the California Verbal Learning Test-II, FAS, and the animal naming tests were the most consistently performed among all 354 subjects in the study (Kalmar et al., 2008). However, many patients with severe TBI may not have met the criteria for the type of intensive inpatient rehabilitation used in this study thus this sample was biased towards patients who were able to participate in intensive inpatient rehabilitation (typically 180 minutes per day). In order to capture all levels of recovery across the continuum, a variety neuropsychological testing should be included in an outcomes battery; global functional outcomes and cognitive-behavioral measures.

The National Institutes of Health Consensus Development Panel on Rehabilitation of persons with TBI (1999) systematically summarizes the sequelae of TBI across all severities. The neurological consequences of TBI include: movement disorders, seizures, headaches, visional changes, and sleep disorders. Pervading medical complications after TBI include: pulmonary, metabolic, nutritional, gastrointestinal, musculoskeletal, and dermatological dysfunction. The neuropsychological impairments include: cognitive, memory, attention, concentration, language, visual perception, executive functioning, abstract reasoning, insight, judgment, planning, information possessing, and organization, all which can impact employment opportunities and community reintegration. Behavioral sequelae include: decreased ability to initiate a response, verbal/ physical aggression, agitation, learning difficulties, impaired self awareness, altered sexual functioning, impulsivity, and social disinhibition. Mood disorders, personality changes, depression, anxiety, and liable emotional control are all common consequences of TBI. The aftermath of TBI can affect physical and psychosocial health and well

being. A concerning 40% of patients in one study report that these neuropsychological impairments are unmet needs requiring for intervention 1 year post injury (Corrigan et al., 2004).

The social consequences and societal burden of the neuropsychological impairments after TBI can lead to an increased risk of suicide, divorce, chronic unemployment, economic strain, substance abuse (NIH, 1999). Caregiver burden is an additional sequelae related to the severity of disability and impairment and is often addressed separately in the literature (Nabors, Seacat, & Rosenthal, 2002). Goals of cognitive-behavioral rehabilitation are to improve the capacity to process and interpret information and improve social interaction (home, family, and community reintegration) (Cicerone, Mott, Azulay, & Friel, 2004). Social and occupational re-integration can be a challenge after severe TBI because the severe neurobehavioral sequelae of cognitive deficits (attention, memory, information processing speed) and self perception tend not to resolve over time ((Lippert-Gruner et al., 2006). In contrast, the functional deficits do improve over time (Lippert-Gruner et al., 2006). The functional/ physical improvements in performing ADL's are an important first step in describing recovery, however, these tests do not tell the whole story of recovery.

The literature stresses the importance that neuropsychological testing needs to include both global functional outcome and cognitive-behavioral measures as to provided a more complete and accurate picture of recovery after TBI, whether mild or severe.

Neuropsychological rehabilitation is often not covered under insurance on an outpatient bases (BIAA, 2007). With more research in this area and interventional studies, the case may be made for neuropsychological rehabilitation to be a vital part of long term recovery from TBI. With empirical evidence, insurance benefits might then extend the outpatient TBI rehabilitation

coverage to encompass not only the physical and occupation therapy domains but to include neuropsychological rehabilitation.

The measurements utilized to assess the neuropsychological outcomes in this study are discussed at length in the Methods section 3.8.2.4.

2.5.2 Biological and Clinical Outcomes

2.5.2.1 Lactate and Pyruvate

Biological predictors are not well defined, but as suggested by the apoptosis cascade of events, lactate and pyruvate levels may inform prognosis (Lu, Ashwell & Waite, 2000). Lactate, pyruvate, and lactate/pyruvate ratio (L/P) are energy- related metabolites and biochemical indicators of brain injury and cerebral anoxia (Kerr et al., 2003; Wagner et al., 2004 ; Wagner et al., 2005 ; Yu et al., 2005). Lactate is a marker for aerobic and anaerobic metabolism and is the end product of glycolysis (Kerr et al., 2003; Wagner et al., 2004 ; Wagner et al., 2005 ; Yu et al., 2005). Elevated lactate is related to poor outcomes, diminished cerebral blood flow, elevated intracranial pressure and ischemia (Kerr et al., 2003; Wagner et al., 2004; Yu et al., 2005). Pyruvate is an intermediate in the metabolism of glucose. It is a potent ROS scavenger. Pyruvate is protective against ROS in neuronal tissue (Kerr et al., 2003; Yu et al., 2005). Increased pyruvate is associated with decreased cerebral ischemia (Yu et al., 2005). Lactate and pyruvate are often considered in ratio because they are antagonistic to each other (Kerr et al., 2003; Wagner et al., 2004; Wagner et al., 2005). Pyruvate inhibits the translocation and activation of p53 proteins, tumor suppressor and regulation of transcription, as is a result of DNA damage secondary to oxidant injury (Lee, Kang, Bunger, & Kang, 2003). Bcl-2 expression was increased as a result of these events (Lee, Y., Kang, Bunger, & Kang, 2004). Pyruvate has anti-apoptotic

influence through over-expression of bcl-2 (Lee, Y. et al., 2004). There have been no studies that examine the relationship between BCL-2 and lactate, pyruvate or L/P ratio after TBI.

2.5.2.2 CBF

Secondary brain injury is one consequence of decreased cerebral blood flow (CBF), cerebral ischemia. Cerebral ischemia is related to the reduction or complete loss of CBF (McLaughlin & Marion, 1996). The cascade continues with a depletion of ATP, anoxic depolarization, and spreading depression depolarization. As a result of ischemic changes cytochrome c release and apoptosis occurs (Zhao, Steinberg, & Sapolsky, 2007). There is evidence of a direct link between bcl-2 and CBF. In a stoke model, bcl-2 knockout mice, homozygous knockout mice has significantly larger infarct area more severe reduction in CBF (Hata, Gillardon, Michaelidis, & Hossmann, 1999). In a transient focal ischemia brain injury stroke model in rats, 3-methyl-1- phenyl-pyrazolin-5-one (Edaravone), a free radical scavenger which inhibits both hydroxyl radical generation and iron-induced peroxidative injuries, was found to be protective against ischemia and reduced apoptotic cell death by upregulating bcl-2 and downregulating bax (Amemiya et al., 2005). Numerous studies have been conducted on the efficacy of hypothermia on ischemia and ischemia related apoptosis with conflicting results (Eberspacher et al., 2003; Yenari, et al., 2002; Zhang, Z., Sobel, Cheng, Steinberg, & Yenari, 2001; Zhao, Yenari, Cheng, Sapolsky, & Steinberg, 2005). Hypothermia increased bcl-2 protein expression after global ischemia but had no influence on bax (Zhang, Z. et al., 2001). However, one study found that hypothermia inhibits bax expression 4 hours after ischemia but had no effect on other proteins (Eberspacher et al., 2003). A difference may be in the type of ischemia. In focal ischemia hypothermia was found to not alter bcl-2 or bax expression, but transiently blocked cytochrome c in addition there was no caspase activity which maybe indicative of

caspase independent apoptosis (Yenari, et al., 2002). In global ischemia, hypothermia blocked caspase activity and the "second large phase" of cytochrome c release, but did not block early cytochrome c release (Zhao et al., 2005). Bcl-2 protein levels and gene knock-out models assert bcl-2 involvement in CBF. BCL-2 genotypes have not been studied in humans.

2.5.2.3 APOE

Apolipoprotein E (APOE) is the most documented genetic marker that has been associated with outcomes after TBI due to its association with numerous cellular activities and, in particular, neuronal plasticity and regulation of neurotoxicity (Chiang, Chang & Hu, 2003). The APOE genotype has been studied extensively in the TBI population. APOE is a lipid clearance and metabolic protein that facilitates neurotransmission (Mauch et al., 2001; Reyland et al., 1991) maintains structural integrity of neuron (Hayashi et al., 2002; Mahley et al., 1988; Weisgraber et al., 1994) is involved with neuronal plasticity (Ignatius et al., 1986; Nathan et al., 1994; Poirier et al., 1991; Poirier et al., 1994) and regulation of neurotoxicity in TBI (Marques et al., 1996; Michikawa et al., 1998; Moulder et al., 1999). In humans it is encoded by a 4-exon gene and is located on chromosome 19. The APOE gene has three alleles (APOEϵ2, APOEϵ3, APOEϵ4). The 4 allele is associated with increase ß-amyloid deposits (Beffert et al.,1998; LaDu et al., 1994; LaDu et al., 1995; Namba et al., 1991) increased circulation of metabolites and excitotoxins (Muller et al., 1998; Tolar et al., 1999; Veinberg et al., 2002) and poor outcomes after neurological insult (Jordan et al., 1997; Liaquat et al., 2002; Liberman et al., 2002; Lynch et al., 2002; Millar et al., 2003 ; Nicoll et al., 1996; Teasdale et al., 1997). However, the relationship between ApoE/APOE and apoptosis markers has not yet been clearly defined

(Belton et al., 2003; Moulder, Narita, Chang, Bu & Johnson, 1999; Ong et al., 2003; Wei, Zhang & Zhou, 1999).

There is some empirical evidence that there is a connection between bcl-2 and apoE proteins. Early in the investigation of APOE and apoptosis, one Alzheimer's disease study suggested that apoE4 protein neurotoxicity is distinct from classical apoptosis or necrosis (Moulder et al., 1999). However, subsequent studies suggest a link. One Alzheimer's disease study of mice bread for senility (SAMP8) or senility resistance (SAMR1) found that mice with SAMP8 had an increase in bcl-2 proteins in astrocytes and very low level of neuronal bcl-2 proteins. The author suggests that this was a compensatory neuroprotective response (Wei et al., 1999). Studies of APOE knockout mice have found increased apoptosis in other disorders (Belton et al., 2003; Ong et al., 2003). Alzheimer's disease studies in mice support that nicotine receptor– mediated protection increases bcl-2 protein levels, which may prevent neuronal cell death via blockade of ß-amyloid and glutamate neurontoxicity (Shimohamma et al., 2001). More research is needed in this area to describe the relationship between BCL-2 and APOE genotypes in humans.

2.6 SUMMARY

Apoptosis plays a pivotal role in cell survival after a neurotrauma. Bcl-2 upregulation is associated with decreased ischemic areas in the brain and increased cerebral blood flow. The literature is sparce on the effect of bcl-2 on functional outcomes after TBI. Bcl-2 is a compeling starting point to futher the investigation of the relationship of BCL-2 genotypes to outcomes as well as biological/ clinical data.

2.7 SIGNIFICANCE AND INNOVATION

As a health care community we can not fully explain why some patients have better outcomes than others after a severe TBI. Nurses are looked to for answers to these questions because we have the most contact hours with TBI patients and their families during their extensive acute care and rehabilitation stays. Recovery from TBI may be influenced by genes. The study of BCL-2 is in its infancy and BCL-2 genotyping has not been addressed in the TBI population, despite the empirical evidence that bcl-2 protein concentrations may affect outcomes. With further research, there is the potential for a better understanding of mechanisms of secondary injury and the development of a BCL-2 related genetic derivative treatment or intervention. These treatments or interventions can potentially decrease the secondary injuries associated with TBI and therefore increasing the odds of survival and improving global functional and cognitive-behavioral neuropsychological outcomes.

3.0 METHODS

3.1 DESIGN

This study utilized a descriptive, longitudinal, prospective between-group, with-in subject design to examine BCL-2 genotypes in DNA extracted from CSF or blood specimens and how they related to outcomes at 3, 6, 12, and 24 months post injury and biological/ clinical data. This study was an ancillary study that utilized biological samples and data collected from federally funded studies that examined genes and gene products in TBI patients and how they related to outcomes attained by the TBI patients (APOE and Outcomes After Traumatic Brain Injury, R01NR04801; Mitochondrial Genetics of Recovery after Brain Injury, NR008424; the Brain Trauma Research Center (BTRC) grant, 5P50NS30318; Injury Control Research Centers: "Evaluating the Impact of Neuroendocrine Hormones on Pathophysiology and Outcomes after Traumatic Brain Injury, CIRCL/CDC grant , R49/CCR 323155-03.)

3.2 SAMPLE

This study used a convenience sample of 230 participants with severe TBI admitted to a level 1 trauma center. The level 1 trauma center at the tertiary care institution admits an average of 40 severe TBI patients each year. The subjects were admitted from May 2000- April 2007. All

patients admitted to the NeuroTrauma or Trauma Intensive Care Units were screened for eligibility based on the following criteria (according to Health Insurance Portability and Accountability Act [HIPAA] regulations since 2003).

3.2.1 Inclusion criteria:

1. Age 16-75 years
2. Initial Glasgow Coma Score (GCS) ≤8 (without the influence of neuromuscular blocking agents, alcohol or drug effects)
3. The presence of an external ventricular drain (EVD) per standard of care for ICP monitoring and CSF drainage and sampling
4. Positive computed tomography (CT) scan for TBI
5. Proxy, signed written consent from next-of-kin

3.2.2 Exclusion criteria include:

1) TBI related to penetrating injury/ open head injuries (gunshot wounds or stab wounds)
2) Pre-existing neurological conditions.

Protected populations: prisoners, and persons with mental retardation/ developmental disabilities, were not recruited. No participant was excluded based upon, gender, race, or ethnicity. Children under the age of 16 were not included in the study because there is evidence that children respond differently to TBI than adults (Chenn et al, 2004).

3.3 RATIONALE FOR SAMPLE SIZE

Rationale: The sample size was based on available subjects in the data base (with both biological samples collected and consented for further genetic testing and outcome measures). Multiple post hoc power analyses were preformed with Power Analysis and Sample Size software [(PASS) NCCS Statistical Software, Kaysville, Utah, 2008] due to the fluctuation in sample sizes. (Refer to table 3-1) Power and effect sizes were calculated for each SNP with a primary analysis (full) model analysis conducted in a particular database.

Table 3-1: Summary of Sample Size

Summary Table of Sample Size		
Database	**Type of Analysis**	
	Preliminary Mixed Models	**Primary Mixed Models**
Outcomes Data	n=	n=
GOS	201	141
DRS	201	141
Mortality	201*	141*
NRS-R	95	61
Trails A	30	27
Trails B	30	27
Biological/ Clinical Outcomes		
Bcl-2 Protein Levels	42	37
Neurometabolites	36	36
CBF	17	17
*Generalized mixed model to account for binary nature of the mortality outcome.		

BCL-2 was dichotomized based on the frequency of the genotype in the database which was being analyzed. Therefore, the homozygous genotype with the least frequency was combined with the heterozygotes and compared against the homozygous with the largest frequency in the particular database.

The power analyses were based on the dichotomized genotypes for each SNP with a primary analysis (full model) for the outcomes indicated in specific aim #1. While specific aim2 was more exploratory in nature it would be helpful for future studies to ascertain power and ES given the current sample size available. Power and ES are presented for the bcl-2 protein and neurometabolite pilot data for specific aim #2. Due to the small sample size of the CBF dataset, power was not able to be calculated using PASS 2008 (SAS 9.1 was unable to calculate rho which was necessary for the analyses.) ES was able to be calculated for the primary analyses.

Effect size (ES) was calculated based on the fixed effects estimate of each SNP and sigma used in the power analyses. Odds ratios were calculated for the mortality outcome variable. Tables of the power and ES analyses were included to illustrate that the power and ES for each of the studies fluctuated based upon the SNP that was analyzed (Refer to tables 3-2 to 3-11). Based on these power calculations, a number of the SNP's had a large enough sample size to reject a false null hypothesis (decreased chance of making a type II error) at least 80% of the time. The ES for some SNP's were adequate for a number of subjects in the given analysis because the degree of difference in the fixed effects estimate was quite large. In contrast, other SNP's would warrant a larger sample size in future studies because the fixed effects estimates were quite small between the genotypes. Therefore, in future studies, the sample size is dependent on the tagging SNP that will be analyzed.

3.3.1 Specific Aim #1

Given a sample size of 141, specific aim #1 detected a power of 0.08 to 0.99 to detect an ES (absolute value) of 0.13 to 0.46 in the relationship between BCL-2 genotype and GOS after severe TBI at 3, 6, 12, and 24 month outcomes at $\alpha = .05$ when conducting two-sided hypothesis

testing controlling for covariates (age, GCS, gender, race, hypothermia, and hypotension). (Refer to table 3-2).

Table 3-2: GOS Post Hoc Power Calculations

Glasgow Coma Scale (GOS) Power Calculation Data (n=141)											
SNP	Database Homozygous WildtypeSample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Number of Time Points	Sigma	Rho	Power	Beta	ES
RS12968517	68 (XX)	73	141	0 (0%)	-0.1623	4	1.2288	0.9	0.12605	0.87395	-0.1321
RS17759659	58 (XX)	80	138	3 (2.1%)	-0.832	4	1.1385	0.8834	0.99339	0.00661	-0.7308
RS1801018	58 (YY)	82	140	1 (0.7%)	-0.7987	4	1.1674	0.8873	0.98642	0.01358	-0.6842
RS1944419	34 (XX	103	137	4(2.8%)	0.3097	4	1.2262	0.8967	0.26427	0.73573	0.25257
RS4941185	52 (XX)	88	140	1 (0.7%)	0.1273	4	1.2326	0.9015	0.08905	0.91095	0.10327
RS7236090	41(XX)	97	138	3 (2.1%)	0.1739	4	1.2314	0.8987	0.12074	0.87926	0.14122
RS949037	42 (YY)	94	136	5 (3.5%)	0.5428	4	1.1906	0.8942	0.7262	0.2738	0.45591

Given a sample size of 141, specific aim #1 had a power of 0.01 to 0.99 to detect an ES (absolute value) of 0.12 to 0.72 in the relationship between BCL-2 genotype and DRS after severe TBI at 3, 6, 12, and 24 month outcomes at $\alpha = .05$ when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, race, hypoxia, seizure, hypothermia, and hypotension). (Refer to table 3-3).

Table 3-3: DRS Post Hoc Power Calculations

Disabilty Rating Scale (DRS) Power Calculation Data (n=141)											
SNP	Database Homozygous WildtypeSample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Number of Time Points	Sigma	Rho	Power	Beta	ES
RS12968517	68 (XX)	73	141	0 (0%)	1.4148	4	10.4475	0.9615	0.12624	0.87376	0.1354
RS17756073	73(XX)	64	137	4(2.8%)	1.892	4	10.4952	0.9612	0.18632	0.81368	0.1803
RS17759659	58 (YY)	80	138	3 (2.1%)	6.949	4	9.7933	0.956	0.98698	0.01302	0.7096
RS1801018	58 (YY)	82	140	1 (0.7%)	7.1039	4	9.8786	0.9565	0.98942	0.01058	0.7191
RS1944419	34 (XX	103	137	4(2.8%)	-2.5393	4	10.3827	0.9597	0.24072	0.75928	-0.2446
RS4941185	52 (XX)	88	140	1 (0.7%)	-1.2376	4	10.4881	0.9613	0.10107	0.89893	-0.1180
RS7236090	41(XX)	97	138	3 (2.1%)	-2.3443	4	10.4451	0.9611	0.23053	0.76947	-0.2244
RS949037	42 (YY)	94	136	5 (3.5%)	-4.4511	4	10.2176	0.9586	0.68468	0.31532	-0.4356

Given a sample size of 141, specific aim #1 had power of 0.18 to 1.0 to detect an odds ratio of 0.412 to 5.051 in the relationship between BCL-2 genotype and mortality after severe TBI at 3, 6, 12, and 24 month outcomes at $\alpha = .05$ when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, race, hypothermia, hypotension, and seizures). (Refer to table 3-4).

Table 3-4: Mortality post Hoc Power Calculations

Mortality Power Calculation Data (n=141)											
SNP	Database Homozygous Wildtype Sample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Number of Time Points	Sigma	Rho	Power	Beta	ES
RS12968517	68 (XX)	73	141	0 (0%)	0.5804	4	0.9023	-0.2538	1	0.0000	0.6432
RS17756073	73(XX)	64	137	4(2.8%)	0.0529	4	0.9093	-0.2413	0.25242	0.7476	0.0582
RS17759659	58 (YY)	80	138	3 (2.1%)	1.6195	4	0.9569	-0.2268	1	0.0000	1.6925
RS1801018	58 (YY)	82	140	1 (0.7%)	1.6109	4	0.9371	-0.2444	1	0.0000	1.7190
RS4941185	52 (XX)	88	140	1 (0.7%)	-0.2074	4	0.9166	-0.2533	0.99955	0.0004	-0.2263
RS7230970	25 (XX)	114	139	2(1.4%)	0.7371	4	0.9587	-0.2465	1	0.0000	0.7688
RS7236090	41(XX)	97	138	3 (2.1%)	-0.0565	4	0.9390	-0.2129	0.18802	0.8120	-0.0602
RS949037	42 (YY)	94	136	5 (3.5%)	-0.8862	4	0.9122	-0.2456	1	0.0000	-0.9715

Given a sample size of 61, specific aim #1 detected a power of 0.06 to 1.0 and an ES (absolute value) of 0.0035 to 0.56 in relation to BCL-2 genotype and NRS-R after severe TBI at 3, 6, and 12 month outcomes at $\alpha = .05$ when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, race, hypothermia, and seizures). (Refer to table 3-5).

Table 3-5: NRS-R Post Hoc Power Calculations

Neurobehavioral Rating Scale-Revised (NRS-R) Power Calculation Data (n=61)											
SNP	Database Homozygous WildtypeSample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Number of Time Points	Sigma	Rho	Power	Beta	ES
RS12454712	32 (YY)	29	61	0 (0%)	2.9552	3	9.6591	0.6584	0.27358	0.7264	0.3060
RS1481031	16 (XX)	44	60	1 (1.6%)	-1.1614	3	9.7497	0.6608	0.06731	0.9327	-0.1191
RS17756073	29 (XX)	31	60	1 (1.6%)	0.0331	3	9.5488	0.6551	0.03538	0.9646	0.0035
RS17759659	30 (XX)	31	61	0 (0%)	1.8206	3	9.7677	0.6684	1	0.0000	0.1864
RS1944419	13(XX)	48	61	0 (0%)	-1.514	3	9.7679	0.6667	0.08108	0.9189	-0.1550
RS4456611	15(YY)	45	60	1 (1.6%)	-4.9777	3	8.8296	0.6073	0.59515	0.4049	-0.5638
RS7236090	19(XX)	41	60	1 (1.6%)	4.2506	3	9.5830	0.6502	0.44638	0.5536	0.4436

Given a sample size of 27, specific aim #1 had power of 0.04 to 0.9 to detect an ES (absolute value) of 0.07 to 0.95 in the relationship between BCL-2 genotype and Trails A after severe TBI at 3, 6, and 12 month outcomes at $\alpha = .05$ when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, race, and hypothermia). (Refer to table 3-6).

Table 3-6: Trails A Post Hoc Power Calculations

Trails A Power Calculation Data (n=27)											
SNP	Database Homozygous WildtypeSample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Number of Time Points	Sigma	Rho	Power	Beta	ES
RS12454712	11(YY)	16	27	0 (0%)	7.4566	3	16.0187	0.4399	0.3233	0.6767	0.4655
RS12968517	15(XX)	12	27	0 (0%)	14.3516	3	15.0280	0.3610	0.9023	0.0977	0.9550
RS1381548	8(YY)	19	27	0 (0%)	12.9308	3	15.1374	0.3694	0.7588	0.2413	0.8542
RS1481031	11(XX)	16	27	0 (0%)	2.4441	3	16.4426	0.4679	0.0684	0.9316	0.1486
RS17756073	15(XX)	12	27	0 (0%)	7.4562	3	15.9141	0.4280	0.3365	0.6635	0.4685
RS17759659	15(XX)	11	26	1 (3.7%)	-7.8583	3	16.3884	0.4473	0.3299	0.6701	-0.4795
RS1801018	16(YY)	11	27	0 (0%)	-6.4113	3	16.2530	0.4635	0.2409	0.7591	-0.3945
RS3810027	10(XX)	17	27	0 (0%)	-9.1309	3	15.7146	0.4116	0.4643	0.5358	-0.5810
RS4941185	10(XX)	17	27	0 (0%)	-7.8852	3	15.9665	0.4344	0.3483	0.6517	-0.4939
RS7236090	11(YY)	15	26	1 (3.7%)	8.7143	3	16.1428	0.4340	0.4065	0.5935	0.5398
RS8083946	9(YY)	18	27	0 (0%)	-1.172	3	16.4636	0.4681	0.0407	0.9593	-0.0712

Given a sample size of 27, specific aim #1 had a power of 0.04 to 0.69 to detect an ES (absolute value) of 0.0009 to 0.896 in the relationship BCL-2 genotype and Trails B after severe TBI at 3, 6, and 12 month outcomes at α= .05 when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, race, and hypothermia). (Refer to table 3-7).

Table 3-7: Trails B Post Hoc Power Calculations

Trails B Power Calculation Data (n=27)											
SNP	Database Homozygous WildtypeSample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Number of Time Points	Sigma	Rho	Power	Beta	ES
RS12454712	11(YY)	16	27	0 (0%)	26.9143	3	43.9547	0.6641	0.4265	0.5735	0.6123
RS12968517	15(XX)	12	27	0 (0%)	-5.8154	3	45.8652	0.6878	0.0557	0.9443	-0.1268
RS1381548	8(YY)	19	27	0 (0%)	37.5431	3	41.8842	0.6267	0.6893	0.3107	0.8964
RS1481031	11(XX)	16	27	0 (0%)	23.0362	3	44.7975	0.6753	0.3168	0.6832	0.5142
RS17756073	15(XX)	12	27	0 (0%)	32.2942	3	42.2470	0.6318	0.6226	0.3774	0.7644
RS17759659	15(XX)	11	26	1 (3.7%)	-0.04064	3	47.1284	0.6918	0.0251	0.9749	-0.0009
RS1801018	16(YY)	11	27	0 (0%)	-14.458	3	45.4778	0.6841	0.1477	0.8523	-0.3179
RS3810027	10(XX)	17	27	0 (0%)	1.3776	3	45.9581	0.6893	0.0304	0.9696	0.0300
RS4941185	10(XX)	17	27	0 (0%)	-16.15	3	45.2483	0.6804	0.1766	0.8234	-0.3569
RS7236090	11(YY)	15	26	1 (3.7%)	7.3982	3	46.6185	0.6943	0.0653	0.9347	0.1587
RS8083946	9(YY)	18	27	0 (0%)	4.7974	3	45.9174	0.6889	0.0472	0.9528	0.1045

3.3.2 Specific Aim #2:

3.3.2.1 Bcl-2 Protein

Given a sample size of 37, specific aim #2 had a power of 0.03 to 0.29 to detect an ES (absolute value) of 0.01 to 0.24 in the relationship BCL-2 genotype and bcl-2 protein with outliers after severe TBI over days1-6 post injury at α= .05 when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, race, hypoxia, and seizure). (Refer to table 3-8).

Table 3-8: Bcl-2 Protein Concentrations WITH Outliers Post Hoc Power Calculations

Bcl-2 Protein WITH Outliers Power Calculation Data (n=42)											
SNP	Database Homozygous Wild-typeSample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Number of Time Points	Sigma	Rho	Power	Beta	ES
RS1026825	11	30	41	6 (14.3%)	0.9364	6	8.515280383	0.0995	0.0909	0.9091	0.109967
RS12454712	17	25	42	5(11.9%)	1.8061	6	8.406241729	0.0872	0.2868	0.7132	0.2148523
RS1481031	18	23	41	5(11.9%)	1.4853	6	8.416923428	0.0895	0.2066	0.7934	0.1764659
RS17756073	22	19	41	6 (14.3%)	1.4255	6	8.539882903	0.0829	0.1943	0.8057	0.1669227
RS4456611	9	32	41	1 (2.4%)	2.0448	6	8.472260619	0.0924	0.2533	0.7467	0.2413523
RS7236090	12	28	40	7 (16.7%)	1.5658	6	8.602435702	0.0647	0.2028	0.7972	0.1820182
RS899968	14	26	40	7 (16.7%)	-0.0828	6	8.582767619	0.1029	0.02858	0.9714	-0.009647

Given a sample size of 37, specific aim #2 had a power of 0.05 to 0.99 to detect an ES of 0.06 to 0.84 in the relationship BCL-2 genotype and bcl-2 protein without outliers after severe TBI over days1-6 post injury at $\alpha = .05$ when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, race, hypoxia, and seizure). (Refer to table 3-9).

Table 3-9: Bcl-2 Protein Concentrations WITHOUT Outliers Post Hoc Power Calculations

Bcl-2 Protein WITHOUT Outliers Power Calculation Data (n=42)											
SNP	Database Homozygous Wild-typeSample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Number of Time Points	Sigma	Rho	Power	Beta	ES
RS1026825	11	30	41	6 (14.3%)	1.2422	6	5.062153692	0.3395	0.1784	0.8217	0.2453896
RS12454712	17	25	42	5(11.9%)	2.6104	6	4.893648945	0.2876	0.7587	0.2413	0.5334261
RS1481031	18	23	41	5(11.9%)	2.4875	6	4.894588849	0.2904	0.7144	0.2856	0.5082143
RS17756073	22	19	41	6 (14.3%)	2.924	6	4.930801152	0.2739	0.8538	0.1462	0.5930071
RS4456611	9	32	41	1 (2.4%)	0.6465	6	5.023166332	0.3294	0.0740	0.9260	0.1287037
RS7236090	12	28	40	7 (16.7%)	3.926	6	4.692184992	0.2058	0.9865	0.0136	0.8367104
RS899968	14	26	40	7 (16.7%)	0.326	6	5.060800332	0.3445	0.04731	0.9527	0.0644167

3.3.2.2 Neurometabolites

Given a sample size of 36, specific aim #2 had a power of 0.05 to 0.54 to detect an ES (absolute value) of 0.07 to 0.43 in the relationship BCL-2 genotype and lactate concentrations over days1-5 post injury at α= .05 when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, and hypothermia). (Refer to table 3-10).

Given a sample size of 36, specific aim #2 had a power of 0.07 to 0.97 to detect an ES (absolute value) of 0.14 to 1.29 in the relationship BCL-2 genotype and pyruvate concentrations over days1-5 post TBI at α= .05 when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, and hypothermia). (Refer to table 3-10).

Given a sample size of 36, specific aim #2 had a power of 0.06 to 0.86 to detect an ES (absolute value) of 0.097 to 0.75 in the relationship BCL-2 genotype LP ratio over days 1-5 post TBI at α= .05 when conducting two-sided hypothesis testing controlling for covariates (age, GCS, gender, and hypothermia). (Refer to table 3-10).

Table 3-10: Neurometabolite Post Hoc Power Calculations

Neurometabolite Power Calculation Data											
SNP	Database Homozygous Wild-type Sample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Number of Time Points	Sigma	Rho	Power	Beta	ES
				Lactate (n=36)							
RS1026825	11	25	36	0 (0%)	-4906.3	5	13219.3041	0.2114	0.3930	0.6070	-0.3711
RS17759659	7	28	35	1 (2.8%)	-6261.9	5	13294.3597	0.2097	0.45147	0.5485	-0.471
RS1801018	6	29	35	1 (2.8%)	-6450.3	5	13381.7039	0.209	0.42608	0.5739	-0.482
RS1944419	12	22	34	2 (5.6%)	-2795.1	5	13748.8181	0.2316	0.14748	0.8525	-0.2033
RS3810027	18	17	35	1 (2.8%)	5682.92	5	13303.0072	0.2148	0.54438	0.4556	0.42719
RS4941185	12	23	35	1 (2.8%)	882.37	5	13587.8622	0.2424	0.04752	0.9525	0.06494
RS7236090	10	26	36	0 (0%)	2233.58	5	13397.7610	0.2355	0.10729	0.8927	0.16671
RS8083946	19	17	36	0 (0%)	4873.33	5	13204.1660	0.2159	0.44072	0.5593	0.36908
RS949037	14	21	35	1 (2.8%)	-961.15	5	13661.2591	0.2363	0.05123	0.9488	-0.0704
				Pyruvate (n=36)							
RS1026825	11	25	36	0 (0%)	27.8983	5	73.2551	0.6623	0.2333	0.7667	0.38084
RS17759659	7	28	35	1 (2.8%)	83.042	5	64.5219	0.556	0.96659	0.0334	1.28704
RS1801018	6	29	35	1 (2.8%)	69.3412	5	69.9734	0.6177	0.75553	0.2445	0.99096
RS1944419	12	22	34	2 (5.6%)	28.5488	5	71.7020	0.646	0.258	0.7420	0.39816
RS3810027	18	17	35	1 (2.8%)	25.3878	5	72.6595	0.6504	0.22884	0.7712	0.34941
RS4941185	12	23	35	1 (2.8%)	-48.843	5	71.0163	0.6339	0.63187	0.3681	-0.6878
RS7236090	10	26	36	0 (0%)	39.4457	5	71.8727	0.6486	0.41278	0.5872	0.54883
RS8083946	19	17	36	0 (0%)	10.092	5	74.1601	0.6705	0.06878	0.9312	0.13608
RS949037	14	21	35	1 (2.8%)	69.1464	5	65.5739	0.5836	0.96266	0.0373	1.05448
				LP Ratio (n=36)							
RS1026825	11	25	36	0 (0%)	-20.463	5	45.8091	0.2223	0.5194	0.4806	-0.4467
RS17759659	7	28	35	1 (2.8%)	-34.001	5	45.0379	0.1864	0.85625	0.1438	-0.7549
RS1801018	6	29	35	1 (2.8%)	-31.449	5	45.6929	0.1974	0.72746	0.2725	-0.6883
RS1944419	12	22	34	2 (5.6%)	-12.335	5	47.6984	0.2442	0.20785	0.7922	-0.2586
RS3810027	18	17	35	1 (2.8%)	17.7995	5	46.6770	0.2429	0.43472	0.5653	0.38133
RS4941185	12	23	35	1 (2.8%)	7.5273	5	47.2491	0.2574	0.10423	0.8958	0.15931
RS7236090	10	26	36	0 (0%)	4.522	5	46.8181	0.2583	0.06022	0.9398	0.09659
RS8083946	19	17	36	0 (0%)	16.3218	5	46.0952	0.2368	0.39728	0.6027	0.35409
RS949037	14	21	35	1 (2.8%)	-11.875	5	47.1140	0.2379	0.21456	0.7854	-0.252

3.3.2.3 Cerebral Blood Flow

Specific aim #2, CBF in relation to BCL-2 genotype over days 1-5 after injury, was a pilot study based on a sub-sample of 17 subjects ES (absolute value) of 0.105 to 1.46 was determined for the right hemisphere CBF. (Refer to table 3-11).

Specific aim #2, CBF in relation to BCL-2 genotype over days 1-5 after injury, was a pilot study based on a sub-sample of 17 subjects ES (absolute value) of 0.25 to1.69 was determined for the left hemisphere CBF. (Refer to table 3-11).

Specific aim #2, CBF in relation to BCL-2 genotype over days 1-5 after injury, was a pilot study based on a sub-sample of 17 subjects ES (absolute value) of 0.14 to 1.56 was determined for the global CBF. (Refer to table 3-11).

Table 3-11: Cerebral Blood Flow (CBF) Post hoc Effect Size Calculations

Cerebral Blood Flow (CBF) Effect Size (ES) Calculation Data

SNP	Database Homozygous Wild-type Sample Size	Database Homozygous Variant & Heterozygous Sample Size	Total Genotyped	Frequency (%) of Missing Data	Fixed Effects Estimate	Sigma	ES
CBF Right Hemisphere (n=17)							
RS1026825	4	13	17	0 (0%)	12.76	13.7996	0.92466
RS12454712	7	10	17	0 (0%)	10.999	14.9211	0.73714
RS12968517	6	11	17	0 (0%)	-10.667	14.7037	-0.7255
RS1381548 XX	3	10	13	4 (23.5%)	-16.633	12.2233	-1.3608
RS1381548 YY	3	10	13	4 (23.5%)	15.73	10.7703	1.46049
RS1481031	10	7	17	0 (0%)	15.415	15.3307	1.0055
RS17756073	9	8	17	0 (0%)	-15.51	12.6463433	-1.2264
RS1801018	6	11	17	0 (0%)	11.881	12.6976376	0.93569
RS3810027	6	10	16	1 (5.9%)	1.5781	14.9843251	0.10532
RS4456611 XX	5	10	15	2 (11.8%)	-9.4863	12.5753728	-0.7544
RS4456611 YY	5	10	15	2 (11.8%)	10.687	11.5896506	0.92212
RS4941185	5	12	17	0 (0%)	-20.845	11.5684917	-1.8019
RS7236090	8	7	15	2 (11.8%)	13.142	14.8502525	0.88497
RS8083946	5	12	17	0 (0%)	11.358	15.645127	0.72598
RS899968	5	10	15	2 (11.8%)	16.296	12.0536302	1.35196
RS949037	8	9	17	0 (0%)	7.2886	14.3150271	0.50916
CBF Left Hemisphere (n=17)							
RS1026825	4	13	17	0 (0%)	11.811	12.3887	0.95337
RS12454712	7	10	17	0 (0%)	8.4772	13.7546	0.61632
RS12968517	6	11	17	0 (0%)	-13.15	13.3417	-0.9856
RS1381548 XX	3	10	13	4 (23.5%)	-17.822	10.5347	-1.6917
RS1381548 YY	3	10	13	4 (23.5%)	14.951	9.4444	1.58306
RS1481031	10	7	17	0 (0%)	13.625	14.8074	0.92015
RS17756073	9	8	17	0 (0%)	-15.234	11.6038787	-1.3128
RS1801018	6	11	17	0 (0%)	11.301	11.6730459	0.96813
RS3810027	6	10	16	1 (5.9%)	3.3574	13.6213068	0.24648
RS4456611 XX	5	10	15	2 (11.8%)	-6.0274	11.4092068	-0.5283
RS4456611 YY	5	10	15	2 (11.8%)	8.8227	10.8295891	0.81468
RS4941185	5	12	17	0 (0%)	-18.369	11.1099055	-1.6534
RS7236090	8	7	15	2 (11.8%)	15.048	13.9183332	1.08116
RS8083946	5	12	17	0 (0%)	9.4463	13.913303	0.67894
RS899968	5	10	15	2 (11.8%)	12.631	12.7671453	0.98934
RS949037	8	9	17	0 (0%)	6.2218	13.2815662	0.46845
CBF Global (n=17)							
RS1026825	4	13	17	0 (0%)	12.174	13.0227	0.93483
RS12454712	7	10	17	0 (0%)	9.5863	14.2531	0.67258
RS12968517	6	11	17	0 (0%)	-11.11	13.9617	-0.7957
RS1381548 XX	3	10	13	4 (23.5%)	-17.5	11.3146	-1.5467
RS1381548 YY	3	10	13	4 (23.5%)	15.633	10.0255	1.55933
RS1481031	10	7	17	0 (0%)	14.914	14.9057	1.00056
RS17756073	9	8	17	0 (0%)	-15.99	11.8249736	-1.3522
RS1801018	6	11	17	0 (0%)	12.028	11.9239255	1.00873
RS3810027	6	10	16	1 (5.9%)	1.9944	14.2098557	0.14035
RS4456611 XX	5	10	15	2 (11.8%)	-8.512	11.8241279	-0.7199
RS4456611 YY	5	10	15	2 (11.8%)	10.37	10.9936345	0.94327
RS4941185	5	12	17	0 (0%)	-19.95	11.1539231	-1.7886
RS7236090	8	7	15	2 (11.8%)	14.095	14.3062923	0.98523
RS8083946	5	12	17	0 (0%)	9.5053	14.5900651	0.65149
RS899968	5	10	15	2 (11.8%)	15.194	12.1519546	1.25033
RS949037	8	9	17	0 (0%)	6.9612	13.6348817	0.51054

3.4 SETTING

Subjects were recruited from a level 1 trauma center in a tertiary care institution; the University of Pittsburgh Medical Center (UPMC), Pittsburgh Pennsylvania. The acute care phase of this study was conducted in the 10 bed Neurotrauma or 10 bed Trauma Intensive Care Units over the first 5 days after injury (over the first 6 days of injury for the bcl-2 protein data collection only). The outcome data were collected at 3, 6, 12, and 24 months intervals post injury; either at UPMC outpatient neurosurgery clinic or the participant's home/ extended care facility. Phone interviews were conducted with either the participant or the primary care giver in the event that distance prevented a face to face visit or participant/ caregivers did not want study staff members in their home. All outcome data was collected by a trained neuropsychological technician under the supervision of a neuropsychologist.

3.5 RECRUITMENT

The parent studies [APOE and Outcomes After Traumatic Brain Injury, R01NR04801; Mitochondrial Genetics of Recovery after Brain Injury , NR008424; Brain Trauma Research Center (BTRC) grant, 5P50NS30318 and Injury Control Research Centers CIRCL/CDC grant, R49/CCR 323155-03] utilized a convenience sample of subjects that were admitted to the UPMC for severe TBI. The nurse researchers on call were contacted by the Neurosurgical resident on call upon the potential admission of a patient with a severe TBI. During the screening procedure potential participants were identified based on the previously described study criteria. Once a potential participant was identified, the nurse researcher approached a proxy to describe the

study, aims, data collection procedures, risks and benefits of participation, and obtain informed consent. Proxy consent was obtained from next-of-kin for the initial consent due to the fact that all of the participants were in a severe coma with GCS≤8. Assent was obtained once the individual's condition permitted. Both the parent studies and this study were reviewed and approved by the University of Pittsburgh Institutional Review Board (IRB) [IRB# 971212; IRB #0607083; see appendix B for this study's IRB approval letter]. Protected populations; children under 16, prisoners, and persons with mental retardation/ developmental disabilities, were not recruited. No participant was excluded based upon, gender, race or ethnicity.

3.6 STANDARD MEDICAL CARE

All potential subjects were admitted to UPMC with a diagnosis of severe TBI within 48 hours of injury. All participants had a routine CT scan on admission and a GCS≤8 without the effects of alcohol, toxins or medications. Patients received aggressive medical treatment as outlined by "The Guidelines for the Management of Severe Head Injury" produced by the Brain Trauma Foundation and endorsed by the American Association of Neurological Surgeons (Bullock et al., 1996). As part of this guideline, external ventricular drains (EVD's) were placed into all participants as part of routine care. In May (2007) the updated guidelines for the management of severe traumatic brain was published (Povlishock, 2007). All of the patients in this study were admitted from May 2000- April 2007.

Blood levels were drawn on admission for alcohol, drug, and other toxin levels. Upon admission (usually placed in the field) all patients had a cervical collar for immobilization. Due to coma state, GCS≤8, therefore unable to protect airway, all participants are on mechanical

ventilation at admission. All of the severe TBI patients were paralyzed and sedated via intravenous medications. Train of Four was used to assess level of the medicated state. Neurological assessments were performed every 1 hour for the first 24 hours and then every 2 hours during the participants ICU admission. The paralytics and sedative medications were held for 15 minutes, every 1 to 2 hours in order to perform the neurological assessments, which included GCS. All of the patients as part of standard of care had an arterial blood line, from which blood pressure was continuously measured. All participants were monitored continuously for physiological parameters and treatment goals: intracranial pressure (ICP) [≤20 mmHg], cerebral perfusion pressure (CPP) [≥60 mmHg], arterial blood pressure (ABP) [≥90 mmHg], central venous pressure (CVP) [>7], pulse oximetry, respiratory rate, heart rate and rhythm, and core temperature. These variables were documented every 1-2 hours and prn. The protocol for the standard of practice which EVD's were drained changed over the course of time which the data were collected. EVD draining by venting for ICP >20mmHg was the practice from 2000-2002. EVD passive drainage method was utilized from 2002-2008. Nursing care; participant to nurse ratio was 1:1 or 2:1 depending on patient's condition. Some of the patients received hypothermia treatment which was controlled for in the analysis. The subjects enrolled from May 2002 to July 2005 were cooled to core temperature 33°C. From November 2005 to present subjects are cooled to core temperature of 32.5- 34°C. The time to target reach the target temperature was 6 hours for a duration of 48 hours for the cooling intervention. The modalities of cooling were; ice bags to groin, armpits, cooling 'blankets' to abdomen/thoracic area/thighs and iced saline lavage (via NG tube). Hypothermia would not change the participants BCL-2 genotypes however, it may alter response to therapy in a gene specific manner.

3.7 DATA COLLECTION

3.7.1 Summary of acute care phase data collection procedures in the parent study

In the acute care phase, data was collected by project personnel over the first 5 days after initial injury (6 days for bcl-2 protein levels). Demographic and medical condition data was collected as part of the study protocol on data collection sheets. The data was electronically transmitted directly into an automated data entry and verification system (Teleform) or recorded on a form that was hand entered and verified. Medical records, electronic medical records, and neurological assessments were downloaded off of the medical center electronic documentation computer system. The data was recorded into the data base. Data from the parent study data base was analyzed in relation to the BCL-2 genotypes.

3.7.1.1 Collection of Bagged CSF Samples

As standard medical care and as an inclusion criterion, each participant in the study had an EVD in for routine care. The EVD was zeroed and calibrated every 1-2 hours and prn. The medical protocol for EVD venting was subject to the chief of neurosurgery at the time the specimen was collected. Therefore some of the CSF samples were collected under a continuous drainage protocol (EVD at 20 mm Hg above midbrain) and others under a venting protocol (EVD at 20 mm Hg above midbrain). The venting protocol required the EVD to remain closed for ICP's ≤ 20 mmHg and vented for 5 minutes should ICP's rise > 20 mmHg. The elevation of the ICP and venting protocol allows for CSF to collect in the drainage bag to gravity. The EVD venting protocol changes would not affect the BCL-2 genotype data but could impact physiologic data parameters being investigated by this study. The research staff changed the

EVD collection bag every 12 hours using sterile technique as per UPMC protocol. Approximately 30 ml of the bag collected CSF was aliquoted into five- 1 ml tubes and five -5 ml tubes every 12 hours. The amount of CSF drainage collected every 12 hours was based on CSF available after drainage based on the EVD venting protocol. DNA obtained from CSF was analyzed for patients for whom blood samples were not available for genotyping BCL-2. This process of CSF collection was also utilized in the collection of CSF samples for Bcl-2 protein levels. Samples were stored via measurement specific protocols.

3.7.1.2 Collection of Fresh CSF Samples

Neurometabolites (lactate, pyruvate, and LP ratio) required the analysis of fresh CSF. Based on the procedure of the parent study; the CSF samples were obtained by turning the stopcock on the ventriculostomy catheter closest to the patient ; turning the stopcock off to the patient; aseptically cleansing the needle insertion port with betadine for 5 minutes per hospital protocol; inserting a needle into the port and withdrawing the CSF (3cc's over 3 minutes) from the tubing into a sterile syringe; injecting the CSF into a tube labeled with the patient identification number, date, and time of fluid removal and placed in a -80'C degree freezer. The samples were thawed and run in batches for lactate and pyruvate concentrations.

3.7.1.3 Collection of Blood Samples

Trained research personnel draw 1 purple top tube (EDTA) of blood (approximately 3 ml) from the participant's arterial line each morning of the first 5 days post injury. The specimen was processed within 48 hours for extraction of the buffy coat and plasma. DNA extracted from blood specimens was utilized for BCL-2 genotyping and was used in the genotyping of the APOE data.

3.7.1.4 CBF

Based upon the parent study protocol; Xenon computed topography (XeCT) CBF measurements were made using standard commercial CT (computed topography) hardware and software scanning system requires for gas delivery and CBF calculations in a subset of subjects. Patients were transported from the emergency department or NTICU to the Radiology department on days 1, 3, and 5 for routine XeCT CBF measurements when medically stable for transport. XeCT CBF data is not available on all subjects due to medical instability and changes in parent study protocol in 2002. All patients were mechanically ventilated using a volume ventilator and paralyzed and sedated. Patients were ventilated using a bag –valve mask during transport to CT and carbon dioxide was monitored and reached a steady state prior to the scan. The arterial pressure was continuously monitored with an intra-arterial catheter attached to a pressure transducer prior to and following the procedure. The peripheral arterial oxygen saturation was monitored continuously with a pulse oximeter and the end-tidal carbon dioxide was monitored and recorded during the procedure.

After patients were positioned within the scanner along the orbitomental plane, the head was immobilized within a vacuum activated cranial mold (Olympic Medical, Seattle, WA, U.S.A.). Two baseline scans were conducted consisting of two or three axial planes 5 cm thick and 20 cm apart. Four to six enhanced scans were obtained at each of three levels following delivery of a mixture of 28% stable xenon and 40% oxygen (XeSCAN: Linde Medical Gases Somerset, NJ, U.S.A.) via the ventilator for 4.5 minutes. The end-tidal Xenon concentration was recorded by a thermoconductivity analyzer. CBF was measured and calculated from sequential CT voxels (series of enhancement values for each voxel 1 x 1 x 10mm3 as a function of time (based on a modified Kety equation assuming a single compartment). Approximately 25,000

CBF values were calculated for each CT slice and the image was analyzed as described by Gur et al., (1982) and Linskey, et al. (1992). The blood flow was averaged within 2 cm circular regions of interest (ROI), by hemisphere and total global flow including white and gray matter. Standard of care in our institution was for a Xe/CT CBF to be conducted on Days 1, 3, and 5 as part of this protocol.

3.7.2 Summary of longitudinal outcome phase data collection procedures

The battery of neuropsychological testing was conducted with each participant at 3, 6, 12, and 24 months post TBI. The tests were conducted by trained neuropsychological technician, under the direction of a neuropsychologist, in a face to face visit; either at the outpatient neurosurgical clinic of the medical center, the participants' home or rehabilitation setting or via phone interview as previously described.

3.8 MEASUREMENT

The independent variable in this study for both specific aims #1 and #2 was BCL-2 genotypes in subjects with severe TBI. The dependent variables for specific aim #1 were DRS, GOS, and mortality at 3, 6, 12, and 24 months after injury. NRS-R, Trails A, and Trails B, at 3, 6, and 12 months post injury. The dependent variables for specific aim #2 are biological/ clinical data (bcl-2 protein, neurometabolites [lactate, pyruvate, and LP ratio] and CBF). The covariates were age, GCS, gender, race, hypothermia versus normothermia. Some of the sub-studies

allowed for additional documented covariates of hypoxia, hypotension, and seizure activity to be added to the statistical models.

3.8.1 Independent Variable: BCL-2 Genotypes

3.8.1.1 DNA Extraction from CSF

DNA extraction was completed by the Conley lab as part of the parent study. The following is the protocol which the Conley lab used.

CSF extraction was conducted using QiAMP midi DNA Kits (Qiagen). The frozen CSF specimens were quick thawed in small batches. Into a 15 ml conical tube 2 ml of CSF were placed. To the CSF, 200 µl of Protease was added. Then 2.4 ml of Buffer AL were added and mixed thoroughly by vortexing 3X 5 seconds each time. The mixture was then incubated at 70ºC for 10 minutes. After the incubation period 2 ml of 96-100% ethanol (ETOH) was added and mixed by vortexing. This was done to separate DNA out from other protein. Half of the solution was then transferred to Midi column resting in a 15 ml conical tube. The tube was then centrifuged at 3000 rpm for 3 minutes. After this process the column was removed from the tube and the filtrate was discarded. The column was placed back into the tube and the rest of the solution into the column. The tube was again centrifuged at 3000 rpms for 3 minutes. The column was then removed and the filtrate was discarded. The column was returned to the tube and 2ml of buffer AW1 was added to the column and centrifuged at 5000 rpm for 1 minute. After this process 2 ml of buffer AW2 was added to the column and centrifuged for 15 minutes. The column was then placed in a clean 15 ml conical tube and the previous conical tube was discarded with wash buffers. To the column 300µl of buffer AE was added then incubated at room temperature for 5 minutes, then centrifuged at 5000 rpm for 5 minutes. The 300µl of buffer

AE that was spun down was reloaded and incubated for 5 minutes and centrifuged for 5 minutes. The next step was to load 300µl of fresh AE buffer into the column and incubated for 5 minutes and centrifuged for 5 minutes. The DNA extraction was then placed into 1.5 ml tube with the appropriate UID number and stored at 4°C.

3.8.1.2 DNA Extraction from Blood

DNA extraction was completed by the Conley lab as part of the parent study. The following is the protocol which the Conley lab used.

The 3ml blood tubes drawn from the participant were centrifuged at 2500 rpm for 5 minutes, the buffy coat was then removed, 1ml of a protective freezing solution added, and frozen in 50 ml conical tubes at -20ºC until batch extraction. The buffy coat specimens were quick thawed in small batches. Into each tube, 3 ml of Lysis solution was placed. The tubes were then vortexed and via transfer pipette, contents of the tubes were transferred to a labeled 15 ml conical tube. To the tubes 200 µl of 10% SDS was added along with 500µl of Proteinase K solution (9 ml batch solution made consists of 900 µl of 10% SDS; 36µl of 0.5 M EDTA; 18mg of Proteinase K; and 8.1 ml of sterile water.) The tubes were covered with parafilm and placed in a 37ºC rotating oven overnight to digest. The tubes were removed from the oven and 1 ml of 6M (saturated) NaCl was added per tube and shaken 15 seconds until foamy. The tubes were then centrifuged 15 minutes at 2500 rpms. The supernatant was then transferred to labeled 15 ml conical tubes. Then absolute ETOH, at 2X the volume of the supernatant, was added and inverted until the DNA precipitated out of the solution. The DNA was removed with sterile loops and placed in labeled flip top tubes. To the tubes, 70% ETOH was added to cover the DNA. The tubes were then microfuged for 10 minutes at the 14 setting. The ETOH was then pippetted off. The tubes were then places in a 37ºC oven until the ETOH evaporated off. Added to each tube

was 1 ml of 1X TE buffer. The tubes were then sealed with parafilm and taped to the sides of wheels in the rotating oven. The tubes were allowed to rotate until the DNA pellet goes into solution. DNA extracted from the buffy coat of the blood was stored at 4° C.

3.8.1.3 Procedure for BCL-2 Genotype Collection

The International HapMap Project is a partnership of scientists and funding agencies from Canada, China, Japan, Nigeria, the United Kingdom and the United States to develop a public resource that assists researchers in finding genes associated with human disease and response to pharmaceuticals. Based on HapMap data (April, 2007) there were 20 high priority tagging single nucleotide polymorphisms (SNPs) for the BCL-2 gene [(RS= reference SNP identification number): RS1026825, RS12454712, RS12968517, RS1381548, RS1481031, RS17756073, RS17759659, RS1801018, RS1944419, RS3810027, RS4456611, RS4941185, RS7230970, RS7236090, RS8083946, RS899968, RS949037]. For many of the currently chosen SNPs, TaqMan® assays on demand were already available through Applied Biosystems Incorporated (ABI). The SNPs for assays that were not available off the shelf were obtained via custom order by ABI design service. ABI was unable to make a successful assay for RS7231914, RS18089538, and RS2850762. Therefore this study analyzed the DNA of the eligible subjects for 17 tagging SNP's. The criterion used in the selection of the tagging SNP's was a minor allele frequency (MAF) of ≥30% and r^2≥.80. The tagging SNP's simply tags the haplotype block and does not address potential function capabilities. The haplotype block includes sizable regions over which there is little evidence for historical recombination and within which only a few common haplotypes are observed. The boundaries of blocks and specific haplotypes they contain are highly correlated across populations (Gabriel, S. et al., 2002; Micklos et al., 2003). Tagging

SNP's with a MAF <30% were not examined at this time because of the low prevalence in the population of the minor allele and limitations of our available sample size.

An ABI Prism® 7000 Sequence Detection System was used to conduct allele discrimination assays using TaqMan® assays. This is a highly automated, high throughput genotyping method for SNPs. Primers flank each polymorphism and polymerase chain reaction (PCR) is conducted using subject DNA. The main difference between TaqMan® PCR and traditional PCR is the use of a probe that is labeled with a reporter and a quencher dye that recognizes a specific allele of a SNP. Each allele of a SNP has its own probe with its own reporter dye (See Appendix C). If the subject's DNA is homozygous for one allele of the SNP, only the probe for that allele will hybridize and only the reporter dye for that allele will be liberated and measured by the ABI Prism® 7000 Sequence Detection System. A heterozygote will have both probes hybridize and both reporter dyes measured. Using this method, each SNP can be genotyped accurately and quickly. For each subject, 200ng of DNA was utilized for each assay.

BCL-2 was dichotomized based on the frequency of the genotype in the database which was being analyzed. Therefore, the homozygous genotype with the least frequency was combined with the heterozygotes and compared against the homozygous with the largest frequency in the particular database.

3.8.2 Dependent Variables

3.8.2.1 Dependent Variable: Bcl-2 Protein

Bcl-2 protein concentration analyses were performed by the Wagner research lab. Based upon the protocol in the parent study; CSF was passively and continuously drained from EVD for 12 hours time periods. CSF collection bags were collected least twelve time period following injury (fewer time periods if the EVD was removed with in this six day window due to the patient's improvement or death). Bagged ventricular CSF was refrigerated and central processed. Samples were then centrifuged at 3000 rpm for 10 minutes and aliquoted. Prior to analysis, samples were stored at -80 degrees Celsius until analysis. The concentrations of Bcl-2 protein were determined by using a commercially available enzyme linked immuno sorbent assay (ELISA) kit [EMD Chemicals Inc., Darmstadt, Germany]. ELISAs were performed according to the instructions which accompany the kit. The detection limit for the Bcl-2 kit used is up to 100 U/mL. All (100%) of the samples were analyzed in duplicate.

3.8.2.2 Dependent Variable: CBF

Xenon CT scan: Cerebral blood flow was typically measured on days 1, 3, and 5 using stable Xenon CT (Xe/CT) scan methodology. A baseline CT scan was obtained. Medical-grade xenon gas (28%) mixed with 40% oxygen (XeScan stable xenon in oxygen USP, Spectra Gas) was administered via the endotracheal tube for 4.3 minutes, during which time rapid sequential CT scanning of four pre-selected levels of the brain was performed. Xenon concentrations of expired gas were also monitored continuously. The data obtained was used in conjunction with time-dependent xenon concentrations measured in tissue to derive blood flow (mL/100 g

brain/min) by solving the Kety-Schmidt equation for each voxel the of CT image (1x1x10 mm3=24,000 voxels/CT level). Regions of interest (ROIs) were plotted on the scans in each of 10 vascular territories for both sides. The blood flow measurement was calculated for each ROI, each hemisphere and the entire brain.

3.8.2.3 Dependent Variable: Neurometabolites

Lactate, pyruvate, and lactate/ pyruvate ratio. Fresh CSF specimens were thawed in batches and processed for analysis of neurometabolites (lactate, pyruvate, and lactate/ pyruvate ratio) utilizing high pressure liquid chromatography (HPLC). Lactate (µmol concentration) and pyruvate (µmol concentration) were measured by HPLC using an ultraviolet detector (Model 486, Waters Corporation, Boston, MA) at 214 nm wavelength. Separation was achieved with a programmable autosampler that injected the sample onto a polymeric guard column in line with an organic acids column that constantly runs a solution of .1N sulfuric acid at a rate of 0.8 ml/min (Varian, Walnut Creek, CA). Peak areas were identified with micro-computer-based control Enpower software (Water corporation) with samples calibrated from standard curves of known standards. Lactate/ pyruvate ratio was calculated via formula driven excel spread sheet by dividing lactate into pyruvate based on the lactate and pyruvate generated/entered by Millennium, the system that drives the HPLC.

3.8.2.4 Dependent Variable: Neuropsychological Outcome Measures

Global Functional Outcomes

Glasgow Outcome Scale (GOS): The GOS, a clinical observation scale, categorizes global functional outcomes into five levels. It is an ordinal level of measurement: 1- Death, 2-

Persistent vegetative state, 3 - Severe disability, 4 - Moderate disability, 5 - Good recovery (Jennett & Bond, 1975; Jennett, 1976). A consensus conference in 1998 recommended evaluating basic functional assessment at 3, 6, and 12 months following a TBI (Wilson, Pettigrew & Teasdale, 1998). Interrater reliability has been reported from 68 - 95% with kappa values from .62 to .79 (Gennarelli et al., 1983; Maas et al., 1983) and correlates well with severity of illness and other measures of function such as DRS (Hall, Cope, & Rappaport, 1985). Interrater reliability is within the acceptable range when there is adherence to assessment guidelines, administration of a structured interview, and training of examiners (Wilson et al, 1998). (See Appendix D). The GOS has wide acceptance and established validity (Jennett & Bond, 1975; Maas et al., 1983).

Disability Rating Scale (DRS)

The DRS was designed to assess disability in patients recovering from severe TBI as they progress from severe coma to community reintegration (Rappaport, Hal, Hopkins, et al., 1982). There are 8 items in the measurement tool. Items 1 to 3 are identical to GCS, except for the points allotted. Items 4-6 assess cognitive ability to complete self care activities; knowing how and when to attend to feeding, toileting, and grooming needs. The motor (physical) capacity to participate in self care activities is accounted for in the seventh item, level of functioning (how dependent or independent a patient is when considering both cognitive and motor [physical] activities). The eighth item refers to employability options. The total score range from 0 (no disability) to 30 (death). The interrater reliability is 0.97- 0.98 (Gouvier, Blanton, et al., 1987 ; Rappaport et al., 1982) and test-retest reliability of 0.95 (Gouvier et al., 1987). (See Appendix E).

Mortality

Mortality is a binary variable (0=alive; 1=dead) and that was coded for by recoding the GOS outcome variable into scores 1 versus all others.

The Neurobehavioral Rating Scale- Revised (NRS-R)

The NRS-R was developed by Levin and colleagues (1990) to increase reliability and content validity of the original 1987 version Neurobehavioral Rating Scale (NRS). The NRS was originally a 27 item measurement modified from the Brief Psychiatric Rating Scale (BPRS) (Overall & Gorham, 1962) specifically for TBI patients. Both the NRS and the NRS-R measurements require a trained examiner. When using the NRS-R, the examiner bases scores from a 15-20 minute interview (two-thirds of the items) and patient observation during the interview (one-third of the items). The 29 items are scored on a 4 point Likert scale (absent, mild, moderate, and severe). NRS-R items address issues such as but not limited to: mental flexibility, irritability, tension/anxiety, alertness, attention, decreased initiation/motivation, suspiciousness, mental fatigability, hallucinating behavior, and motor retardation. The interrater reliability reported ranges from Kappa equal to 0.22 (difficulty planning) to 0.77 (memory difficulties); (median Kappa =0.4) (Vanier, Mazaux et al., 2000). Therefore inter-rater agreement is dependent on the use of the tool/sub-scale. NRS total score correlates with 6 month GOS (r=0.72) and DRS (r=0.74) (Lezek, Howieson, & Loring, 2004).

Trails Making Tests

The Trails Making Test was developed by United States Army psychologists as part of the Army Individual Test Battery (1944) and is in the public domain. The test is administered in two parts; Trails A which assesses attention and Trails B which assesses mental flexibility. There are several options for the administration for this test. This study utilized the commonly used Reitan (1958) method of the test administration (Lezek et al. , 2004).

Trails A

Attention was evaluated with the Trail Making Test-A (Trails A) which consists of a white sheet of paper on which 25 circles are distributed. Each circle contains a number from 1 to 25 (Basso, Bornstein, & Lang, 1999; Reitan & Wolfson, 1985). Subjects were asked to draw a line connecting all circles in numerical sequence as quickly and accurately as possible without lifting the pencil from the paper. Errors were immediately pointed out by the test administrator and corrected by the subject. The score is the amount of time, in seconds, to complete the test and includes any time needed for corrections. The number of errors committed were also recorded. Administration time is 3 minutes. This test may also be used to evaluate motor processing speed. (See Appendix F).

Trails B

Mental Flexibility was evaluated with the Trail Making Test-B (Trails B). The Trails-B consists of a white sheet of paper with 25 circles containing numbers 1 to 13 and letters A to L (Basso et al.,1999; Reitan & Wolfson, 1985). Subjects are asked to quickly draw a line consecutively connecting all the circles alternating between numbers and letters. The score is the amount of time to complete the task. Administration time is 3 minutes. This test may also be used to evaluate executive functioning. (See Appendix G).

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Trails A & Trails B Test Characteristics

The reliability coefficients vary based on method administration and population tested r= .60-.90 (Spreen & Strauss, 1998). Among the general population, performance may decline with age and improve with greater years of education (Fullerton, Wu, Zhao, & Johnston, 2003; Raudenbush, 1997; Raudenbush & Bryk, 2001; Ryan & Geckle, 2000). Test-retest reliability was r = .75 in 23 healthy adults (14 women, 9 men) with a mean age of 32.3 years (Davies, 1968). Among the TBI population, mild TBI patients versus controls have a slowing test times with increased severity of injury Leininger, Gramling, Farrell, Kreutzer, & Peck, 1990). Patients with moderate to severe TBI, have slower test times than controls on Trails B 2-5 years following injury (Spikeman, Deelman, & van Zomeren, 2000). Both Trails A and Trails B significantly contribute to the prediction as to what degree patients recovering from a moderately to severe TBI are independent in their living situation; patients with high Trails A and Trails B scores were found to have decreased independence (Acker & Davis, 1989).

3.8.3 Covariates

3.8.3.1 Covariate: severity of injury: Glasgow Coma Score (GCS)

The GCS is an instrument developed at the Glasgow Neurological Institute in Scotland by Teasdale and Jennett (Teasdale & Jennett, 1974) for a consistent general scale to assess depth and duration of impaired consciousness and coma. The GCS has had extensive worldwide testing for its usefulness in the assessment and prognosis of head injured patients. It is the clinical parameter closely linked to acuity in the head injured patient. The GCS score has been shown to

be reliable with minimal observer deviation when nurses and physicians tested the scale (Teasdale, Knill-Jones & Jennett, 1974). The 15 point scale has 3 aspects of behavioral response that are evaluated: best verbal response, best motor response and eye opening. Patients with high scores have the best chance of good recovery or moderate disability, and those with the lowest scores have an 80% chance of death or persistent vegetative state (Finklestein & Ropper, 1979). The predications of outcomes using GCS improves within 72 hours of injury (Jennett et al, 1976). The GCS at time of admission as documented by the neurosurgeon was be utilized. GCS recorded is without the impairment of alcohol, substance or medications. While GCS was be used as a covariate in this study, it was also a component of the inclusion criteria. All subjects had a GCS <9. GCS was dichotomized; 3-5 versus 6-8. Dichotomized GCS was used as the measure of severity of injury for statistical analysis. (See Appendix H).

3.8.3.2 Covariate: demographic characteristics

Demographic information (age, gender, race) was collected from the medical record upon entry into the study. Age was used as a continuous variable. Race was dichotomized as Caucasians vs non-Caucasians due to the lack of variability among the distribution of race. Race was omitted from the neurometabolite and CBF analyses due to non-Caucasians cell size of n=1.

3.8.3.3 Covariate: hypoxia

Hypoxia is defined as an oxygen saturation saO_2 of ≤80% for >30 minutes at the scene of the accident or during transport to the hospital. Subjects were coded as 0=no confirmed event and 1= confirmed event.

3.8.3.4 Covariate: hypotension

Hypotension was defined as sustained systolic blood pressure <90 mmHg for >30minutes at the scene of the accident or during transport. Subjects were coded as 0=no confirmed event and 1= confirmed event.

3.8.3.5 Covariate: seizure

Seizure was defined as a witnessed seizure at the scene of the accident or during transport. Subjects were coded as 0=no confirmed event and 1= confirmed event.

3.8.3.6 Covariate: hypothermia treatment

A subgroup of the sample used were enrolled in a randomized control trial to determine efficacy of therapeutic hypothermia in improving outcomes after severe TBI. Therapeutic hypothermia decreases metabolic needs of the brain and may protect the brain from secondary injury. As such, it was important to consider this therapy as a potential covariate in all analysis. Hypothermia was defined as follows: 1= subjects who were maintained in a hypothermic state (May 2002 to July 2005 cooled to core temperature 33°C; November 2005 to present cooled to core temperature of 32.5- 34°C) for the first 48 hours after injury or 0= subjects who were maintained in a non-hypothermic state for the first 48 hours after injury.

3.8.3.7 Covariate: APOEε4

Restriction Fragment Length Polymorphism (RFLP) analysis was utilized. First, a polymerse chain reaction (PCR) procedure was be set up as follows: 200ng of DNA was combined with 0.9nM of each primer flanking the variants (forward: 5' - TAA GCT TGG CAC GGC TGT CCA AGG A -3' and reverse: 5' - ACA GAA TTC GCC CCG GCC TGG TAC AC -

3')., 0.6U of taq polymerase, 1.5mM MgC12, IX reaction buffer and 200uM of each dNTP in a total reaction volume of 25ul. Each sample was exposed to thermal cycling with a programmable thermal cycler (MJ Research, model PTC-200). Thirty cycles of denaturation at 95oC for one minute, annealing at 57^{o}C for thirty seconds, and extension at 72^{o}C for one minute, amplified the PCR product, which was then exposed to HhaI restriction endonuclease to perform the RFLP analysis. The digested products were electrophoresed on a 6% polyacrylamide gel stained with ethidium bromide for DNA band detection and genotype assignment based on presence or absence of the original. A computer generated picture of the gel was recorded. Two independent researchers reviewed the pictures and assign an APOE genotype based on appropriate banding. Genotyping will be repeated in 10% of the samples to assure reliability. (See Appendix I).

3.9 DE-IDENTIFICATION PROCESS

Each patient screened for the parent study was assigned a unique identification number (UID). This number was the mechanism by which the data collected (raw data form and specimens) was de-identified. Throughout the study the UID number was used. Under the design of this study the primary investigator did not have access to link the UID's to any identifiable information.

3.10 STORAGE

The consent forms, which contain identity information, are kept separate from the data in a locked file cabinet. The data records are kept in a locked file cabinet and the database information are kept secure by password protection. The consent forms, data records, and databases are also secured in a locked office.

3.11 DATA MANAGEMENT

The data are linked by the UID's. The data base for this study did not contain any personal identification data. The data base was compiled by the personnel at the Brain Trauma Research Center at the University of Pittsburgh. In the parent study the APOE genotype data was hand entered and verified. Demographic and severity of injury data was extracted from the chart by study personnel, documented on data entry forms and entered electronically. Lactate and Pyruvate were electronically entered into an excel file by the Millennium software. For this study the BCL-2 genotyping data was the only new variable for which data needed to be entered. The TAQ MAN report data was exported to an Excel file, from which the data was merged with the pertinent variables from the parent study. All of the databases were in Excel spreadsheet. The excel spreadsheet were exported into SPSS [SPSS Inc., Chicago, Ill., versions 15 and 16] and SAS [SAS institute Inc., Cary, NC., version 9.1] statistical packages. SPSS was utilized for descriptive statistics and recoding the data. SAS was employed for preliminary and primary mixed models and generalized mixed models analyses.

3.12 DATA ANALYSIS PLAN

3.12.1 Preliminary Analyses

3.12.1.1 Accuracy

Accuracy of the data was assessed by screening for missing or out of range data. Frequency tables were performed for all categorical and ordinal variables (all 17 of the BCL-2 SNP's, GOS, mortality, age, GCS, gender, race, hypothermia, hypoxia, hypotension, seizures, and APOEε4 allele presence). Descriptive statistics (range, mean, median, mode) were preformed on the continuous variables (DRS, NRS-R, Trails A, Trails B, Bcl-2 protein, neurometabolites [lactate, pyruvate, LP ratio], and CBF) to assess the accuracy of those data. In order to accommodate missing data on the continuous variables mixed models analysis was used; GOS, while an ordinal variables, was treated as a continuous variable (Wagner et al., 2004). To accommodate the missing data in the Mortality (binary outcome) generalized mixed models was employed (i.e. participant lost to follow-up). Overall, missing data was examined but no attempt was made to impute missing values. NRS-R and Trails Making Tests can not accommodate an outcome for subjects who died or those who are too cognitively impaired to complete the measures. These subjects were removed from the overall data set to form a subset as it is not meaningful to include these people in the analyses. With this said, the data is not missing at random and the results are only generalizable to people who have had a severe TBI and have made a degree of cognitive recovery that they are able to participate in the measure. Other dependent variable missing data were assumed to be missing at random (lost to follow-up, patient/family refused, incarceration, the data were stopped being collected on all patients).

3.12.1.2 Distribution assumptions

Normality

Normality was assessed by examining histograms, skewness, and kurtosis for the continuous variables (DRS, GOS, NRS-R, Trails A, Trails B, Bcl-2 protein, neurometabolites [lactate, pyruvate, LP ratio], and CBF). Outliers or extreme values were noted in the Bcl-2 protein database as indicated by scatter plots. The extreme values were verified with the lab and protocol. Analyses were run with and without outliers. No gross non-normality was found among the other continuous variables.

HWE

Hardy-Weinberg Equilibrium was used to assess the distribution of the 17 BCL-2 SNP genotypes. Based upon this theory from population genetics there is an expected distribution of genotypes (where p2= genotype frequency of homozygous wild type; 2pq= genotype frequency of each alleles contribution from the heterozygous genotype; q2= frequency of homozygous variant). (See figure 3-1).

$$p2 + 2pq + q2 = 1$$

Figure 3-1: Hardy-Weinberg Equation

This formula was adapted to accommodate TAQ MAN assay labeling and is mathematically accurate. (See figure 3-2).

$$X^2 + 2XY + Y^2 = 1$$

Figure 3-2: Adapted Hardy-Weinberg Equation

HWE's were calculated for each SNP using a formula driven Excel spreadsheet. This was completed for the overall sample genotyped, global functional outcomes (GOS, DRS, and mortality) analyses as well as for all of the sub-analyses (NRS-R, Trails Making Tests, Bcl-2 protein, Neurometabolites, and CBF.). Violations of HWE were confirmed with Chi Square tests

to ascertain if the disequilibrium was statistically significant. For detailed information on HWE for the overall genotyping and each subsequent dependent variable please see tables 4-2, 4-4, 4-15, 4-19, 4-27, 4-33, and 4-43.

3.12.1.3 Preliminary (Abbreviated) Model Analyses: Continuous Dependent Variables

An abbreviated mixed models analysis was performed for each of the continuous/ordinal dependent variables (GOS, DRS, NRS-R, Trails A, Trails B, Bcl-2 protein, neurometabolites [lactate, pyruvate, and LP ratio] and CBF [right hemisphere, left hemisphere, and global) independently with each individual SNP (17) and the time point variable. Each of the continuous/ ordinal dependent variables were also analyzed independently with each individual covariate and time. This was done to select the SNP's and covariates of interest for the Primary Full Mixed Models analyses. The conservative criterion used for the selection of a SNP or covariate into the Primary analysis was Type 3 tests with $P \leq 0.2$. (Bendel & Afifi, 1977) recommend a less conservative $P \leq 0.1$.) This was a conservative significance level that was set a priori in order to focus the analyses. In addition, covariates that were deemed prudent through the evidence in the literature (age, gender, race, GCS, and hypothermia) were chosen a priori to be used in the Primary Full Mixed Models analyses regardless of significance for Type 3 tests. These predetermined covariates were added to each model unless there was a cell size of one or less for that covariate.

3.12.1.4 Preliminary (Abbreviated) Model Analyses: Binary Dependent Variable

The process of the preliminary (abbreviated) model analysis of the binary variable of mortality mirrors that of the process for the continuous variables with exception that a

generalized mixed model was used to accommodate for the binary nature of mortality (alive vs dead) and a conservative Type 3 $X^2 \leq 0.3$ criterion (Hosmer & Lemeshow , 2000)

3.12.2 Primary Analysis

Analysis of specific aims:

1. Specific Aim 1: Compare the relationship between BCL-2 genotype and global functional outcomes, cognitive-behavioral outcomes, and mortality attained by patients who have sustained a severe TBI.

RQ1. Is there a relationship between BCL-2 genotype and global functional outcomes (GOS and DRS), cognitive-behavioral outcomes (NRS-R, Trails A, and Trails B) and mortality attained by patients who have sustained a severe TBI?

Global Functional Outcomes

GOS: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, race, hypothermia, and hypotension were included in the model versus GOS.

DRS: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, race, hypothermia, hypoxia, hypotension, and seizure were included in the model versus DRS.

Mortality: A generalized mixed model analysis of the SNP of interest, time point, age, GCS, gender, race, hypothermia, hypoxia, hypotension, and seizure were included in the model versus mortality.

Cognitive-behavioral Outcomes

NRS-R: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, race, hypothermia, and seizure were included in the model versus NRS-R.

Trails Making Tests

Trails A: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, race, and hypothermia were included in the model versus Trails A. Hypoxia and hypotension were not included in the model despite Type 3 test significance due to only 1 subject meeting criteria in each of those covariates.

Trails B: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, race, and hypothermia were included in the model versus Trails A. Hypoxia and hypotension were not included in the model despite Type 3 test significance due to only 1 subject meeting criteria in each of those covariates.

2. Specific Aims 2: Compare the relationship between BCL-2 genotype and biological/ clinical data.

RQ2. Is there a relationship between BCL-2 genotype and biological/ clinical data (bcl-2 protein, neurometabolites [lactate, pyruvate, LP ratio], and CBF [right hemisphere, left hemisphere, and global] from patients who have sustained a severe TBI?

Bcl-2 Protein

Bcl-2 Protein with outliers: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, race, hypoxia, and seizure were included in the model versus Bcl-2 Protein level. None of the subjects in this subgroup were treated with hypothermic protocol.

Bcl-2 Protein without outliers: There is evidence that bcl-2 protein peaks between days 3 and 4 after injury (Lee et al., 2004; Xiong et al., 2001)). Based on preliminary descriptive analysis there are 2 potential extreme values. A separate analysis was performed without the 2 extreme values. A mixed models analysis of the SNP of interest, time point, age, GCS, gender,

race, hypoxia, and seizure were included in the model versus Bcl-2 Protein level. None of the subjects in this subgroup were treated with the hypothermic protocol

Neurometabolites

Lactate: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, and hypothermia were included in the model versus lactate concentration. There was only one non-caucasian subject in this subgroup; therefore, race was not used in this analysis.

Pyruvate: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, \and hypothermia were included in the model versus pyruvate concentration. There was only one non-caucasian subject in this subgroup; therefore, race was not used in this analysis.

L/P ratio: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, and hypothermia were included in the model versus L/P ratio. There was only one non-caucasian subject in this subgroup; therefore race was not used in this analysis.

CBF

Right Hemisphere: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, and hypothermia were included in the model versus right hemisphere. There was only one non-caucasian subject in this subgroup; therefore, race was not used in this analysis.

Left Hemisphere: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, and hypothermia were included in the model versus left hemisphere. There was only one non-caucasian subject in this subgroup; therefore, race was not used in this analysis.

Global: A mixed models analysis of the SNP of interest, time point, age, GCS, gender, and hypothermia were included in the model versus global. There was only one non-caucasian subject in this subgroup; therefore race was not used in this analysis.

4.0 RESULTS

4.1 SAMPLE DESCRIPTION FOR OVERALL GENOTYPES

The subjects enrolled in this study were injured between May 2000 and April 2007. The mean age of the overall sample of 230 subjects genotyped was 34.4 year old (range 16-73; SD± 14.8). (see figure 4-1). The genotype information is reported in table 4-1.

Table 4-1: BCL-2 Genotype Information

	BCL-2 Genotype Information										
	SNP RS Number	Alleles Base Δ	Ancestral Allele	Homozygous XX Allele	Homozygous YY Allele	Heterozygous XY Allele	n=	Study Minor Allele Frequency	Hap-Map Minor Allele Frequency	Location of the Tagging SNP	Position
3' end	RS17756073	A/G	A	111 (AA)	27 (GG)	83 (A/G)	221	0.310	0.3	intron	58960763
I	RS4456611	C/T	T	57 (CC)	50 (TT)	113(C/T)	220	0.484	0.467	intron	58961432
I	RS1026825	A/G	T	65(AA)	52 (GG)	112 (A/G)	229	0.472	0.458	intron	58971255
I	RS7230970	C/T	T	44 (CC)	79 (TT)	93(C/T)	220	0.411	0.473	intron	58972056
I	RS899968	A/C	A	40 (AA)	83 (CC)	99(A/C)	221	0.405	0.425	intron	58973244
I	RS4941185	A/G	A	77 (AA)	45 (GG)	102(A/G)	225	0.427	0.4	intron	58974534
I	RS12454712	C/T	T	31(CC)	99 (TT)	98 (C/T)	228	0.351	0.362	intron	58996864
I	RS1481031	A/G	A	95 (AA)	24 (GG)	107(A/G)	226	0.343	0.325	intron	59003065
I	RS3810027	C/G	G	99 (CC)	28(GG)	98(C/G)	225	0.342	0.331	intron	59054958
I	RS8083946	A/G	A	47 (AA)	87(GG)	95(A/G)	216	0.438	0.333	intron	59056901
I	RS1944419	A/T	T	58 (AA)	51(TT)	111(A/T)	220	0.484	0.458	intron	59075593
I	RS12968517	C/T	T	99 (CC)	35(TT)	94(C/T)	228	0.360	0.292	intron	59092951
I	RS7236090	C/T	T	63(CC)	55(TT)	103(C/T)	224	0.475	0.45	intron	59098091
I	RS1381548	A/G	A	40 (AA)	81(GG)	101(A/G)	222	0.408	0.392	intron	59108376
I	RS17759659	A/G	A	83 (AA)	31(GG)	110(A/G)	224	0.384	0.483	intron	59109624
I	RS949037	C/T	C	49 (CC)	70(TT)	103(C/T)	229	0.439	0.4	intron	59129993
5' end	RS1801018	A/G	A	44 (AA)	82(GG)	102(A/G)	228	0.417	0.475	exon 2 synonymous	59136859

The sample was primarily Caucasian (n=213; 92.6%) and male (n=178; 77.4%) (see figure 4-2 and 4-3). The majority of the sample genotyped had admission GCS of 6-8 (n=136;

59.1%) (see figure 4-4). The mechanism of injury for the majority of the sample was related to an automobile crash (n=114; 49.6%) (see figure 4-5). Of the subjects enrolled in the study n=40 (17.4%) received a hypothermia intervention. In the overall sample the presence of sustained hypoxia (n=28; 12.7%), hypotension (n=19; 8.3%), documented pre-admission seizures (n=8; 3.5%), and the presence of APOE ε4 (n=58; 25.2%) were considered as covariates. Refer to table 4-2 for sample description.

All of the SNP's genotyped for the overall sample met or approached the Hardy-Weinberg Equilibrium (HWE) criteria for genotype representation in a population. For the SNP's (RS4941185, RS7230970, RS 7236090, RS8083946, RS899968, and RS 949037) that had attained HWE values that were not equal to 1, a Chi Square test was performed to assess if there was a significant difference. Only RS8083946 significantly deviated from anticipated HWE (X^2=4.76139; P=0.02911). Refer to table 4-2 for the Chi Square Results. Refer to specific tables (4-2, 4-4, 4-15, 4-19, 4-27, 4-33, and 4-43) for genotype frequencies for each SNP and HWE calculations for the overall sample and each portion of specific aims 1 and 2. From this overall sample, subjects were "selected" for inclusion in the outcomes data and/ or clinical/ biological data analyses based on the availability of the data being analyzed in comparison to genotypes. Refer to table 4-3 for a summary of the sample sizes for each of the analyses.

Table 4-2: BCL-2 Genotypes Overall Summary Descriptive Data

BCL-2 Genotypes Overall Summary Descriptive Data					
	Variable	**n=**	**Mean**	**Std. Deviation**	**Range**
	Age	230	34.38	14.836	16-73
				Frequency (%)	**Frequency (%) of Unknown**
	GCS Scores		3	24 (10.4%)	4 (1.7%)
			4	37 (16.1%)	
			5	29 (12.6%)	
			6	42 (18.3%)	
			7	74 (32.2%)	
			8	20 (8.7%)	
	Dichotomized GCS	226	score 3-5	90 (39.1%)	4 (1.7%)
			score 6-8	136 (59.1%)	
	Gender	230	Male	178 (77.4%)	0
			Female	52 (22.6%)	
	Race	230	Caucasian	213 (92.6%)	0
			African American	13 (5.7%)	
			Asian	1 (0.43%)	
			Hispanic	1 (0.43%)	
	Hypothermic	230		40 (17.4%)	0
	Hypoxia	176		28 (12.7%)	54 (23.5%)
	Hypotensive	176		19 (8.3%)	54 (23.5%)
	Seizures	176		8 (3.5%)	54 (23.5%)
	APOEe4	225		58 (25.2%)	5 (2.2%)

Hardy-Weinberg Equilibrium										
SNP	**n=**	**XX Allele**	**YY Allele**	**XY Allele**	**Undetermined**	**X**	**Y**	**HWE**	**X^2**	**P**
RS1026825	229	65	52	112	1 (0.4%)	0.528384279	0.47162	1	.	.
RS12454712	228	31	99	98	2 (0.9%)	0.350877193	0.64912	1	.	.
RS12968517	228	99	35	94	2 (0.9%)	0.640350877	0.35965	1	.	.
RS1381548	222	40	81	101	8 (3.5%)	0.407657658	0.59234	1	.	.
RS1481031	226	95	24	107	4 (1.7%)	0.657079646	0.34292	1	.	.
RS17756073	221	111	27	83	9 (3.9%)	0.690045249	0.30995	1	.	.
RS17759659	224	83	31	110	6 (2.6%)	0.616071429	0.38393	1	.	.
RS1801018	228	44	82	102	2 (0.9%)	0.416666667	0.58333	1	.	.
RS1944419	220	58	51	111	10 (4.3%)	0.515909091	0.48409	1	.	.
RS3810027	225	99	28	98	5 (2.2%)	0.657777778	0.34222	1	.	.
RS4456611	220	57	50	113	10 (4.3%)	0.515909091	0.48409	1	.	.
RS4941185	225	77	45	102	6 (2.6%)	0.568888889	0.42667	0.99113	1.107	0.2926
RS7230970	220	44	79	93	14 (6.1%)	0.411363636	0.57045	0.96397	2.8899	0.0891
RS7236090	224	63	55	103	9 (3.9%)	0.511160714	0.47545	0.97339	0.98174	0.3218
RS8083946	216	47	87	95	1 (0.4%)	0.4375	0.62269	1.12399	4.76139	0.0291*
RS899968	221	40	83	99	8 (3.5%)	0.404977376	0.59955	1.00907	1.19416	0.2745
RS949037	229	49	70	103	8 (3.5%)	0.438864629	0.53057	0.9398	0.90063	0.3426

*** X^2 p≤ 0.05**

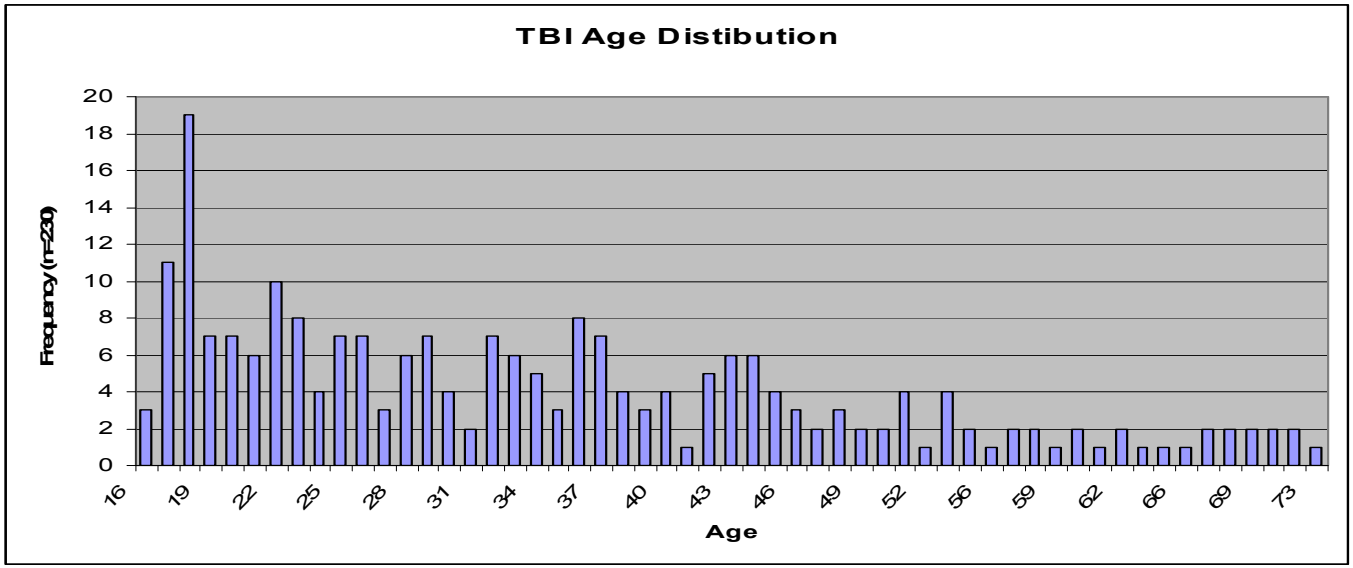


Figure 4-1: TBI Age Distribution

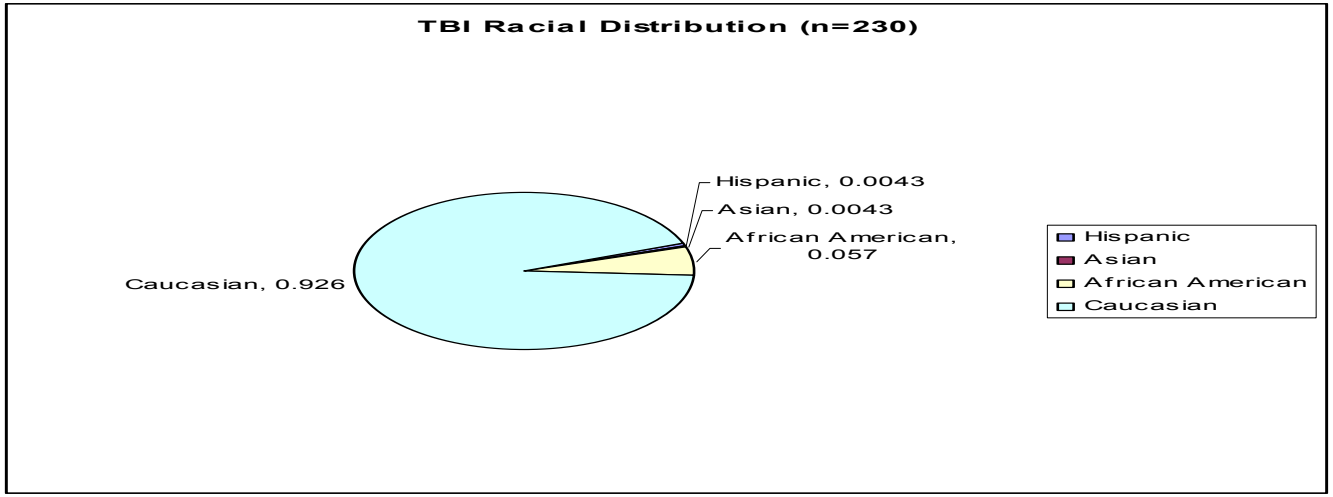


Figure 4-2: TBI Racial Distribution

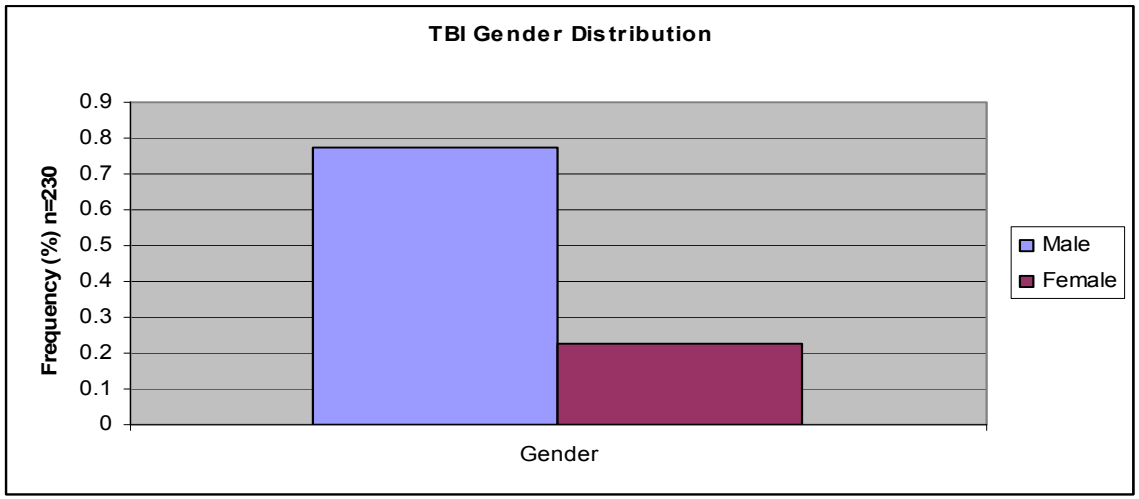


Figure 4-3: TBI Gender Distribution

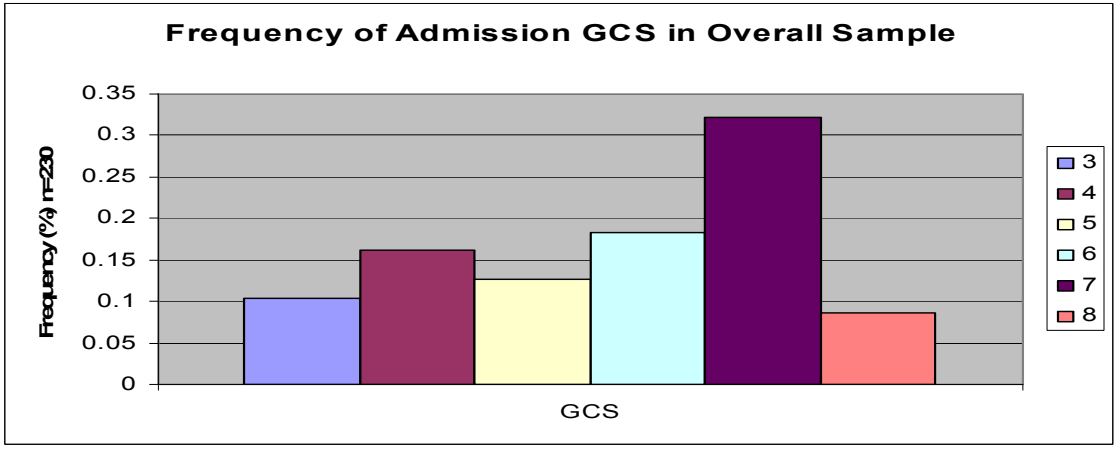


Figure 4-4: Frequency of Admission GCS in Overall Sample

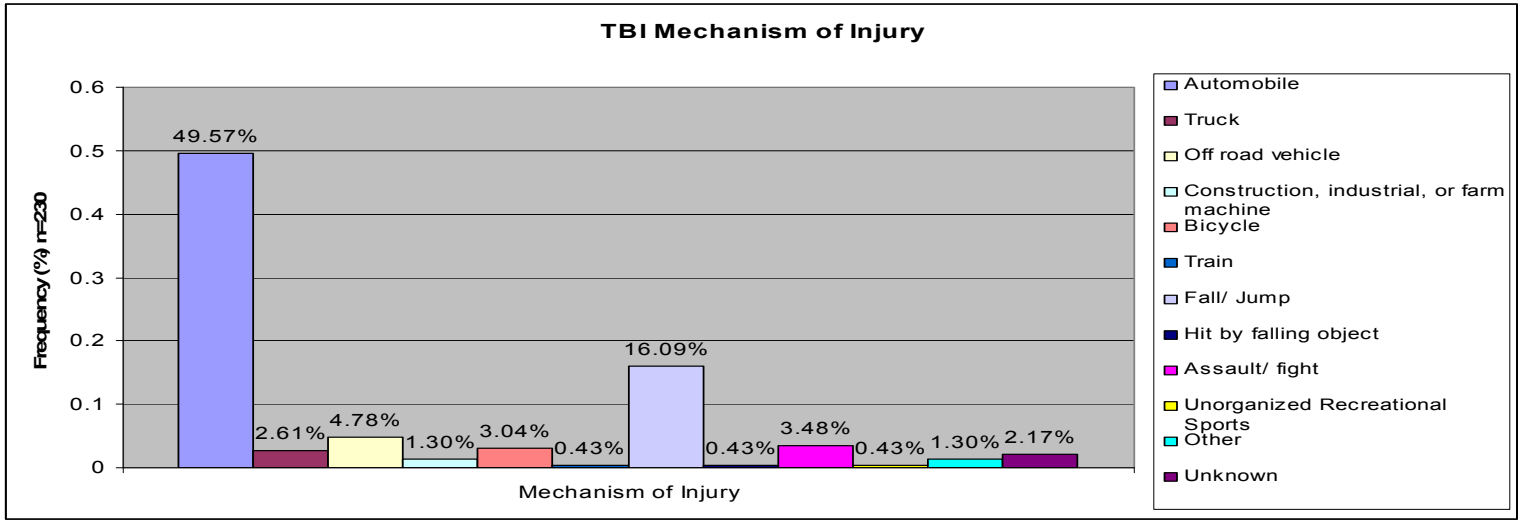


Figure 4-5: TBI Mechanism of Injury

Table 4-3: Summary Table of Sample Size

Summary Table of Sample Size		
Database	**Type of Analysis**	
	Preliminary Mixed Models	**Primary Mixed Models**
Outcomes Data	n=	n=
GOS	201	141
DRS	201	141
Mortality	201*	141*
NRS-R	95	61
Trails A	30	27
Trails B	30	27
Biological/ Clinical Outcomes		
Bcl-2 Protein Levels	42	37
Neurometabolites	36	36
CBF	17	17
*Generalized mixed model to account for binary nature of the mortality outcome.		

4.2 RESEARCH QUESTIONS

4.2.1 The Specific Aims and the Research Questions

1. Specific Aim 1: Compare the relationship between BCL-2 genotype and global functional outcomes, cognitive-behavioral outcomes, and mortality attained by patients who have sustained a severe TBI.

RQ1. Is there a relationship between BCL-2 genotype and global functional outcomes (GOS and DRS), cognitive-behavioral outcomes (NRS-R, Trails A, and Trails B), and mortality attained by patients who have sustained a severe TBI?

4.2.1.1 Global Functional Outcomes

Sample Description

The mean age of the overall sample of 201 subjects was 34.2 year old (range 16-72; SD± 14.4). The sample was primarily Caucasian (n=188; 93.5%) and male (n=159; 79.1%). The majority of the sample genotyped had admission GCS of 6-8 (n=117; 58.2%). Of the subjects enrolled in the study 38 (18.9%) received a hypothermia intervention. In the overall sample the presence of sustained hypoxia (n=26;12.94%), hypotension (n=12; 5.97%), documented pre-admission seizures (n=8; 3.98%), and the presence of APOE ε4 (n=46; 22.89%) were considered as covariates. Refer to table 4-4 for sample description. All of the SNP's genotyped for the overall sample met the HWE criteria for genotype representation in a population. Refer to table 4-4 for genotype frequencies for each SNP and HWE calculations.

When reviewing the available data, a trend of decreasing number of subjects with data available in each of the outcome time points was noted. This can be explained by the following rationales; the subject did not reach the time point milestone evaluation based on date of injury and loss to attrition due to refused the assessment, unable to contact/find, or in prison.

At the 3 month outcomes data collection of the 201 subjects in this analysis 198 (98.5%) of the subjects were captured for a mortality rate approximately one quarter of the sample (n=51; 25.4%). The mortality rate stabilizes in reference to the 6, 12, and 24 month outcome data with adding seven confirmed subject deaths by the 24 month outcome time point. (See table 4-4).

Interestingly at a 12 months post injury according to GOS scores; 27.9% of the subjects had died (GOS=1), 20.4% had a poor outcome (GOS= 2-3), 30.8% (GOS= 4-5) had a good outcome, and 21.4% of the subjects outcome was either too early to evaluate or unknown. (See table 4-4).

The DRS means at the 3, 6, and 12 months outcomes evaluation classify the disability of the subjects as having severe limitations (DRS score 12-16). At the 24 months outcomes evaluation, 41.3% of the subjects outcomes are unknown, and the mean score of the known subjects was 17.3 placing the outcomes in the extremely severe limitations category, however, all of the scores from the patients who had died, 58 of the 118 subjects or 49.2% known outcomes, are included in the 24 months statistics. (See table 4-4).

Preliminary Analysis (Abbreviated Mixed Models)

In the preliminary mixed model analyses, each of the SNP's was analyzed individually for a relationship to each of the global functional outcome variables (GOS and DRS) over time. Each of the covariates was analyzed for potential relationship with the global functional outcome

variable over time. By doing the preliminary analyses the SNP's and covariates of interest were ascertained and were used in building the larger model. (Refer to tables 4-5 and 4-6).

The preliminary mixed models analyses for GOS indicated that the SNP's and covariates of interest, meeting the criterion (P for Type-3 Tests P<0.2) were: RS12968517 (genotypes P=0.0899; dichotomized genotype P=0.5371); RS 17759659 (genotypes P=0.0524; dichotomized genotype P=0.0153); RS 1801018 (genotypes P=0.0923; dichotomized genotype P=0.18273); RS1944419 (genotypes P=0.3303; dichotomized genotype P=0.1465); RS4941185 (genotypes P=0.0376; dichotomized genotype P=0.4693); RS 7236090 (genotypes P=0.0966; dichotomized genotype P=0.2195); RS949037 (genotypes P=0.197; dichotomized genotype P=0.437); Time (months) (P <.0001); Age (P <.0001); GCS (P <.0001); and Gender (P= 0.1085). Race (P=0.3485) and hypothermia (P= 0.6511) were not indicated in the preliminary analyses, however, they were added to the full model as discussed in the methods section. (Refer to tables 4-5 and 4-6).

The preliminary mix models analyses for DRS indicated that the SNP's and covariates of interest, meeting the criterion were: RS12968517 (genotypes P=0.0722; dichotomized genotype P=0.4163); RS17756073 (genotypes P=0.1981; dichotomized genotype P=0.6903); RS17759659 (genotypes P=0.0325; dichotomized genotype P=0.0089); RS1801018 (genotypes P=0.0298; dichotomized genotype P=0.0813); RS1944419 (genotypes P=0.3075; dichotomized genotype P=0.1244); RS4941185 (genotypes P=0.0272; dichotomized genotype P=0.7106); RS7236090 (genotypes P=0.0121; dichotomized genotype P=0.061); RS949037 (genotypes P=0.1441; dichotomized genotype P=0.508); Time (months) (P <.0001); Age (P <.0001); GCS (P <.0001); Gender (P= 0.1696); Hypoxia (P= 0.1972); Hypotension (P= 0.1131); and Seizures (P= 0.1676). Race (P=0.6144) and hypothermia (P= 0.3417) were not indicated in the preliminary analysis,

however, were added to the full model as discussed in the methods section. Unlike GOS, the preliminary analyses of DRS data, hypoxia, hypotension, and seizure covariates did meet the criterion. (Refer to tables 4-5 and 4-6).

Table 4-4: Bcl-2 Genotypes Versus Global Outcomes Descriptive Data

BCL-2 Genotypes Versus GOS, DRS, and Mortality Descriptive Data							
Variable	**n=**	**Mean**	**Std. Deviation**	**Range**			
Age	201	34.16	14.395	16-72			
			Frequency (%)	**Frequency (%) of Unknown**			
GCS	201	score 3 to 5	84(41.8%)	0			
		score 6 to 8	117(58.2%)	0			
Gender (Male)	201		159(79.1%)	0			
Race (Caucasian)	201		188(93.5%)	0			
Hypothermic	198		38(19.2%)	3 (1.5%)			
Hypoxia	148		26(17.6%)	53 (24.6%)			
Hypotensive	148		12(8.1%)	53 (24.6%)			
Seizures	148		8(5.4%)	53 (24.6%)			
APOEε4	196		46(23.5%)	5 (2.5%)			
				Frequency (%)			
GOS		**Death (1)**	**Vegitative State (2)**	**Severe (3)**	**Moderate (4)**	**Good (5)**	**Unknown**
3 months	182	51(25.4%)	16(7.96%)	74(36.8%)	32(15.9%)	9(4.5%)	19 (9.5%)
6 months	182	55(27.4%)	11(5.5%)	54(26.9%)	37(18.4%)	25(12.4%)	19 (9.5%)
12 months	159	56(27.9%)	9(4.5%)	32(15.9%)	34(16.9%)	28(13.9%)	43 (21.4%)
24 months	118	58(28.9%)	4 (1.99%)	16(7.96%)	13 (6.5%)	27(13.4%)	83 (41.3%)
DRS		**Mean**	**Std. deviation**	**Range**	**Frequency (%) of Unknown**		
3 months	178	15.39	11.62	0-30	23 (11.44%)		
6 months	181	13.96	12.358	0-30	20 (9.95%)		
12 months	155	14.72	13.025	0-30	44 (22.9%)		
24 months	118	17.33	13.595	0-30	83 (41.3%)		
		Frequency (%)					
Mortality		**Death**	**Unknown**				
3 months	198	51(25.4%)	3 (1.5%)				
6 months	186	55(27.4%)	15 (7.5%)				
12 months	162	56(27.9%)	39 (19.4%)				
24 months	118	58(28.9%)	83 (41.3%)				
Hardy-Weinberg Equilibrium							
SNP	**n=**	**XX**	**YY**	**XY**	**X**	**Y**	**HW E**
RS1026825	201	59	49	93	0.524875622	0.47512438	1.0000
RS12454712	199	25	91	83	0.334170854	0.66582915	1.0000
RS12968517	200	90	31	79	0.6475	0.3525	1.0000
RS1381548	195	34	70	91	0.407692308	0.59230769	1.0000
RS1481031	198	87	21	90	0.666666667	0.33333333	1.0000
RS17756073	196	98	26	72	0.683673469	0.31632653	1.0000
RS17759659	197	74	28	95	0.616751269	0.38324873	1.0000
RS1801018	199	41	72	86	0.422110553	0.57788945	1.0000
RS1944419	192	50	46	96	0.510416667	0.48958333	1.0000
RS3810027	196	85	25	86	0.653061224	0.34693878	1.0000
RS4456611	193	49	48	96	0.502590674	0.49740933	1.0000
RS4941185	195	66	39	90	0.569230769	0.43076923	1.0000
RS7230970	193	40	67	86	0.430051813	0.56994819	1.0000
RS7236090	193	57	47	89	0.525906736	0.47409326	1.0000
RS8083946	200	42	77	81	0.4125	0.5875	1.0000
RS899968	195	36	76	83	0.397435897	0.6025641	1.0000
RS949037	194	44	64	86	0.448453608	0.55154639	1.0000

Preliminary Analysis (Abbreviated) Generalized Mixed Models: Mortality

The preliminary generalized mixed models analysis for Mortality indicated that the SNP's and covariates of interest, meeting the criterion (Type 3 X^2 <0.3) were: RS12968517 (genotypes P=0.1177, OR= 2.5; dichotomized genotype P=0.3732; OR=1.3); RS 17756073 (genotypes P=0.2853, OR= 2.1; dichotomized genotype P=0.6031, OR=0.84); RS 17759659 (genotypes P=0.1953, OR=0.86; dichotomized genotype P=0.0737, OR=1.8); RS 1801018 (genotypes P=0.2143, OR=0.69; dichotomized genotype P=0.6498, OR=1.2); RS4941185 (genotypes P=0.2763, OR=1.73; dichotomized genotype P=0.3528, OR=0.72); RS7230970 (genotypes P=0.2953, OR=0.78; dichotomized genotype P=0.8845, OR=1.05); RS7236090 (genotypes P=0.0994, OR=1.42; dichotomized genotype P=0.136, OR=0.59); Time (months) (P <.0001); Age (P <.0001, OR=1.05); GCS (P <.0001, OR=0.198); Gender (P= 0.3014, OR=1.5), Race (P=0.2963, OR=.049); and seizures (P= 0.1658, OR=3.32). Hypothermia (P= 0.9) was not indicated in the preliminary analysis, however, it was added to the full model as discussed in the methods section. Unlike DRS and/or GOS, RS1944419, RS949037, hypoxia, and hypotension did not meet the criterion. (Refer to table 4-10 and 4-11).

Primary Mixed Models Analysis for Global Functional Outcomes: Overview

Primary full mixed models were built for GOS and DRS using the SNP of interest from the preliminary analyses (RS12968517, RS17756073, RS 17759659, RS 1801018, RS1944419, RS4941185, RS7236090, and RS949037), time (month), age, GCS, gender, race, hypothermia, and hypotension. (Refer to tables 4-7 to 4-9). In the DRS model additional covariates of seizure and hypoxia were indicated to be added by the preliminary analysis. While SNP RS17756073 was not indicated in GOS preliminary analysis, models were built using this SNP's as to provide

comparison across the global functional outcome measures. The covariates of seizure and hypoxia were not included in the GOS because the P for type 3 test criterion were not significant for those covariates and could decrease the power of the analysis.

Primary full generalized mixed models were built for mortality using the SNP of interest from the preliminary analyses RS12968517, RS17756073, RS17759659, RS1801018, RS4941185, RS7230970, RS7236090, time (months), age, GCS, gender, race, seizures and hypothermia. While SNP RS949037, was not indicated in the mortality preliminary analysis, models were built using this SNP's because it was significant in the GOS and DRS analyses and as to provide comparison across the global functional outcome measures. (RS1944419 was not significant in GOS or DRS and therefore was omitted in the mortality analysis). The covariates of seizure and hypoxia were not included in the mortality analyses because they did not meet the set criterion. Overall in the analyses, time, age, and GCS remained significant across all of the full models for DRS, GOS, and mortality.

Primary Mixed Models for GOS

The mixed model with SNP RS12968517, genotype YY is significant (P= 0.0242) with overall genotype marginally significant (P for type 3 F tests =0.0591). YY genotype is associated with a decrease in GOS scores and is therefore associated with poorer outcomes. The significance of this SNP was not evident in the dichotomized analysis (P=0.455). See figures 6 and 4-7 for the adjusted GOS means over time versus genotype/ dichotomized genotype for this SNP. Gender was found to been marginally significant (genotypes P=0.0728; dichotomized genotype P=0.0845) with females having lower GOS scores, therefore, poorer outcomes. (Refer to table 4-7).

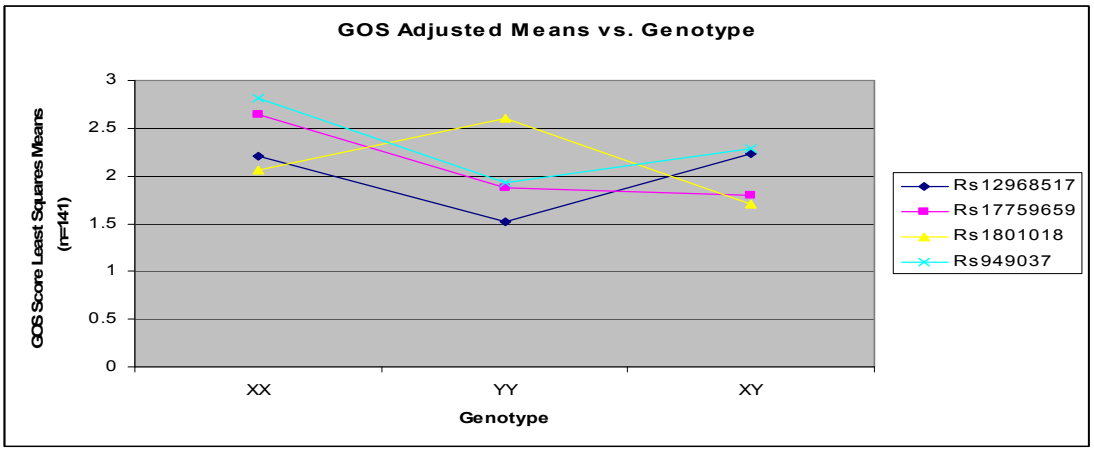


Figure 4-6: GOS Adjusted Means vs. Genotypes

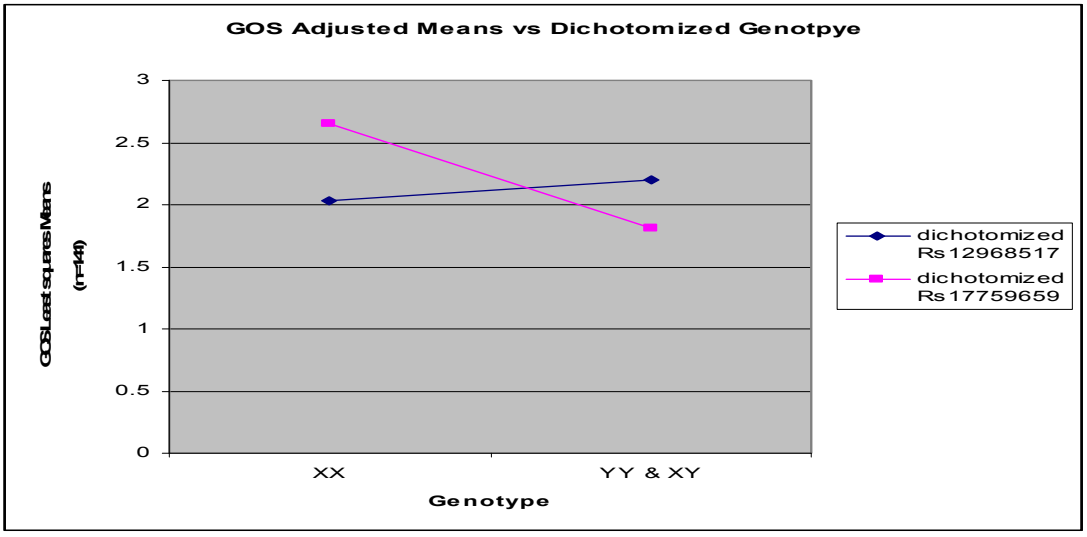


Figure 4-7: GOS Adjusted Means vs. Dichotomized Genotypes rs12968517 & rs17759659

The mixed model with RS17759659, genotype XX is significant (P<.0001) with overall genotype significant (P for type 3 F tests =0.0003). XX genotype is associated with increased GOS scores and is therefore associated with good outcomes (two copies of X) (See figures 4-6 and 4-7). This SNP was significant in the dichotomized analysis (P<.0001); subjects with 0 copies of X or heterozygous, have significantly lower GOS scores and poorer outcomes. Race was found to been marginally significant genotypes (P=0.0521) and significant for dichotomized

genotype (P=0.0477). Caucasians tend to have higher GOS scores and therefore better outcomes. (Refer to table 4-7).

The mixed model with RS1801018 genotype YY is significant (P< .0001) with overall genotype significant (P for type 3 F tests =0.0004). YY genotype is associated with higher GOS scores and is therefore associated with good outcomes (See figure 4-6). This SNP was significant in the dichotomized analysis (P=.0002); subjects with 0 copies of Y or heterozygous, have significantly lower GOS scores and poorer outcomes (See figure 4-8). Race was found to been significant in the models (genotypes P=0.0392; dichotomized genotype (P=0.0277). Caucasians have higher GOS scores and therefore better outcomes in this sample. (Refer to table 4-8).

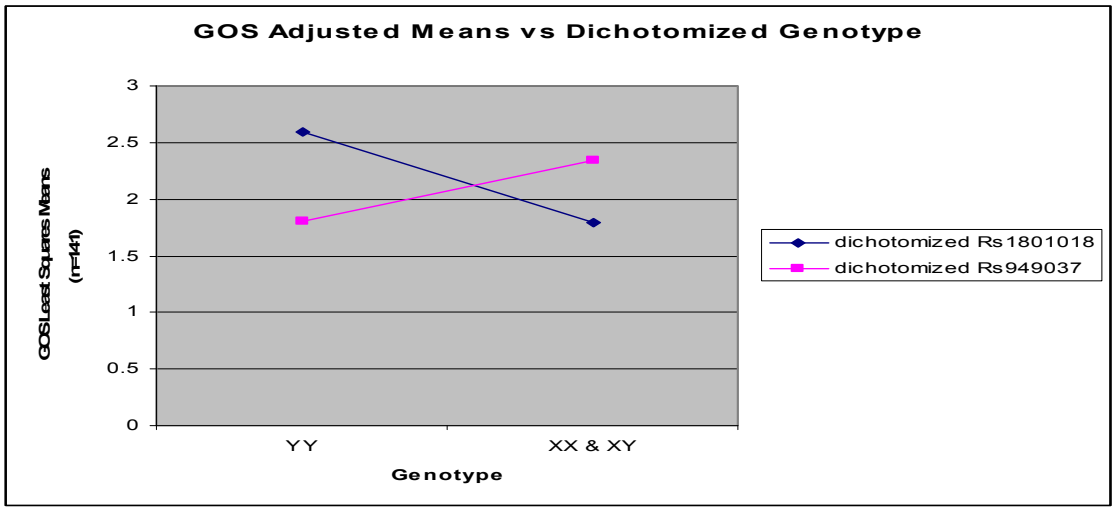


Figure 4-8: GOS Adjusted Means vs Dichotomized Genotypes rs1801018 and rs949037

The mixed model with RS949037 genotype XX is significant (P=0.0425) with overall genotype significant (P for type 3 F tests =0.0074). XX genotype is associated with increased GOS scores and is therefore associated with good outcomes (See figures 4-6 and 4-8). This SNP was significant in the dichotomized analysis YY (P=0.0168) subjects with 2 copies of X or heterozygous, have significantly higher GOS scores and hence better outcomes. There were no significant trends in gender or race in this model. (Refer to table 4-9).

The mixed models analyses indicated that the following SNP's were not significant RS17756073 (genotypes P=0.736; dichotomized genotype P=0.3851); RS1944419 (genotypes P=0.3673; dichotomized genotype P=0.1973); RS4941185 (genotypes P=0.2874; dichotomized genotype P=0.5567); and RS7236090 (genotypes P=0.138; dichotomized genotype P=0.4572). (Refer to tables 4-7 to 4-9).

Primary Mixed Models with DRS as the Global Functional Outcome

The primary models with SNP RS12968517 genotype YY was significant (P= 0.0277) with overall genotype marginally significant (P for type 3 F tests =0.0667). YY genotype is associated with increased DRS scores and is therefore associated with poor outcomes. This trend was not evident in the dichotomized analysis (P=0.4585). There was a trend towards statistical significance of the therapeutic hypothermia variable (genotypes P=0.0546; dichotomized genotype P=0.0924) with the absence of therapeutic hypothermia associated with lower DRS scores (better outcomes). (Refer to table #4-7).

The mixed model with RS17759659, genotype XX (P= 0.0002) with overall genotype significant (P for type 3 F tests =0.0006). XX genotype is associated with decreased DRS scores and is therefore associated with good outcomes. This SNP was significant in the dichotomized analysis (P=.0001); subjects with 0 copies of X or heterozygous, have an increase in DRS, and therefore poorer outcomes. (Refer to table 4-7).

The mixed model with RS1801018 genotype YY (P<.0001) with overall genotype significant (P for type 3 F tests =0.0002). YY genotype is associated with decreased DRS scores and is therefore associated with good outcomes. This SNP was significant in the dichotomized analysis (P=0.0002) subjects with 0 copies of Y or heterozygous, have an increase in DRS, and therefore poorer outcomes. There was a trend towards significance when evaluating the covariate race (genotypes P=0.0862; dichotomized genotype P=0.0599). Caucasian subjects had lower DRS scores and therefore better outcomes. There was a trend towards significance when evaluating therapeutic hypothermia (P=0.0759). The absence of therapeutic hypothermia was associated with a trend of lower DRS scores. (Refer to table 4-8).

The mixed model with RS949037 genotype XX (P=0.037) with overall genotype significant (P for type 3 F tests =0.0094). XX genotype was associated with decreased DRS scores and is therefore associated with good outcomes. This SNP was significant in the dichotomized analysis YY (P=0.0255) subjects with 2 copies of X or heterozygous, lower DRS scores, hence 1 or 2 copies of X are related to better outcomes There was a trend towards significance when assessing the therapeutic hypothermia variable in the dichotomized analysis with the dichotomized genotypes (P=0.0609). The absence of therapeutic hypothermia was associated with a trend of lower DRS scores. (See figure 4-9 and table 4-9).

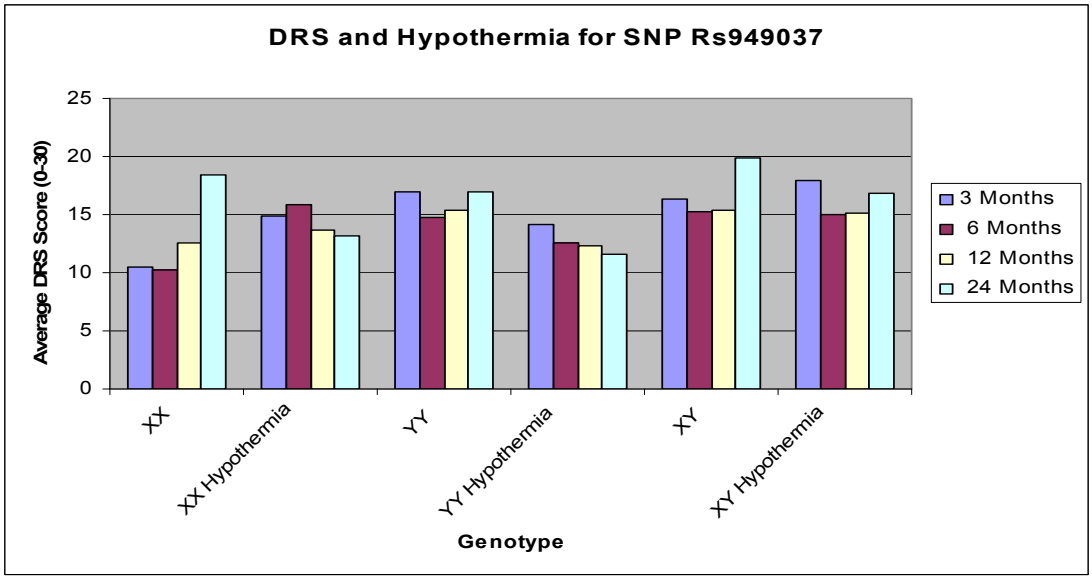


Figure 4-9: DRS and Hypothermia for SNP rs949037

The mixed models analyses indicated that the following SNP's were not significant: RS17756073 (genotypes P=0.227; dichotomized genotype P=0.2291); RS1944419 (genotypes P=0.4339; dichotomized genotype P=0.2293); RS4941185 (genotypes P=0.3357; dichotomized genotype P=0.5137); and RS7236090 (genotypes P=0.1167; dichotomized genotype P=0.249). (Refer to tables 4-7 to 4-9).

Table 4-5: Preliminary Mixed Models Analysis for Global Functional Outcomes

Preliminary Mixed Models Analysis for Global Funcational Outcomes								
			GOS			DRS		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS1026825	201	xx	-0.1143	0.6178	0.8819	0.8631	0.6734	0.8945
		yy	-0.0352	0.8852		0.7578	0.7256	
		xy						
		yy & xy	0.1021	0.6308	0.6308	-0.6012	0.752	0.752
		xx						
RS12454712	199	xx	-0.3608	0.2462	0.3122	2.2884	0.4096	0.6374
		yy	0.1076	0.6042		-0.2825	0.8787	
		xy						
		xx & xy	-0.1928	0.3215	0.3215	0.8128	0.6392	0.6392
		yy						
RS12968517	200	xx	-0.053	0.8017	0.0899*	0.1417	0.9396	0.0722*
		yy	-0.6093	0.0354*		5.5154	0.0323*	
		xy						
		yy & xy	-0.1209	0.5371	0.5371	1.4154	0.4163	0.4163
		xx						
RS1381548	195	xx	-0.0157	0.955	0.9804	0.5343	0.8276	0.9747
		yy	-0.0432	0.8429		0.03393	0.9861	
		xy						
		xx & xy	0.03897	0.8486	0.8486	0.1114	0.9513	0.9513
		yy						
RS1481031	198	xx	-0.1783	0.3891	0.5321	1.7951	0.3294	0.5027
		yy	0.1394	0.6751		-0.9535	0.7469	
		xy						
		yy & xy	0.2051	0.2968	0.2968	-1.9755	0.259	0.259
		xx						
RS17756073	196	xx	-0.1358	0.525	0.3823	1.9944	0.2916	0.1981*
		yy	-0.4394	0.1673*		4.8816	0.0797*	
		xy						
		yy & xy	0.02052	0.9171	0.9171	-0.6964	0.6903	0.6903
		xx						
RS17759659	197	xx	0.4732	0.0249*	0.0524*	-4.7556	0.0114*	0.0325*
		yy	-0.0504	0.8615		-0.4299	0.8673	
		xy						
		yy & xy	-0.4848	0.0153*	0.0153*	4.6576	0.0089*	0.0089*
		xx						
RS1801018	199	xx	0.4464	0.084*	0.0923*	-4.5709	0.0458*	0.0298*
		yy	0.4156	0.084*		-4.6159	0.0174*	
		xy						
		xx & xy	-0.2704	0.1827*	0.1827*	3.1398	0.0813*	0.0813*
		yy						
RS1944419	192	xx	-0.2987	0.2096	0.3303	3.125	0.1424*	0.3075
		yy	0.0826	0.7374		0.1354	0.9503	
		xy						
		yy & xy	0.3253	0.1465*	0.1465*	-3.0811	0.1244*	0.1244*
		xx						
RS3810027	196	xx	0.1009	0.6318	0.8667	-0.667	0.7217	0.8271
		yy	0.1206	0.7036		-1.6432	0.554	
		xy						
		yy & xy	-0.0746	0.7068	0.7068	0.2722	0.9967	0.9967
		xx						
* p≤ 0.2								

Table 4-5 continued

Preliminary Mixed Models Analysis for Global Funcational Outcomes								
			GOS			DRS		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS4456611	193	xx	-0.2262	0.3468	0.5268	2.3024	0.2866	0.4656
		yy	-0.2216	0.3655		2.0398	0.3446	
		xy						
		yy & xy	0.1541	0.4965	0.4965	-1.6217	0.4255	0.4255
		xx						
RS4941185	195	xx	-0.3443	0.1188*	0.0376*	2.5731	0.1886*	0.0272*
		yy	-0.6379	0.0141*		6.1868	0.0079*	
		xy						
		yy & xy	0.1509	0.4693	0.4693	-0.6868	0.7106	0.7106
		xx						
RS7230970	193	xx	0.1165	0.6602	0.8506	-2.0889	0.3702	0.6176
		yy	0.1102	0.6198		-1.4009	0.4821	
		xy						
		xx &xy	-0.0744	0.7183	0.7183	0.7376	0.6899	0.6899
		yy						
RS7236090	193	xx	-0.1148	0.6197	0.0966*	1.8843	0.3546	0.0121*
		yy	0.4436	0.0753*		-5.0602	0.0211*	
		xy						
		yy & xy	0.2661	0.2195	0.2195	-3.6046	0.061*	0.061*
		xx						
RS8083946	200	xx	0.2847	0.2802	0.543	-2.4921	0.2877	0.4966
		yy	0.1421	0.5159		0.08201	0.9663	
		xy						
		xx &xy	-0.0457	0.819	0.819	-0.9192	0.6051	0.6051
		yy						
RS899968	195	xx	-0.2583	0.3395	0.6309	1.7189	0.4837	0.7668
		yy	-0.0939	0.6636		0.8627	0.655	
		xy						
		xx &xy	0.01604	0.936	0.936	-0.3535	0.8431	0.8431
		yy						
RS949037	194	xx	0.4098	0.1042*	0.197*	-4.1966	0.064*	0.1441*
		yy	-0.0214	0.9238		-0.1662	0.9335	
		xy						
		xx &xy	0.1611	0.437	0.437	-1.2295	0.508	0.508
		yy						
Covariates								
Time (Months)	201	3	-0.3347	<.0001*	<.0001*	1.8984	<.0001*	<.0001*
		6	-0.1686	0.0008*		0.5673	0.0325*	
		12	-0.0887	0.0806*		0.03159	0.906	
		24						
Age	201		-0.0352	<.0001*	<.0001*	0.2919	<.0001*	<.0001*
GCS	201		1.0036	<.0001*	<.0001*	-10.058	<.0001*	<.0001*
Gender	201		-0.3766	0.1085*	0.1085*	2.8824	0.1696*	0.1696*
Race	201		-0.3677	0.3485	0.3485	-1.7531	0.6144	0.6144
Hypothermic	200		0.1095	0.6511	0.6511	-2.0581	0.3417	0.3417
Hypoxia	142		0.2509	0.3814	0.3814	-3.2762	0.1972*	0.1972*
Hypotensive	142		0.4556	0.2242	0.2242	-5.2691	0.1131*	0.1131*
Seizures	148		-0.558	0.2639	0.2639	6.1357	0.1676*	0.1676*
APOEε4	196		0.03907	0.8645	0.8645	-0.6825	0.7389	0.7389
* p≤ 0.2								

Table 4-6: Primary Mixed Models Analyses of Global Functional Outcomes and BCL-2 SNP's

Primary Mixed Models Analyses of Global Functional Outcomes and BCL-2 SNP																
Variable	**GOS**								**DRS**							
		coefff	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**
RS12968517 (n=141)																
Genotype	xx	-0.0256	0.9111	0.0591	yy & xy	-0.1623	0.455	0.455	xx	0.2511	0.9012	0.0667	yy & xy	1.4148	0.4585	0.4585
	yy	-0.7091	0.0242*		xx				yy	6.1021	0.0277*		xx			
	xy								xy							
Month	3	-0.3395	<.0001***	<.0001***	3	-0.3396	<.0001***	<.0001***	3	1.7602	<.0001***	<.0001***	3	1.7613	<.0001***	<.0001***
	6	-0.1419	0.0205*		6	-0.142	0.0204*		6	0.5209	0.1083		6	0.522	0.1076	
	12	-0.1112	0.0728		12	-0.1113	0.0726		12	-0.0309	0.9252		12	-0.0312	0.9247	
	24				24				24				24			
Age		-0.0337	<.0001***	<.0001***		-0.0344	<.0001***	<.0001***		0.2853	<.0001***	<.0001***		0.2938	<.0001***	<.0001***
GCS		0.9993	<.0001***	<.0001***		1.0457	<.0001***	<.0001***		-10.14	<.0001***	<.0001***		-10.58	<.0001***	<.0001***
Gender		-0.4678	0.0728	0.0728		-0.4566	0.0845	0.0845		3.1476	0.1687	0.1687		3.1514	0.1745	0.1745
Race		0.5239	0.152	0.152		0.5277	0.1553	0.1553		-3.5941	0.2589	0.2589		-3.7406	0.247	0.247
Hypoxia										-2.7634	0.2361	0.2361		-3.2292	0.1712	0.1712
Seizure										3.2549	0.4401	0.4401		1.6909	0.6883	0.6883
Hypothermia		0.4552	0.1556	0.1556		0.4129	0.2035	0.2035		-5.6043	0.0546	0.0546		-4.9462	0.0924	0.0924
Hypotension		0.3315	0.3471	0.3471		0.3602	0.3143	0.3143		-1.7397	0.58	0.58		-2.0276	0.5249	0.5249
RS17756073 (n=138)																
Genotype	xx	0.08323	0.7165	0.736	yy & xy	-0.1832	0.3851	0.3851	xx	-0.8247	0.6757	0.227	yy & xy	1.892	0.2991	0.2991
	yy	-0.396	0.27077		xx				yy	4.1805	0.1699		xx			
	xy								xy							
Time	3	-0.3296	<.0001***	<.0001***	3	-0.0329	<.0001***	<.0001***	3	1.6994	<.0001***	<.0001***	3	1.6986	<.0001***	<.0001***
	6	-0.1281	0.0386*		6	-0.1276			6	0.4555	0.1651		6	0.4534	0.1671	
	12	-0.0978	0.1201		12	0.0977			12	-0.1023	0.7597		12	-0.1033	0.7575	
	24	.			24				24				24			
Age		-0.0364	<.0001***	<.0001***		-0.0363	<.0001***	<.0001***		0.3143	<.0001***	<.0001***		0.3125	<.0001***	<.0001**
GCS		1.0489	<.0001***	<.0001***		1.0736	<.0001***	<.0001***		-10.4	<.0001**	<.0001***		-10.638	<.0001***	<.0001***
Gender		-0.3757	0.158	0.158		-0.334	0.2047	0.2047		2.5735	0.2642	0.2642		2.105	0.3571	0.3571
Race		0.4952	0.1795	0.1795		0.4835	0.19	0.19		-3.6254	0.2557	0.2557		-3.4581	0.2795	0.2795
Hypoxia										-3.7757	0.1128	0.1128		-3.5179	0.1394	0.1394
Seizure										1.5069	0.7207	0.7207		1.4733	0.7276	0.7276
Hypothermia		0.3822	0.2417	0.2417		0.4047	0.2146	0.2146		-4.4813	0.1265	0.1265		-4.7371	0.1069	0.1069
Hypotension		0.3955	0.2592	0.2592		0.387	0.2697	0.2697		-2.4304	0.4382	0.4382		-2.4173	0.4422	0.4422
RS17759659 (n=139)																
Genotype	xx	0.847	<.0001***	0.0003**	yy & xy	-0.832	<.0001***	<.0001***	xx	-7.2388	0.0002**	0.0006**	yy & xy	6.949	0.0001***	0.0001***
	yy	0.07353	0.8062		xx				yy	-1.4389	0.5877		xx			
	xy								xy							
Time	3	-0.3475	<.0001***	<.0001***	3	-0.3474	<.0001***	<.0001***	3	1.7625	<.0001***	<.0001***	3	1.7627	<.0001***	<.0001***
	6	-0.1297	0.0343*		6	-0.1296	0.0344*		6	0.4667	0.1513		6	0.4666	0.1514	
	12	-0.1082	0.0814		12	-0.1082	0.0813		12	-0.0741	0.8226		12	-0.0736	0.8239	
	24				24				24				24			
Age		-0.0405	<.0001***	<.0001***		-0.0406	<.0001***	<.0001***		0.3381	<.0001***	<.0001***		0.339	<.0001***	<.0001***
GCS		0.9565	<.0001***	<.0001***		0.9562	<.0001***	<.0001***		-9.6293	<.0001***	<.0001***		-9.6263	<.0001***	<.0001***
Gender		-0.2488	0.3096	0.3096		-0.2416	0.3185	0.3185		1.2488	0.5638	0.5638		1.1075	0.6051	0.6051
Race		0.6728	0.0521	0.0521		0.6806	0.0477*	0.0477*		-4.8495	0.1111	0.1111		-4.9981	0.0984	0.0984
Hypoxia										-2.5131	0.2557	0.2557		-2.4304	0.2692	0.2692
Seizure										3.0807	0.4385	0.4385		2.8996	0.463	0.463
Hypothermia		0.2778	0.3603	0.3603		0.2843	0.3456	0.3456		-4.0601	0.1455	0.1455		-4.1747	0.1324	1324
Hypotension		0.334	0.307	0.307		0.3272	0.3135	0.3135		-2.1823	0.4647	0.4647		-2.0922	0.4815	0.4815

*** ≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001**

Table 4-6 contined

Primary Mixed Models Analyses of Global Functional Outcomes and BCL-2 SNP																
Variable	**GOS**								**DRS**							
		coefff	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**
RS1801018 (n=141)																
Genotype	xx	0.3537	0.2009	0.0004**	xx & xy	-0.7987	0.0002**	0.0002**	xx	-3.8674	0.11	0.0002**	xx & xy	7.1039	0.0002**	0.0002**
	yy	0.8987	<.0001***		yy				yy	-8.1741	<.0001**		yy			
	xy								xy							
Time	3	-0.3376	<.0001***	<.0001***	3	-0.3383	<.0001***	<.0001***	3	1.7266	<.0001***	<.0001***	3	1.7285	<.0001***	<.0001***
	6	-0.1346	0.0292*		6	-0.1349	0.0289*		6	0.4829	0.1383		6	0.4836	0.1377	
	12	-0.1121	0.073		12	-0.1125	0.0721		12	-0.0581	0.8605		12	-0.0567	0.864	
	24				24				24				24			
Age		-0.0376	<.0001***	<.0001***		-0.0384	<.0001***	<.0001***		0.3126	<.0001***	<.0001***		0.3221	<.0001***	<.0001***
GCS		1.0147	<.0001***	<.0001***		1.0106	<.0001***	<.0001***		-10.221	<.0001***	<.0001***		-10.186	<.0001***	<.0001***
Gender		-0.3251	0.1872	0.1872		-0.2755	0.2587	0.2587		1.942	0.3659	0.3659		1.3785	0.5177	0.5177
Race		0.7376	0.0392*	0.0392*		0.7862	0.0277*	0.0277*		-5.273	0.0862	0.0862		-5.794	0.0599	0.0599
Hypoxia										-1.8446	0.4064	0.4064		-1.488	0.5034	0.5034
Seizure										4.8055	0.2325	0.2325		4.037	0.3151	0.3151
Hypothermia		0.2665	0.3902	0.3902		0.3404	0.2659	0.2659		-4.1436	0.1352	0.1352		-4.8929	0.0759	0.0759
Hypotension		0.3574	0.2763	0.2763		0.3316	0.3127	0.3127		-2.2932	0.4328	0.4328		-2.1867	0.4571	0.4571
RS1944419 (n=138)																
Genotype	xx	-0.2594	0.3096	0.3673	yy & xy	0.3097	0.1973	0.1973	xx	2.1712	0.335	0.4339	yy & xy	-2.5393	0.2293	0.2293
	yy	0.1485	0.5565		xx				yy	-1.0388	0.6321		xx			
	xy								xy							
Time	3	-0.3374	<.0001***	<.0001***	3	-0.3376	<.0001***	<.0001***	3	1.7457	<.0001***	<.0001***	3	1.7461	<.0001***	<.0001***
	6	0.135	0.0331*		6	-0.1347	0.0335*		6	0.4545	0.1764		6	0.4534	0.1774	
	12	-0.1031	0.1081		12	-0.103	0.1083		12	-0.0989	0.7723		12	-0.099	0.7721	
	24				24				24				24			
Age		-0.0329	<.0001***	<.0001***		-0.0335	<.0001***	<.0001***		0.2818	<.0001***	<.0001***		0.2856	<.0001***	<.0001***
GCS		1.0488	<.0001***	<.0001***		1.051	<.0001***	<.0001***		-10.799	<.0001***	<.0001***		-10.817	<.0001***	<.0001***
Gender		-0.2678	0.3191	0.3191		-0.2695	0.3148	0.3148		1.1567	0.6213	0.6213		1.1545	0.6209	0.6209
Race		0.4843	0.1868	0.1868		0.4823	0.1875	0.1875		-3.3098	0.2976	0.2976		-3.2846	0.2998	0.2998
Hypoxia										-2.7125	0.2611	0.2611		-2.6122	0.2758	0.2758
Seizure										2.1386	0.6145	0.6145		2.2926	0.5871	0.5871
Hypothermia		0.3696	0.2642	0.2642		0.3822	0.2462	0.2462		-4.5781	0.1278	0.1278		-4.713	0.1143	0.1143
Hypotension		0.3401	0.3493	0.3493		0.3247	0.3691	0.3691		-1.6289	0.615	0.615		-1.5405	0.6328	0.6328
RS4941185 (n=140)																
Genotype	xx	-0.2457	0.2868	0.2874	yy & xy	0.1273	0.5567	0.5567	xx	2.1697	0.2824	0.3357	yy & xy	-1.2376	0.5137	0.5137
	yy	-0.4326	0.1433		xx				yy	3.4122	0.1857		xx			
	xy								xy							
Time	3	-0.3426	<.0001***	<.0001***	3	-0.3417	<.0001***	<.0001***	3	1.7592	<.0001***	<.0001***	3	1.7564	<.0001***	<.0001***
	6	-0.1339	0.0291		6	-0.1335	0.0296*		6	0.5149	0.1167		6	0.5141	0.1173	
	12	-0.1122	0.0711		12	-0.1116	0.0727		12	-0.0325	0.9223		12	-0.0339	0.9191	
	24				24				24				24			
Age		-0.0358	<.0001***	<.0001***		-0.0349	<.0001***	<.0001***		0.3063	<.0001***	<.0001***		0.2992	<.0001***	<.0001***
GCS		0.9982	<.0001***	<.0001***		1.0453	<.0001***	<.0001***		-10.255	<.0001***	<.0001***		-10.629	<.0001***	<.0001***
Gender		-0.3401	0.1877	0.1877		-0.392	0.1272	0.1272		2.2038	0.3268	0.3268		2.5998	0.245	0.245
Race		0.5014	0.1784	0.1784		0.4423	0.234	0.234		-3.4924	0.2789	0.2789		-3.0207	0.3472	0.3472
Hypoxia										-3.2135	0.1825	0.1825		-3.3843	0.1612	0.1612
Seizure										1.2542	0.7683	0.7683		1.5083	0.7237	0.7237
Hypothermia		0.3896	0.2324	0.2324		0.3847	0.2402	0.2402		-4.7146	0.1128	0.1128		-4.6755	0.1169	0.1169
Hypotension		0.3751	0.2961	0.2961		0.3656	0.3103	0.3103		-2.2304	0.4867	0.4867		-2.0742	0.5186	0.5186

*** p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001**

Table 4-6 continued

Primary Mixed Models Analyses of Global Functional Outcomes and BCL-2 SNP																
Variable	**GOS**								**DRS**							
		coefff	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**
RS7236090 (n=139)																
Genotype	xx	-0.0219	0.9288	0.138	yy & xy	0.1739	0.4572	0.4572	xx	1.1494	0.5893	0.1167	yy & xy	-2.3443	0.249	0.249
	yy	0.5052	0.0652		xx				yy	-4.0751	0.0852		xx			
	xy								xy							
Time	3	-0.3378	<.0001***	<.0001***	3	-0.3366	<.0001***	<.0001***	3	1.7236	<.0001***	<.0001***	3	1.7208	<.0001***	<.0001***
	6	-0.1292	0.0354*		6	-0.1287	0.0363*		6	0.4639	0.1526		6	0.4611	0.1551	
	12	-0.1072	0.0847		12	-0.1075	0.0842		12	-0.0653	0.8427		12	-0.0644	0.8449	
	24				24				24				24			
Age		-0.0363	<.0001***	<.0001***		-0.0356	<.0001***	<.0001***		0.305	<.0001***	<.0001***		0.2999	<.0001***	<.0001***
GCS		1.0229	<.0001***	<.0001***		1.0539	<.0001***	<.0001***		-10.181	<.0001***	<.0001***		-10.501	<.0001***	<.0001***
Gender		-0.4313	0.0921	0.0921		-0.4046	0.1164	0.1164		3.022	0.1755	0.1755		2.7781	0.2153	0.2153
Race		0.7414	0.0588	0.0588		0.5252	0.1629	0.1629		-5.7685	0.0902	0.0902		-3.9841	0.2215	0.2215
Hypoxia										-3.0492	0.1929	0.1929		-2.9755	0.2071	0.2071
Seizure										1.5105	0.7177	0.7177		1.7048	0.6854	0.6854
Hypothermia		0.3722	0.2531	0.2531		0.4257	0.1937	0.1937		-4.711	0.1082	0.1082		-5.1979	0.0775	0.0775
Hypotension		0.2937	0.3992	0.3992		0.3483	0.3202	0.3202		-1.5677	0.6156	0.6156		-1.9938	0.5251	0.5251
RS949037 (n=137)																
Genotype	xx	0.5303	0.0425*	0.0074**	xx & xy	0.5428	0.0168*	0.0168*	xx	-4.8164	0.037*	0.0094**	xx & xy	-4.4511	0.0255*	0.0255*
	yy	-0.3604	0.1333		yy				yy	2.9064	0.164		yy			
	xy								xy							
Time	3	-0.3343	<.0001***	<.0001***	3	-0.3332	<.0001***	<.0001***	3	1.7356	<.0001***	<.0001***	3	1.7335	<.0001***	<.0001***
	6	-0.1298	0.0348*		6	-0.1282	0.0371*		6	0.4818	0.1457		6	0.4763	0.1503	
	12	-0.1025	0.0997		12	-0.1025	0.0998		12	-0.0592	0.8605		12	-0.0579	0.8634	
	24				24				24				24			
Age		-0.0388	<.0001***	<.0001***		-0.0387	<.0001***	<.0001***		0.3244	<.0001***	<.0001***		0.325	<.0001***	<.0001***
GCS		1.0187	<.0001***	<.0001***		1.0357	<.0001***	<.0001***		-10.263	<.0001***	<.0001***		-10.436	<.0001***	<.0001***
Gender		-0.3143	0.2144	0.2144		-0.3036	0.2361	0.2361		1.7571	0.4327	0.4327		1.7117	0.4509	0.4509
Race		0.3388	0.3389	0.3389		0.4523	0.2024	0.2024		-1.8825	0.5444	0.5444		-2.9236	0.3473	0.3473
Hypoxia										-1.381	0.5509	0.5509		-2.0047	0.3896	0.3896
Seizure										3.031	0.4568	0.4568		2.4372	0.5539	0.5539
Hypothermia		0.3156	0.3197	0.3197		0.4405	0.163	0.163		-4.5249	0.1153	0.1153		-5.4111	0.0609	0.0609
Hypotension		0.2392	0.4743	0.4743		0.3288	0.3279	0.3279		-1.532	0.6118	0.6118		-2.1826	0.4736	0.4736

*** p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001**

Primary Generalized Mixed Models Analyses for Mortality

Among the full models analyzed, time was consistently found to be related to an increase in mortality the further from the time of the injury (i.e. 24 months post injury compared to 3 months post injury (Type 3 X^2 <.0001; OR= 3.75- 4.36). Increasing age was consistently related associate with an increase in mortality (P<.0001; Type 3 X^2 =0.0002; OR=1.1). A statistical trend was found for the dichotomized GCS covariate; people who are admitted with a higher GCS had a decrease in mortality GCS (P<.0001; Type 3 X^2 <.0001; OR= 0.08). (Refer to tables 4-12 to 4-14),

The mixed model with SNP RS12968517, genotype YY is significant (P=0.0115; OR= 6.38) with overall genotype marginally significant (Type 3 X^2 =0.0493). YY genotype was associated with increase in mortality. A significant statistical trend was not present in the dichotomized genotype analysis (P=0.2544; OR= 1.79). Hypothermia was found to been marginally significant in the models (genotypes Type 3 X^2=0.0534; OR= 0.24; dichotomized genotype Type 3 X^2=0.0863; OR= 0.27) having higher mortality. (Refer to table 4-12).

The mixed model with RS17759659, genotype XX is significant (P=.0092; OR= 0.17) with overall genotype significant (Type 3 X^2=0.0075). XX genotype was associated with decreased mortality. This SNP was significant in the dichotomized analysis (Type 3 X^2=0.0031; OR=5.05); subjects with 0-1 copies of X had an increase in mortality. (Refer to table 4-12).

The mixed model with RS1801018 genotype YY is significant (P=0.0026; OR= 0.14) with overall genotype significant (Type 3 X^2=0.0055). YY genotype was associated with survival. This SNP was significant in the dichotomized analysis (Type 3 X^2=.0035; OR= 5.01); subjects with 1-2 copies of Y are associated with mortality. Race was found to been marginally significant in the models (genotypes Type 3 X^2=0.0621; OR= 0.18; dichotomized genotype Type

3 X^2=0.056; OR= 0.16). Caucasians have higher survival rate. Hypothermia was found to been marginally significant in the models [genotypes Type 3 X^2=0.1057; OR= 1/0.26 (3.8); dichotomized genotype Type 3 X^2=0.0702; OR= 1/0.23 (4.3)] having lower mortality. (Refer to table 4-13).

The mixed model with RS7230970 genotype XX (P=0.0548; OR= 0.19) and YY (P=0.0582; OR= 0.30) showed trends towards significance with overall genotype (Type 3 X^2=0.054). XX and YY genotypes are both associated with a decrease in mortality. These trends were not significant in the XX dichotomized analysis (Type 3 X^2=0.17; OR= 2.09). Hypothermia was found to been significant in the models [genotypes Type 3 X^2=0.0227, OR= 1/0.09 (11.1); dichotomized genotype Type 3 X^2=0.0446; OR= 1/0.17 (5.89)] having higher mortality. (Refer to table 4-13).

The mixed model with RS949037 overall genotypes showed trends towards significance (Type 3 X^2=0.0551) while YY was not statically significant, the OR indicated a trend (P=0.2827; OR= 1.9) and was related to increased mortality. This trend was not significant in the dichotomized analysis [1-2 copies of YY] (Type 3 X^2=0.1112, OR= 0.41). There was also a trend towards statistical significance when evaluating therapeutic hypothermia in the models [genotypes Type 3 X^2=0.0688, OR= 1/0.24 (4.2)]; dichotomized genotype Type 3 X^2=0.0599, OR= 1/0.22 (4.5)] having higher mortality. (Refer to table 4-14).

The mixed models analyses indicated that the following SNP's were not significant: RS17756073 (genotypes Type 3 X^2=0.7177; dichotomized genotype Type 3 X^2=0.9098, OR= 1.05); RS4941185 (genotypes Type 3 X^2=0.8593; dichotomized genotype Type 3 X^2=0.6581, OR= 0.81); and RS7236090 (genotypes Type 3 X^2=0.5572; dichotomized genotype Type 3 X^2=0.9116, OR= 0.95). (Refer to tables 4-12 to 4-14).

Summary of Global Functional Outcome Results

In analyzing the 17 BCL-2 SNP's genotype and dichotomized genotype data there are four SNP's which have significant findings. In the GOS, DRS, and mortality models RS12968517 was associated with poor outcomes. The 2 SNP's (RS17759659 andRS1801018) were associated with good outcomes across the GOS, DRS, and mortality rates. SNP RS949037 was significantly related to good outcomes for GOS and DRS, however a trend was identified in the mortality outcome. Time points of the assessment, age, and GCS on admission were consistently significant across all of the models. Gender, race, and hypothermia were on occasion implicated as potential significant covariates in the mixed models/ generalized mixed models. (Refer to tables 4-7 to 4-9 and 4-12 to 4-14).

Table 4-7: Preliminary Generalized Mixed Models of Mortality Outcomes

Preliminary Generalized Mixed Models of Mortality Outcomes								
BCL-2 SNP	n=	Genotype	coefff	Odds Ratio	95% Confidence Limits		Z	Type 3 X^2
RS1026825	201	xx	0.2626	1.3003065	-0.4744	0.9996	0.4849	0.7371
		yy	-0.0299	0.9705426	-0.809	0.7491	0.94	
		xy						
		yy & xy	-0.2737	0.7605602	-0.9504	0.4029	0.4278	0.4373
		xx						
RS12454712	199	xx	0.299	1.3485096	-0.6821	1.2802	0.5502	0.7525
		yy	-0.0883	0.9154862	-0.7734	0.5969	0.8006	
		xy						
		xx & xy	0.1625	1.1764483	-0.4733	0.7984	0.6163	0.6155
		yy						
RS12968517	200	xx	0.009	1.0090406	-0.7045	0.7226	0.9802	0.1177*
		yy	0.9176	2.5032753	0.0415	1.7937	0.0401	
		xy						
		yy & xy	0.2864	1.331625	-0.3488	0.9216	0.3769	0.3732
		xx						
RS1381548	195	xx	0.1099	1.1161664	-0.7869	1.0066	0.8103	0.8293
		yy	0.2207	1.2469493	-0.4843	0.9258	0.4843	
		xy						
		xx & xy	-0.1907	0.8263805	-0.8518	0.4703	0.5718	0.5757
		yy						
RS1481031	198	xx	-0.0576	0.9440275	-0.7207	0.6054	0.8648	0.9355
		yy	-0.2009	0.8179942	-1.3258	0.9239	0.7262	
		xy						
		yy & xy	0.0212	1.0214263	-0.6147	0.6571	0.948	0.948
		xx						
RS17756073	196	xx	0.4096	1.5062152	-0.3216	1.1409	0.2722	0.2853*
		yy	0.74	2.0959355	-0.2159	1.6958	0.1292	
		xy						
		yy & xy	-0.1686	0.8448468	-0.8039	0.4668	0.6031	0.6031
		xx						
RS17759659	197	xx	-0.6401	0.5272397	-1.3673	0.0871	0.0845	0.1953*
		yy	-0.1457	0.864417	-1.0464	0.755	0.7512	
		xy						
		yy & xy	0.6064	1.8338178	-0.0883	1.3012	0.0871	0.0737*
		xx						
RS1801018	199	xx	-0.7312	0.481331	-1.6051	0.1428	0.1011	0.2143*
		yy	-0.3758	0.6867397	-1.0883	0.3368	0.3013	
		xy						
		yy & xy	0.1535	1.1659078	-0.517	0.8239	0.6537	0.6498
		xx						
RS1944419	192	xx	0.1924	1.2121553	-0.5649	0.9498	0.6185	0.882
		yy	0.0333	1.0338607	-0.7899	0.8565	0.9368	
		xy						
		yy & xy	-0.1813	0.8341851	-0.8912	0.5286	0.6166	0.6219
		xx						
RS3810027	196	xx	-0.3122	0.7318351	-0.9922	0.3679	0.3683	0.6354
		yy	-0.3237	0.7234673	-1.3871	0.7397	0.5507	
		xy						
		yy & xy	0.2419	1.2736668	-0.4052	0.8889	0.4638	0.4615
		xx						
* = X^2 p≤ 0.3								

Table 4-7 contiued

Preliminary Generalized Mixed Models of Mortality Outcomes								
BCL-2 SNP	**n=**	**Genotype**	**coefff**	**Odds Ratio**	**95% Confidence Limits**		**Z**	**Type 3 X^2**
RS4456611	193	xx	0.434	1.5434189	-0.3481	1.2162	0.2768	0.4351
		yy	0.4092	1.5056128	-0.3555	1.1739	0.2943	
		xy						
		yy & xy	-0.2862	0.7511124	-0.10075	-0.4351	0.4367	0.449
		xx						
RS4941185	195	xx	0.5056	1.65798	-0.2195	1.2307	0.1718	0.2763*
		yy	0.5488	1.7311744	-0.2873	1.385	0.1983	
		xy						
		yy & xy	-0.3199	0.7262217	-0.9819	0.3421	0.3436	0.3528
		xx						
RS7230970	193	xx	-0.6641	0.5147366	-1.533	0.2048	0.1341	0.2953*
		yy	-0.2481	0.7802819	-0.9758	0.4795	0.5039	
		xy						
		xx & xy	0.0503	1.0515865	-0.63	0.7305	0.8849	0.8845
		yy						
RS7236090	193	xx	0.3519	1.4217663	-0.3616	1.0654	0.3337	0.0994*
		yy	-0.6465	0.5238761	-1.5802	0.2872	0.1747	
		xy						
		yy & xy	-0.5295	0.5888993	-1.2037	0.1447	0.1237	0.136*
		xx						
RS8083946	200	xx	-0.4074	0.665378	-1.2951	0.4803	0.3684	0.6376
		yy	-0.078	0.9249644	-0.7697	0.6137	0.8252	
		xy						
		xx & xy	-0.0497	0.9515148	-0.6899	0.5905	0.8791	0.8793
		yy						
RS899968	195	xx	0.6605	1.93576	-0.2241	1.5452	0.1434	0.366
		yy	0.2676	1.3068243	-0.4461	0.9813	0.4624	
		xy						
		xx & xy	-0.0646	0.9374424	-0.7078	0.5787	0.844	0.8443
		yy						
RS949037	194	xx	-0.3035	0.7382299	-1.2092	0.6021	0.5113	0.579
		yy	0.1797	1.1968583	-0.5278	0.8872	0.6187	
		xy						
		xx & xy	-0.2646	0.7675129	-0.9291	0.4	0.4352	0.4417
		yy						
Covariates								
Time (Months)	201	24	0.9395	1	0.7023	1.1768	<.0001	<.0001*
		12	0.3544	2.5587017	0.19	0.5188	<.0001	
		6	0.1366	1.4253252	0.007	0.2662	0.0388	
		3						
Age	201		0.0485	1.0496954	0.0263	0.0707	<.0001	<.0001*
GCS	201		-1.6171	0.1984734	-2.2825	-0.9517	<.0001	<.0001*
Gender	201		0.4001	1.4919739	-0.328	1.1282	0.2814	0.3014
Race	201		-0.7055	0.4938616	-1.8623	0.4513	0.232	0.2963
Hypothermic	200		-0.1005	0.9043851	-0.8372	0.6362	0.7892	0.7911
Hypoxia	142		0.2899	1.3362939	-0.5809	1.1607	0.5141	0.5055
Hypotensive	142		-0.5568	0.5730399	-1.654	0.5404	0.3199	0.3437
Seizures	148		1.2025	3.3284276	-0.9324	3.3373	0.2696	0.1658*
APOEε4	196		-0.0238	0.976481	-0.7643	0.7167	0.9497	0.9499
* $=X^2$ p≤ 0.3								

Table 4-8: Primary Generalized Mixed Models Analyses of Mortality Outcomes and BCL-2 SNP's

Primary Generalized Mixed Models Analyses of Mortality Outcome and BCL-2 SNP														
	Analysis of GEE Parameter Estimates							Analysis of GEE Parameter Estimates						
Variables	Genotype	coefff	Odds Ratio	95% Confidence Limits		Z	Type 3 X^2	Genotype	coefff	Odds Ratio	95% Confidence Limits		Z	Type 3 X^2
RS12968517 (n=141)														
Genotype	xx	-0.0382	0.96252	-1.1453	1.0690	0.9461	0.0493*	yy & xy	0.5804	1.786753	-0.4325	1.5934	0.2614	0.2544
	yy	1.8525	6.37574	0.4154	3.2897	.0115*		xx						
	xy													
Time (Months)	24	1.4739	4.36623	1.0512	1.8966	<.0001***	<.0001***	24	1.4125	4.106208	0.9897	1.8353	<.0001***	<.0001***
	12	0.5011	1.65054	0.2187	0.7835	.0005**		12	0.4979	1.645263	0.2187	0.7771	.0005**	
	6	0.1718	1.18744	-0.0747	0.4184	0.1719		6	0.1675	1.182345	-0.0712	0.4062	0.169	
	3							3						
Age		0.0772	1.08026	0.0370	0.1174	.0002**	0.0005**		0.0709	1.073474	0.0356	0.1062	<.0001***	0.0005**
GCS		-2.5193	0.08052	-3.6508	-1.3878	<.0001***	<.0001***		-2.518	0.080621	-3.5229	-1.5131	<.0001***	<.0001***
Gender		0.7537	2.12485	-0.3053	1.8127	0.163	0.1179		0.762	2.142557	-0.3224	1.8464	0.1684	0.1837
Race		-1.1605	0.31333	-2.5421	0.2212	0.0997*	0.1569		-1.233	0.291417	-2.5915	0.1256	0.0753	0.139
Hypothermic		-1.4352	0.23807	-2.6872	-0.1831	.0247*	0.0534		-1.312	0.269281	-2.6522	0.0282	0.055	0.0863
Hypotensive		-0.6907	0.50123	-1.9734	0.5920	0.2912	0.3018		-0.6924	0.500374	-2.0532	0.6685	0.3187	0.3269
Seizures		0.2778	1.32022	-1.7165	2.2721	0.7848	0.7731		0.1926	1.212398	-1.9718	2.357	0.8615	0.8571
RS17756073 (n=138)														
Genotype	xx	0.1646	1.17892	-0.8534	1.1825	0.7513	0.7177	yy & xy	0.0529	1.054324	-0.8617	0.9675	0.9098	0.9098
	yy	0.6714	1.95698	-0.9339	2.2767	0.4123		xx						
	xy													
Time (Months)	24	1.3487	3.85241	0.9261	1.7714	<.0001***	<.0001***	24	1.3447	3.837035	0.9301	1.7954	<.0001***	<.0001***
	12	0.4792	1.61478	0.1981	0.7602	.0008**		12	0.4883	1.629544	0.2039	0.7726	.0008**	
	6	0.1667	1.1814	-0.0810	0.4143	0.1871		6	0.1615	1.175272	-0.0871	0.4101	0.2028	
	3							3						
Age		0.0758	1.07875	0.0416	0.1101	<.0001***	0.0002**		0.0751	1.077992	0.0399	0.1102	<.0001***	0.0002**
GCS		-2.5665	0.0768	-3.6133	-1.5197	<.0001***	<.0001***		-2.6023	0.074103	-3.6346	-1.57	<.0001***	<.0001***
Gender		0.6053	1.8318	-0.4828	1.6935	0.2756*	0.2938		0.4987	1.646579	-0.5748	1.5721	0.3625	0.3787
Race		-1.08	0.3396	-2.5153	0.3553	0.1403	0.1973		-1.0514	0.349448	-2.4062	0.3033	0.1282	0.1949
Hypothermic		-1.395	0.24783	-2.7864	-0.0036	0.0494*	0.0751		-1.4306	0.239165	-2.7794	-0.0817	0.0376*	0.0645
Hypotensive		-0.4364	0.64636	-1.7413	0.8685	0.5122	0.5133		-0.3877	0.678616	-1.7081	0.9327	0.565	0.5645
Seizures		0.3646	1.43994	-1.8449	2.5740	0.7464	0.7331		0.4045	1.498553	-1.7852	2.5942	0.7173	0.7015
RS17759659 (n=139)														
Genotype	xx	-1.7814	0.1684	-3.1219	-0.4408	0.0092**	0.0075**	yy & xy	1.6195	5.050564	0.3189	2.9202	.0147*	0.0031**
	yy	-0.6233	0.53617	-1.9120	0.6655	0.3432		xx						
	xy													
Time (Months)	24	1.3191	3.74005	0.8818	1.7564	<.0001***	<.0001***	24	1.2805	3.598438	0.8419	1.719	<.0001***	<.0001***
	12	0.5505	1.73412	0.2228	0.8782	0.001**		12	0.5353	1.707961	0.2136	0.857	.0011**	
	6	0.1561	1.16894	-0.1029	0.4151	0.2375		6	0.1477	1.159165	-0.1043	0.3998	0.2506	
	3							3						
Age		0.0918	1.09615	0.0471	0.1366	<.0001***	<.0001***		0.0909	1.095159	0.0469	0.1348	<.0001***	<.0001***
GCS		-2.6174	0.07299	-3.7840	-1.4509	<.0001***	<.0001***		-2.5867	0.075268	-3.7247	-1.4487	<.0001***	<.0001***
Gender		0.5786	1.78354	-0.5629	1.7202	0.3205	0.3399		0.5144	1.672635	-0.6347	1.6634	0.3803	0.394
Race		-1.2239	0.29408	-2.5108	0.0629	0.0623	0.1087		-1.3282	0.264954	-2.6139	-0.0425	.0429*	0.0961
Hypothermic		-1.2639	0.28255	-2.7850	0.2572	0.1034	0.1241		-1.3064	0.270793	-2.8266	-0.2138	0.0921	0.1172
Hypotensive		-0.4876	0.6141	-1.8369	0.8616	0.4788	0.479		-0.4479	0.638969	-1.8426	0.9469	0.5291	0.5275
Seizures		0.4462	1.56236	-1.5836	2.4760	0.6666	0.6503		0.3358	1.399059	-1.6832	2.3548	0.7444	0.7329

* X^2 p≤ 0.05; ** X^2 p≤ 0.01; *** X^2 p≤ 0.0001

Table 4-8 continued

Primary Generalized Mixed Models Analyses of Mortality Outcome and BCL-2 SNP														
	Analysis of GEE Parameter Estimates							Analysis of GEE Parameter Estimates						
Variables	Genotype	coefff	Odds Ratio	95% Confidence Limits		Z	Type 3 X^2	Genotype	coefff	Odds Ratio	95% Confidence Limits		Z	Type 3 X^2
RS1801018(n=141)														
Genotype	xx	-1.0477	0.35074	-2.3834	0.2879	0.1242	0.0055**	yy & xy	1.6109	5.007316	0.4024	2.8194	.009**	.0035**
	yy	-1.9454	0.14293	-3.2108	-0.6801	.0026**		xx						
	xy													
Time (Months)	24	1.3458	3.84126	0.9214	1.7702	<.0001***	<.0001***	24	1.275	3.578701	0.862	1.688	<.0001***	<.0001***
	12	0.5362	1.7095	0.2363	0.8361	0.0005**		12	0.4924	1.636238	0.2022	0.7827	.0009**	
	6	0.2108	1.23467	-0.0432	0.4649	0.1039		6	0.1805	1.197816	-0.0638	0.4248	0.1476	
	3							3						
Age		0.0852	1.08893	0.0449	0.1256	<.0001***	0.0004**		0.0842	1.087846	0.0451	0.1233	<.0001***	0.0002**
GCS		-2.7356	0.06486	-3.8590	-1.6122	<.0001***	<.0001***		-2.6201	0.072796	-3.6544	-1.5858	<.0001***	<.0001***
Gender		0.5469	1.72789	-0.6603	1.7542	0.3746	0.3928		0.4031	1.496457	-0.7714	1.5775	0.5012	0.5121
Race		-1.7043	0.1819	-3.1224	-0.2862	0.0185	0.0621		-1.8294	0.16051	-3.2378	-0.421	.0109*	0.056
Hypothermic		-1.3365	0.26276	-2.8328	0.1598	0.08	0.1057		-1.4903	0.225305	-2.946	-0.0346	.0448*	0.0702
Hypotensive		-0.4179	0.65843	-1.7319	0.8961	0.5331	0.5354		-0.3439	0.709	-1.7248	1.037	0.6255	0.6245
Seizures		0.9825	2.67113	-1.4457	3.4107	0.4278	0.4155		0.7289	2.072799	-1.595	3.0529	0.5387	0.522
RS4941185 (n=140)														
Genotype	xx	0.2696	1.30944	-0.6961	1.2354	0.5843	0.8593	yy & xy	-0.2074	0.812695	-1.1236	0.7087	0.6572	0.6581
	yy	0.2039	1.22618	-1.2899	1.6977	0.7891		xx						
	xy													
Time (Months)	24	1.4091	4.09227	0.9832	1.8351	<.0001***	<.0001***	24	1.4153	4.117722	0.9915	1.839	<.0001***	<.0001***
	12	0.4927	1.63673	0.2158	0.7697	0.0005**		12	0.4914	1.634603	0.2134	0.7694	.0005**	
	6	0.1562	1.16906	-0.0792	0.3915	0.1934		6	0.1556	1.168359	-0.0812	0.3925	0.1978	
	3							3						
Age		0.0744	1.07724	0.0402	0.1086	<.0001***	0.0002**		0.0739	1.076699	0.0387	0.1092	<.0001***	0.0002**
GCS		-2.524	0.08014	-3.5678	-1.4802	<.0001***	<.0001***		-2.549	0.07816	-3.5453	-1.5527	<.0001***	<.0001***
Gender		0.5355	1.7083	-0.5311	1.6022	0.3251	0.3358		0.5677	1.764205	-0.4515	1.587	0.275	0.2903
Race		-0.9892	0.37187	-2.3365	0.3581	0.1502	0.2067		-0.9665	0.380412	-0.2305	0.3721	0.157	0.2144
Hypothermic		-1.2773	0.27879	-2.6708	0.1163	0.0724	0.1085		-1.2792	0.27826	-2.6711	0.1127	0.0717	0.1075
Hypotensive		-0.6475	0.52335	-2.0495	0.7545	0.3653	0.3713		-0.6433	0.525555	-2.0401	0.7535	0.3667	0.3724
Seizures		0.2266	1.25433	-1.9994	2.4525	0.8419	0.8372		0.2784	1.321014	-1.9475	2.5043	0.8064	0.7997
RS7230970 (n=140)														
Genotype	xx	-1.6522	0.19163	-3.3382	0.0339	0.0548	0.054	yy & xy	0.7371	2.089866	-0.3661	1.8404	0.1904	0.17
	yy	-1.2079	0.29882	-2.4578	0.0420	0.0582		xx						
	xy													
Time (Months)	24	1.4528	4.27507	0.9700	1.9356	<.0001***	<.0001***	24	1.4012	4.060069	0.9589	1.8436	<.0001***	<.0001***
	12	0.4944	1.63951	0.1801	0.8087	0.0021**		12	0.4863	1.626288	0.198	0.7745	.0009**	
	6	0.1776	1.19435	-0.1128	0.4681	0.2306		6	0.1512	1.163229	-0.1028	0.4051	0.2433	
	3							3						
Age		0.0971	1.10197	0.0585	0.1357	<.0001***	<.0001***		0.0882	1.092207	0.0523	0.1241	<.0001***	<.0001***
GCS		-3.4687	0.03116	-4.9089	-2.0285	<.0001***	<.0001***		-2.9738	0.051109	-3.9907	-1.957	<.0001***	<.0001***
Gender		0.5176	1.678	-0.6071	1.6423	0.3671	0.3852		0.5945	1.812125	-0.539	1.728	0.304	0.3246
Race		-1.7047	0.18183	-3.1846	-0.2248	0.024*	0.0856		-1.0361	0.354836	-2.4629	0.3907	0.1547	0.2165
Hypothermic		-2.3842	0.09216	-4.1698	-0.5986	0.0089**	0.0227*		-1.749	0.173948	-3.2171	-0.281	.0195*	0.0446*
Hypotensive		0.1158	1.12277	-1.4269	1.6585	0.883	0.8835		-0.465	0.628135	-1.8723	0.9422	0.5172	0.5136
Seizures		0.4182	1.51922	-1.5573	2.3936	0.6782	0.6676		0.1233	1.131224	-1.8931	2.1396	0.9046	0.9025

*** X^2 p≤ 0.05; **X^2 p≤ 0.01; ***X^2 p≤ 0.0001**

Table 4-8 continued

Primary Generalized Mixed Models Analyses of Mortality Outcome and BCL-2 SNP														
	Analysis of GEE Parameter Estimates							Analysis of GEE Parameter Estimates						
Variables	Genotype	coefff	Odds Ratio	95% Confidence Limits		*Z*	Type 3 X^2	Genotype	coefff	Odds Ratio	95% Confidence Limits		*Z*	Type 3 X^2
RS7236090 (n=139)														
Genotype	xx	-0.1195	0.88736	-1.1331	0.8942	0.8173	0.5572	yy & xy	-0.0565	0.945066	-1.0552	0.9421	0.9117	0.9116
	yy	-0.7971	0.45063	-2.3619	0.7678	0.3181		xx						
	xy													
Time (Months)	24	1.2943	3.64844	0.8887	1.6999	<.0001***	<.0001***	24	1.3305	3.782934	0.9203	1.7408	<.0001***	<.0001***
	12	0.4459	1.5619	0.1797	0.7121	0.001**		12	0.4796	1.615428	0.1968	0.7625	.0009**	
	6	0.1367	1.14648	-0.0975	0.3709	0.2526		6	0.156	1.168826	-0.0902	0.4022	0.2144	
	3							3						
Age		0.0781	1.08123	0.0405	0.1158	<.0001***	0.0002**		0.0764	1.079394	0.0413	0.1114	<.0001***	0.0002**
GCS		-2.5966	0.07453	-3.6382	-1.5549	<.0001***	<.0001***		-2.617	0.073022	-3.6345	-1.5995	<.0001***	<.0001***
Gender		0.6854	1.98457	-0.4002	1.7711	0.2159	0.2234		0.5763	1.779442	-0.4628	1.6154	0.277	0.2903
Race		-1.384	0.25057	-3.0015	0.2335	0.0935	0.1336		-1.0701	0.342974	-2.4741	0.3338	0.1352	0.1885
Hypothermic		-1.4222	0.24118	-2.8239	-0.0205	.0467*	0.0691		-1.4428	0.236265	-2.8362	-0.0494	.0424*	0.0634
Hypotensive		-0.3188	0.72702	-1.7484	1.1109	0.6621	0.6636		-0.3783	0.685025	-1.7204	0.9639	0.5807	0.5801
Seizures		0.5124	1.66929	-1.6629	2.6877	0.6443	0.6301		0.4144	1.513462	-1.769	2.5977	0.7099	0.6954
RS949037 (n=137) *this was an add on b/c of the drs/ gos results*														
Genotype	xx	-1.101	0.33254	-2.4680	0.2661	0.1145	0.0551	yy & xy	-0.8862	0.412219	-1.9451	0.1728	0.101	0.1112
	yy	0.6168	1.85299	-0.5086	1.7422	0.2827		xx						
	xy													
Time (Months)	24	1.2459	3.47606	0.8257	1.6661	<.0001***	<.0001***	24	1.2937	3.646253	0.872	1.7154	<.0001***	<.0001***
	12	0.4171	1.51755	0.1368	0.6974	0.0035**		12	0.446	1.562051	0.1626	0.7294	.002**	
	6	0.1257	1.13394	-0.1126	0.3640	0.3011		6	0.1289	1.137576	-0.1116	0.3695	0.2935	
	3							3						
Age		0.0802	1.0835	0.0443	0.1162	<.0001***	0.0002**		0.078	1.081123	0.0427	0.1133	<.0001***	0.0003**
GCS		-2.4704	0.08455	-3.5463	-1.3945	<.0001***	<.0001***		-2.4448	0.086743	-3.4938	-1.3958	<.0001***	<.0001***
Gender		0.5398	1.71566	-0.6561	1.7356	0.3764	0.3943		0.5063	1.659141	-0.6514	1.6641	0.3913	0.4088
Race		-0.911	0.40212	-2.4244	0.6025	0.2381	0.2738		-1.1254	0.324523	-2.6401	0.3892	0.1453	0.1996
Hypothermic		-1.4116	0.24375	-2.7640	-0.0592	.0408*	0.0688		-1.5125	0.220358	-2.8862	-0.1388	.0309*	0.0599
Hypotensive		-0.2086	0.81172	-1.5624	1.1451	0.7626	0.7621		-0.3431	0.709567	-1.6722	0.9859	0.6129	0.6125
Seizures		0.4395	1.55193	-1.7440	2.6230	0.6932	0.6782		0.2914	1.3383	-1.8547	2.4375	0.7902	0.7807

*** X^2 p≤ 0.05; **X^2 p≤ 0.01; ***X^2 p≤ 0.0001**

4.2.1.2 Cognitive Behavioral Outcomes

Sub-sample description; Cognitive-Behavioral Outcomes: NRS-R

The mean age of the overall sample of 94 subjects was 31.24 years old (range 16-67; SD± 12.07). The sample was primarily Caucasian (n=91; 96.8%) and male (n=75; 79.8%). The majority of the sample genotyped had admission GCS of 6-8 (n=71; 75.5%). Of the subjects enrolled in the study (n=21; 22.3%) received therapeutic hypothermia. In the overall sample, the presence of hypoxia (n=12; 12.77%), hypotension (n=6; 6.38%), and documented seizures (n=6; 6.98%), and the genotype of APOE ε4 (n=22; 23.4%) were considered. Refer to table 4-15 for sample description. All of the SNP's genotyped for the overall sample met the HWE criteria for genotype representation in a population. Refer to table 4-15 for genotype frequencies for each SNP and HWE calculations.

NRS-R was analyzed at the 3, 6, and 12 month outcome time points. The time point of 24 months was excluded due to low sample size. Descriptive statistics for the NRS-R score are included in table #. The mean of the NRS-R scores consistent, ranging from 40.3-41.9 over the year of outcome measures. There was a consistent level of mild to moderate cognitive-behavioral issues among the subjects.

Preliminary Mixed Models Analysis for Cognitive-Behavioral Outcomes: NRS-R

In the preliminary mixed models analyses, each SNP was analyzed individually for a relationship with NRS-R over time. Each of the covariates was analyzed for potential relationship with NRS-R. By doing this the SNP's and covariates of interest in building the larger model were ascertained. The criterion used was a P for Type 3 tests of ≤0.2 in addition to covariates that were deemed prudent through the evidence in the literature (age, gender, race, GCS, and hypothermia). (Refer to table 4-16).

The preliminary mixed model analyses for NRS-R indicated that the SNP's and covariates of interest, meeting the criterion were: RS12454712 (genotypes P=0.0456; dichotomized genotype P=0.233); RS1481031 (genotypes P=0.1249; dichotomized genotype P=0.3432); RS17756073 (genotypes P=0.2039; dichotomized genotype P=0.0881); RS17759659 (genotypes P=0.0776; dichotomized genotype P=0.02668); RS 1944419 (genotypes P=0.1585; dichotomized genotype P=0.1382; RS4456611 (genotypes P=0.18888; dichotomized genotype P=0.0765); RS7236090 (genotypes P=0.0272; dichotomized genotype P=0.0072); age (P= 0.0019); and seizures (P= 0.0748). Time (months) (P=0.8966), GCS (P=0.3761), gender (P= 0.9465), race (P=0.2342), and hypothermia (P= 0.2865) were not indicated to be significant in the preliminary analysis, however, they were controlled for in the full model. (Refer to table 4-16).

Primary Mixed Models Analyses for Cognitive-Behavioral Outcomes: NRS-R

The sample size for the primary analysis of the full model was n=61 compared to the n=94 in the preliminary analyses. The decrease in the sample size was due to unknown/ missing data for the seizure variable. In analyzing the 7 BCL-2 SNP's of interest there were two SNP's which had significant findings. (Refer to tables 4-17 and 4-18).

The mixed model with SNP RS17756073: genotype XX (P= 0.0514) with overall genotype marginally significant (P for type 3 F tests =0.1041). XX genotype was associated with a decrease in NRS-R scores and therefore is associated with better outcomes. This trend was significantly evident in the dichotomized analysis (P=0.0331); subjects with no X alleles or only 1 X allele(YY and XY) had significantly higher NRS-R scores, worse outcomes. A documented presence of seizures preadmission were found to been marginally significant (genotypes

P=0.0544; dichotomized genotype P=0.0519) and associated with higher NRS-R scores. (Refer to table 4-17).

The mixed models analysis with RS4456611 in the full model resulted in trends towards significance on all accounts, overall genotype (P for type 3 F tests =0.0992). This trend was evident in the dichotomized analysis (P=0.065) with YY being associated with higher NRS-R scores, indicating poorer outcomes; Subjects with 0-1 copies of the Y allele have trend of better outcomes. Age was found to trend towards significance (genotypes P=0.0805; dichotomized genotype P=0.0684). Increasing age was associated with having higher NRS-R scores and therefore poor outcomes. Seizure was found to trend towards significance (genotypes P=0.0812; dichotomized genotype P=0.0732). A documentation of pre-admission seizures was related to having higher NRS-R scores compared to those without known seizures and therefore poorer outcomes. (Refer to table 4-18).

The mixed models analyses indicated that the following SNP's were not significant predictors of NRS-R: RS12454712 (genotypes P=0.4012; dichotomized genotype P=0.2214); RS1481031 (genotypes P=0.9115; dichotomized genotype P=0.6693); RS17759659 (genotypes P=0.3314; dichotomized genotype P=0.4579); RS1944419 (genotypes P=0.8539; dichotomized genotype P=0.6165) and RS7236090 (genotypes P=0.2497; dichotomized genotype P=0.0959). Among the models for SNP's that were not significant, age and seizures on pre admission were covariates of interest in the model with trending statistical significance (age, P= 0.0379-0.0789; seizures, P=0.0388-0.0783). Increasing age and preadmission seizures were consistently associated with poor outcomes in this subsample. (Refer to tables 4-17 and 4-18).

Table 4-9: BCL-2 Genotypes and NRS-R Descriptive Data

BCL-2 Genotypes and NRS-R Descriptive Data					
Variable	**n=**	**Mean**	**Std. Deviation**	**Range**	
Age	94	31.24	12.07	16-67	
			Frequency (%)	**Frequency (%)of Unknown**	
GCS	94	score 3 to 5	23(24.5%)	0	
		score 6 to 8	71(75.5%)		
Gender (male)	94		75(79.8%)	0	
Race (Caucasian)	94		91(96.8%)	0	
Hypothermic	94		21(22.3%)	0	
Hypoxia	59		12(12.77%)	35 (37.23%)	
Hypotensive	59		6(6.38%)	35 (37.23%)	
Seizures	61		6(6.38%)	31 (35.12)	
APOE4	90		22(23.4%)	4 (4.26%)	
NRS-R	**n=**	**Mean**	**Std. Deviation**	**Range**	**Frequency (%)of Unknown**
3 months	64	40.3	9.89	29-75	30(31.91%)
6 months	74	40.74	9.41	29-74	20 (21.28%)
12 months	69	41.9	12.97	29-85	25 (26.6%)

Hardy-Weinberg Equilibrium							
SNP	**n=**	**XX**	**YY**	**XY**	**X**	**Y**	**HWE**
RS1026825	94	25	26	43	0.494680851	0.50531915	1.0000
RS12454712	94	9	46	39	0.303191489	0.69680851	1.0000
RS12968517	94	44	11	39	0.675531915	0.32446809	1.0000
RS1381548	90	15	37	38	0.377777778	0.62222222	1.0000
RS1481031	92	35	11	46	0.630434783	0.36956522	1.0000
RS17756073	93	39	13	41	0.639784946	0.36021505	1.0000
RS17759659	94	38	15	41	0.622340426	0.37765957	1.0000
RS1801018	93	26	32	35	0.467741935	0.53225806	1.0000
RS1944419	91	22	20	49	0.510989011	0.48901099	1.0000
RS3810027	93	42	11	40	0.666666667	0.33333333	1.0000
RS4456611	90	16	25	49	0.45	0.55	1.0000
RS4941185	90	28	14	48	0.577777778	0.42222222	1.0000
RS7230970	88	20	25	43	0.471590909	0.52840909	1.0000
RS7236090	90	26	25	39	0.505555556	0.49444444	1.0000
RS8083946	93	16	38	39	0.38172043	0.61827957	1.0000
RS899968	92	13	39	40	0.358695652	0.64130435	1.0000
RS949037	91	22	33	36	0.43956044	0.56043956	1.0000

Table 4-10: Preliminary Mixed Models Analyses BCL-2 vs. NRS-R

			Preliminary Mixed Models Anaylsis BCL-2 versus NRS-R		
SNP	**n=**	**Genotype**	**coefff**	**P**	**P for Type-3 Test**
RS1026825	94	xx	-3.1409	0.1986*	0.3753
		yy	0.04537	0.9849	
		xy			
		xx & xy	-1.2178	0.5832	0.5832
		yy			
RS12454712	94	xx	3.582	0.3079	0.0456*
		yy	-3.8089	0.0644*	
		xy			
		xx & xy	4.4656	0.233	0.233
		yy			
RS12968517	94	xx	-1.3235	0.5359	0.7665
		yy	0.4897	0.8823	
		xy			
		yy & xy	1.4329	0.4731	0.4731
		xx			
RS1381548	90	xx	-2.7593	0.3421	0.5796
		yy	-1.6521	0.4563	
		xy			
		xx & xy	0.8825	0.6686	0.6686
		yy			
RS1481031	92	xx	-0.877	0.688	0.1249*
		yy	6.0508	0.0707*	
		xy			
		yy & xy	1.9983	0.3432	0.3432
		xx			
RS17756073	93	xx	-3.8707	0.076*	0.2039
		yy	-1.6042	0.5996	
		xy			
		yy & xy	3.4654	0.0881*	0.0881*
		xx			
RS17759659	94	xx	-0.7398	0.7279	0.0776*
		yy	5.6594	0.0492*	
		xy			
		yy & xy	2.2554	0.2668	0.2668
		xx			
RS1801018	93	xx	0.4225	0.8659	0.8039
		yy	-1.1793	0.623	
		xy			
		xx & xy	1.3612	0.5222	0.5222
		yy			
RS1944419	91	xx	2.6426	0.2906	0.1585*
		yy	-3.138	0.2226	
		xy			
		yy & xy	-3.5683	0.1382*	0.1382*
		xx			
RS3810027	93	xx	3.0567	0.1419*	0.2948
		yy	3.1578	0.32	
		xy			
		yy & xy	-2.3638	0.2273	0.2273
		xx			
***p≤ 0.2**					

			Preliminary Mixed Models Anaylsis BCL-2 versus NRS-R		
SNP	**n=**	**Genotype**	**coefff**	**P**	**P for Type-3 Test**
RS4456611	90	xx	-1.2087	0.6544	0.1888*
		yy	3.6873	0.1175*	
		xy			
		xx & xy	-3.9777	0.0765*	0.0765*
		yy			
RS4941185	90	xx	0.4017	0.8649	0.7641
		yy	-1.8885	0.5287	
		xy			
		yy & xy	-0.8372	0.7096	0.7096
		xx			
RS7230970	88	xx	0.1218	0.9639	0.8531
		yy	1.3635	0.5881	
		xy			
		xx & xy	-1.3255	0.5726	0.5726
		yy			
RS7236090	90	xx	-5.9396	0.0121*	0.0272*
		yy	-0.287	0.9051	
		xy			
		yy & xy	5.8278	0.0072*	0.0072*
		xx			
RS8083946	93	xx	2.408	0.4125	0.7025
		yy	0.3246	0.8831	
		xy			
		xx & xy	0.357	0.8613	0.8613
		yy			
RS899968	92	xx	-2.7203	0.3862	0.6626
		yy	-1.1659	0.5885	
		xy			
		xx & xy	0.5295	0.7931	0.7931
		yy			
RS949037	91	xx	-2.2179	0.402	0.4586
		yy	1.1366	0.6239	
		xy			
		xx & xy	-1.9536	0.3526	0.3526
		yy			
Covariates					
Time (Months)	94	3	0.373	0.8006	0.8966
		6	-0.2864	0.835	
		12			
Age	94		0.07604	0.0019*	0.0019*
GCS	94		2.0633	0.3761	0.3761
Gender	94		0.1679	0.9465	0.9465
Race	94		-6.9721	0.2342	0.2342
Hypothermic	94		-2.4932	0.2865	0.2865
Hypoxia	59		1.2398	0.6379	0.6379
Hypotensive	59		2.3247	0.5359	0.5359
Seizures	61		-6.7635	0.0748*	0.0748*
APOEε4	90		0.4914	0.8378	0.8378
***p≤ 0.2**					

Table 4-11: Primary Mixed Models Analyses of NRS-R and BCL-2 SNP's

Primary Mixed Models Analyses of NRS-R as Cognitive-Behavioral Outcome and BCL-2 SNP								
Model Variables		**NRS-R**						
		coefff	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**
RS12454712 (n=61)								
Genotype	xx	3.1112	0.5562	0.4012	xx & xy	2.9552	0.2214	0.2214
	yy	-2.5692	0.3078		yy			
	xy							
Time (Months)	3	2.0285	0.1496	0.2935	3	2.0103	0.1536	0.2946
	6	1.6257	0.2009		6	1.6449	0.1958	
	12				12			
Age		0.1824	0.0789	0.0789		0.1979	0.0475*	0.0475*
GCS		0.6564	0.8316	0.8316		0.9711	0.7473	0.7473
Gender		0.0768	0.9811	0.9811		0.08747	0.9783	0.9783
Race		-4.627	0.4224	0.4224		-4.1425	0.4639	0.4639
Seizure		-5.8315	0.1719	0.1716		-6.2428	0.1354	0.1354
Hypothermia		-0.7293	0.8252	0.8252		-0.466	0.8857	0.8857
RS1481031 (n=60)								
Genotype	xx	1.1161	0.6928	0.9115	yy & xy	-1.1614	0.6693	0.6693
	yy	-0.2513	0.9449		xx			
	xy							
Time (Months)	3	2.1546	0.1312	0.2548	3	2.124	0.1366	0.2631
	6	1.7452	0.1738		6	1.7296	0.1778	
	12				12			
Age		0.1934	0.0575	0.0575		0.1932	0.055	0.055
GCS		1.6591	0.5957	0.5957		1.6413	0.5948	0.5948
Gender		1.4179	0.6579	0.6579		1.4	0.6582	0.6582
Race		-3.5959	0.5406	0.5406		-3.6597	0.5265	0.5265
Seizure		-9.8397	0.0407*	0.0407*		-9.8262	0.0388*	0.0388*
Hypothermia		0.6396	0.8489	0.8489		0.6503	0.8445	0.8445
RS17756073 (n=60)								
Genotype	xx	-5.22	0.0514	0.1041	yy & xy	5.248	0.0331*	0.0331*
	yy	0.1657	0.9648		xx			
	xy							
Time (Months)	3	2.2143	0.1156	0.2332	3	2.1842	0.1204	0.2412
	6	1.7656	0.1635		6	1.7497	0.1674	
	12				12			
Age		0.1639	0.1026	0.1026		0.1639	0.099	0.099
GCS		-0.5927	0.8491	0.8491		-0.5964	0.8462	0.8462
Gender		1.2356	0.6912	0.6912		1.215	0.6922	0.6922
Race		-3.1898	0.5752	0.5752		-3.2362	0.5636	0.5636
Seizure		-8.0352	0.0544	0.0544		-8.0318	0.0519	0.0519
Hypothermia		-0.15	0.9633	0.9633		-0.1669	0.9583	0.9583
*** p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001**								

Table 4-11 continued

Primary Mixed Models Analyses of NRS-R as Cognitive-Behavioral Outcome and BCL-2 SNP								
Model		**NRS-R**						
Variables		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**
RS17759659 (n=61)								
Genotype	xx	-0.6033	0.8172	0.3314	yy & xy	1.8206	0.4579	0.4579
	yy	5.1123	0.1987		xx			
	xy							
Time (Months)	3	2.0576	0.1426	0.2658	3	2.0428	0.1462	0.2735
	6	1.7384	0.1698		6	1.7181	0.1756	
	12				12			
Age		0.213	0.0379*	0.0379*		0.2065	0.0444*	0.0444*
GCS		2.2396	0.4796	0.4796		1.5404	0.6226	0.6226
Gender		0.2679	0.9329	0.9329		0.9047	0.7746	0.7746
Race		-5.1003	0.3748	0.3748		-4.8255	0.4024	0.4024
Seizure		-7.7964	0.0701	0.0701		-6.7008	0.1122	0.1122
Hypothermia		0.6227	0.8503	0.8503		-0.0621	0.9849	0.9849
RS1944419 (n=61)								
Genotype	xx	1.3005	0.681	0.8539	yy & xy	-1.514	0.6165	0.6165
	yy	-0.7463	0.7962		xx			
	xy							
Time (Months)	3	2.0911	0.138	0.2689	3	2.0543	0.1449	0.2796
	6	1.6992	0.1813		6	1.6787	0.1867	
	12				12			
Age		0.1839	0.0783	0.0783		0.1893	0.0617	0.0617
GCS		1.4943	0.6451	0.6451		1.4049	0.6545	0.6545
Gender		0.7488	0.8176	0.8176		0.8273	0.7958	0.7958
Race		-4.4672	0.4406	0.4406		-4.3924	0.4431	0.4431
Seizure		-6.8657	0.1076	0.1076		-6.8218	0.106	0.106
Hypothermia		-0.1416	0.966	0.966		-0.1802	0.9562	0.9562
RS4456611 (n=60)								
Genotype	xx	-3.2884	0.2627	0.0992	xx & xy	-4.9777	0.065	0.065
	yy	3.9228	0.1673		yy			
	xy							
Time (Months)	3	2.2506	0.1017	0.1905	3	2.2222	0.1066	0.1992
	6	1.9197	0.1128		6	1.8983	0.1277	
	12				12			
Age		0.1567	0.0805	0.0805		0.1632	0.0684	0.0684
GCS		0.6409	0.8222	0.8222		-0.2486	0.9277	0.9277
Gender		-1.9713	0.5119	0.5119		-1.0876	0.7073	0.7073
Race		-2.1103	0.707	0.707		-1.532	0.784	0.784
Seizure		-6.5108	0.0812	0.0812		-6.6863	0.0732	0.0732
Hypothermia		-1.1029	0.7041	0.7041		-0.9036	0.7551	0.7551
RS7236090 (n=60)								
Genotype	xx	-4.0415	0.1535	0.2497	yy & xy	4.2506	0.0959	0.0959
	yy	0.5587	0.8588		xx			
	xy							
Time (Months)	3	2.0173	0.1554	0.2967	3	1.9991	0.1584	0.3002
	6	0.16508	0.196		6	1.6451	0.1972	
	12				12			
Age		0.1857	0.0634	0.0634		0.1859	0.0601	0.0601
GCS		0.8952	0.7673	0.7673		0.9049	0.7622	0.7622
Gender		0.5163	0.8694	0.8694		0.5334	0.8635	0.8635
Race		-2.8704	0.6293	0.6293		-3.1854	0.5729	0.5729
Seizure		-6.4849	0.1209	0.1209		-6.5703	0.1096	0.1096
Hypothermia		0.3886	0.9076	0.9076		0.5308	0.8688	0.8688

*** p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001**

Sub-sample Description Cognitive-Behavioral Outcomes: Trails Making Tests

The mean age of the overall sample of 30 subjects was 33.17 years old (range 17-60; SD± 11.002). The sample was primarily Caucasian (n=24; 88.9%) and male (n=22; 81.5%). The majority of the sample had admission GCS of 6-8 (n=19; 70.4%) (See figure 4-10). Only 2 (7.4%) of the subjects in this sub-sample received therapeutic hypothermia. In the overall sample the presence of sustained hypoxia (n=1; 4 %), hypotension (n=1; 4 %), documented pre-admission seizures (n=0) and the presence of APOE ε4 (n=6; 23.1%) were considered. Refer to table 4-19 for sample description. All of the SNP's genotyped for the overall sample met the HWE criteria for genotype representation in a population. Refer to table 4-19 for genotype frequencies for each SNP and HWE calculations.

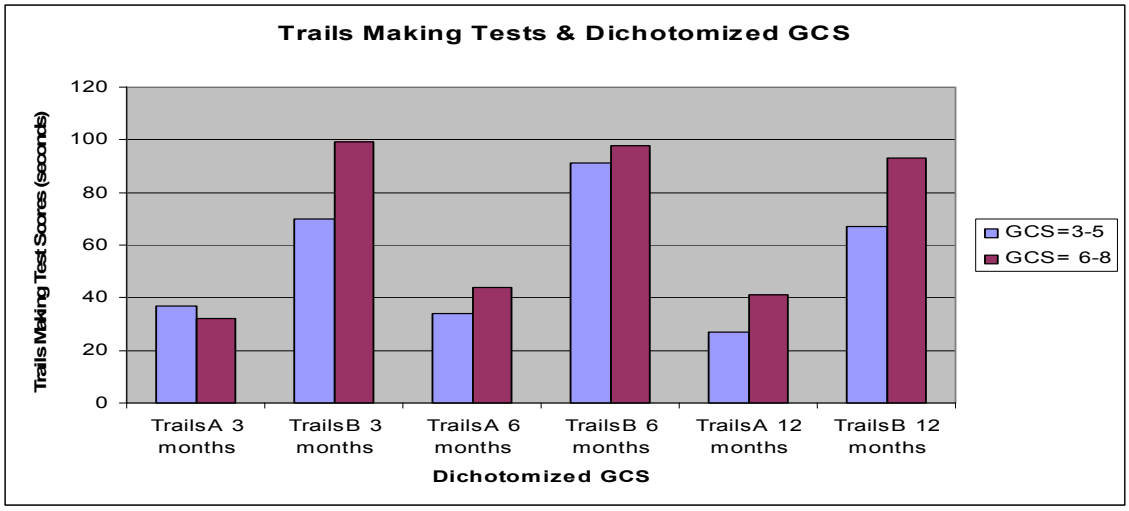


Figure 4-10: Trails Making Tests & Dichotomized GCS

Trails A and Trails B were analyzed at the 3, 6, and 12 month outcome time points. The sample size at the 3 month outcomes evaluation is low (n=6; 77.8% missing data). In summer of 2005, the decision to stop collecting Trails Making Test data at the 3 month time point was made due to inability of subjects to participate at this early stage of recovery. The higher percentage of subjects in vegetative state and severe disability as indicated in the 3 months GOS scores (ref to

table 4-19) effected eligibility to perform the test. The sample size for the 6 month and 12 month outcomes are equal (n=22; 18.5% missing data). Trails A scores (measured in seconds) improve with time, indicating that the subject's attention to task improves. Trails B scores (measured in seconds) improve with time, indicating that the subject's mental flexibility improves with time as well. (Refer to table 4-19).

Preliminary Mixed Models Analysis for Cognitive-Behavioral Outcomes: Trails Making Tests

In the preliminary mixed models analyses, each SNP was analyzed individually for a relationship with Trails A and Trails B over time. Each of the covariates were analyzed for potential relationship with Trails A and Trails B. By doing this the SNP's and covariates of interest in building the larger model were ascertained. The criterion used was a P for Type 3 tests of ≤ 0.2 in addition to covariates that are deemed prudent through the evidence in the literature (age, gender, race, GCS, hypothermia).

Trails A

The preliminary mixed model analyses for Trails A indicated that the SNP's of interest meeting the significance criterion were: RS12454712 (genotypes P=0.3136; dichotomized genotype P=0.1229); RS12968517(genotypes P=0.2941; dichotomized genotype P=0.1281); RS 1381548 (genotypes P=0.0695; dichotomized genotype P=0.0225); RS1481031 (genotypes P=0.024; dichotomized genotype P=0.7551); RS 17756073 (genotypes P=0.0916; dichotomized genotype P=0.2669); RS 17759659 (genotypes P=0.2924; dichotomized genotype P=0.1766); RS 1801018 (genotypes P=0.1043; dichotomized genotype P=0.0475); RS3810027 (genotypes P=0.0238; dichotomized genotype P=0.1893); RS4941185 (genotypes P=0.0373; dichotomized genotype P=0.0838); RS7236090 (genotypes P=0.0215; dichotomized genotype P=0.1866); and

RS8083946 (genotypes P=0.0709; dichotomized genotype P=0.3083). The covariates of interest were: GCS (P= 0.1314), hypothermia (P= 0.0191), hypoxia (P= 0.1535), and hypotension (P= 0.1535), however hypoxia and hypotension were not included in the models due to low sample size in each group (n=1). (Refer to table 4-20 and 4-21).

The covariates not meeting the statistical criterion, but included in the full models were: time (months) (P=0.6923); age (P= 0.7936); Gender (P= 0.467) and Race (P=0.4104). (Refer to table 4-20 and 4-21).

Trails B

The preliminary mixed model analyses for Trails B indicated that the SNP's of interest meeting the criterion were: RS12454712 (genotypes P=0.26; dichotomized genotype P=0.1013); RS 1381548 (genotypes P=0.1167; dichotomized genotype P=0.037);); RS 17756073 (genotypes P=0.1129; dichotomized genotype P=0.1133); RS 1801018 (genotypes P=0.1835; dichotomized genotype P=0.1008); RS4941185 (genotypes P=0.3783; dichotomized genotype P=0.1825); RS7236090 (genotypes P=0.1592; dichotomized genotype P=0.4147); and RS8083946 (genotypes P=0.1664; dichotomized genotype P=0.1606). The covariates of interest were: time (months) (P=0.1135), hypothermia (P= 0.0549), hypoxia (P= 0.1682), and hypotension (P= 0.1682), however hypoxia and hypotension were not included in the models due to low sample size in each group (n=1). (Refer to table 4-20 and 4-21).

The covariates not meeting the statistical criterion, but included in the full models were: age (P= 0.3197), GCS (P= 0.2635), Gender (P= 0.8871) and Race (P=0.52). (Refer to table 4-20 and 4-21).

While the following SNP's did not meet the criterion for Trails B, but did meet the criterion for Trails A, RS12968517, RS1481031, RS17759659, and RS3810027. Full models were considered for these SNP's in the primary analyses for comparison to Trails A results.

Primary Mixed Models Analysis for Trails Making Tests

Primary Mixed Models Analysis for Trails A

The mixed model with SNP RS12968517: genotype XX (P= 0.0305) with overall genotype trending towards significance (P for type 3 F tests =0.087). XX genotype is associated with decrease Trails A scores and was therefore associated with better outcomes (attention). This trend was evident in the dichotomized analysis (P=0.0289). Subjects with 0-1 copies of the X allele have higher Trails A scores and therefore poorer outcomes. The covariates in this model that were significant to Trails A were; GCS was found to been significantly associated with Trails A (genotypes P=0.0119; dichotomized genotype P=0.0108), with higher GCS scores on admission being associated with higher Trails A scores (longer times); poor outcomes. Compared to the subjects in the hypothermia protocol, those who were not hypothermic were found to have significantly higher Trails A scores (genotypes P=0.0218; dichotomized genotype P=0.0208); poor outcomes. (Refer to table 4-22).

The mixed model with SNP RS13815487: genotype YY (P= 0.0515) with overall genotype not significant (P for type 3 F tests =0.1203). YY genotype is associated with decrease Trails A scores and was therefore associated with better outcomes (attention). This trend was evident in the dichotomized analysis (P=0.0366). Subjects with 0-1 copies of the Y allele have higher Trails A scores and therefore poorer outcomes. The covariates in this model that were significant to Trails A were; GCS was found to been significant (genotypes P=0.0529;

dichotomized genotype P=0.0438), with higher GCS scores on admission being associated with higher Trails A scores (longer times); poor outcomes. Compared to the subjects in the hypothermia protocol, those who were not hypothermic were found to have significantly higher Trails A scores (genotypes P=0.0338; dichotomized genotype P=0.0291); poor outcomes. (Refer to table 4-23).

The mixed model with SNP RS3810027: genotype XX (P= 0.0196); and YY (P= 0.0351) with overall genotype significant (P for type 3 F tests =0.032). Compared to subjects with heterozygous (XY), subjects who were homozygous for wild type or he variant, had significantly higher Trails A scores and was therefore associated with poorer outcomes (attention). GCS was found to been significant (genotypes P=0.4196; dichotomized genotype P=0.0669), with higher GCS scores on admission being associated with higher Trails A scores (longer times); poor outcomes. Compared to the subjects in the hypothermia protocol, those who were not hypothermic were found to have significantly higher Trails A scores (genotypes P=0.0079; dichotomized genotype P=0.0064); poor outcomes. (Refer to table 4-25).

The mixed model with SNP RS7236090: genotype XX (P= 0.0396); and YY (P= 0.0387) with overall genotype marginally significant (P for type 3 F tests =0.0577). Compared to subjects with heterozygous (XY), subjects who were homozygous for wild type or he variant, had significantly lower Trails A scores and was therefore associated with good outcomes. (Refer to table 4-26).

The mixed model with SNP RS8083946: genotype XX (P= 0.0285) with overall genotype showed trends towards statistical significance (P for type 3 F tests =0.0835). XX genotype was associated with higher Trails A scores and was therefore associated with poor outcomes; decreased attention. This trend was not evident in the dichotomized analysis

(P=0.8682). The covariates in this model that showed trends towards significance in relation to Trails A were; GCS (genotypes P=0.1211; dichotomized genotype P=0.0657), with higher GCS scores on admission being associated with higher Trails A scores (longer times); poor outcomes. Compared to the subjects in the hypothermia protocol, those who were not hypothermic were found to have trends towards significantly higher Trails A scores (genotypes P=0.0079; dichotomized genotype P=0.0064); poor outcomes. (Refer to table 4-26).

Primary Mixed Models Analysis for Trails B

The mixed model with SNP RS1381548: genotype YY (P= 0.044) with overall genotype was non-significant (P for type 3 F tests =0.1195). However, in the dichotomized analysis, YY genotype was associated with decrease Trails B scores and was therefore associated with better outcomes (mental flexibility) (P=.0391). In contrast, subjects with 0-1 copies of the Y allele have significantly higher Trails B scores. The covariates in this model that showed trends towards significance to Trails B were; time (genotypes P=0.0733; dichotomized genotype P=0.069) as Trails B tests administered closer to time of injury (3 month evaluation compared to 12 months post injury) had longer Trails B time scores indicating that less mental flexibility. GCS was found to been marginally significant (genotypes P=0.0809; dichotomized genotype P=0.0788), with higher GCS scores on admission being associated with higher Trails B scores (longer times); poor outcomes. Compared to the subjects in the hypothermia protocol, those who were not hypothermic were found to have marginally significantly higher Trails B scores (genotypes P=0.0681; dichotomized genotype P=0.0655); poor outcomes. (Refer to table 4-23).

The mixed model with SNP RS17756073: genotype XX (P= 0.0532) with overall genotype was non-significantly associated with Trails B scores (P for type 3 F tests =0.1456). Individuals with two copies of the X allele had lower Trails B scores and therefore associated

with better outcomes (mental flexibility). This trend was evident in the dichotomized analysis with a marginal significant relationship between the SNP and Trails B scores (P=0.0516). Individuals with 0-1 copies of X had higher Trails B time and was associated with decreased mental flexibility. The covariates in this model that were marginally /significant to Trails B were; GCS (genotypes P=0.0736; dichotomized genotype P=0.0714), with higher GCS scores on admission being associated with higher Trails B scores (longer times), higher GCS are associated with poor outcomes. Compared to the subjects in the hypothermia protocol, those who were not hypothermic were found to have marginally significantly higher Trails B scores (genotypes P=0.1415; dichotomized genotype P=0.0319); poor outcomes. (Refer to table 4-24).

In summary, among the SNP analyzed for scores on the Trails Making Tests (Trails A and Trails B), RS1381548 was implicated as a SNP of interest. Higher GCS on admission was found to have statistically significantly higher Trails A and Trails B scores or poorer outcomes compared to subjects admitted with a lower GCS score.

Table 4-12: BCL-2 Genotypes Versus Trails Making Tests Descriptive Data

BCL-2 Genotypes Versus Trails Making Tests Descriptive Data					
Variables	**n=**	**Mean**	**Std. Deviation**	**Range**	
Age	27	33.17	11.002	17-60	
			Frequency (%)	**Frequency % of Unknown**	
GCS	27	score 3 to 5	8 (29.6%)	0	
		score 6 to 8	19(70.4%)		
Gender (male)	27		22(81.5%)	0	
Race (Caucasian)	27		24(88.9%)	0	
Hypothermic	27		2(7.4%)	0	
Hypoxia	25		1(4%)	2 (7.41%)	
Hypotensive	25		1(4%)	2 (7.41%)	
Seizures	27		0	0	
APOEε4	26		6(23.1%)	1 (3.7%)	
Trails Making Tests	**n=**	**Mean**	**Std. Deviation**	**Range**	**Frequency % of Unknown**
Trails A					
3 months	6	33.5	13.31	19-54	21(77.8%)
6 months	22	41.05	16.66	15-79	5(18.5%)
12 months	22	36.91	18.96	10-83	5(18.5%)
Trails B					
3 months	6	89.17	36.68	44-131	21(77.8%)
6 months	22	95.87	57.35	3-300	5(18.5%)
12 months	22	85.95	38.91	33-179	5(18.5%)

Hardy-Weinberg Equilibrium							
SNP	**n=**	**XX**	**YY**	**XY**	**X**	**Y**	**HWE**
RS1026825	10	5	12	27	0.592592593	0.40740741	1.0000
RS12454712	4	11	12	27	0.37037037	0.62962963	1.0000
RS12968517	15	2	10	27	0.740740741	0.25925926	1.0000
RS1381548	5	8	14	27	0.444444444	0.55555556	1.0000
RS1481031	11	4	12	27	0.62962963	0.37037037	1.0000
RS17756073	15	1	11	27	0.759259259	0.24074074	1.0000
RS17759659	15	2	9	26	0.75	0.25	1.0000
RS1801018	4	16	7	27	0.277777778	0.72222222	1.0000
RS1944419	5	6	16	27	0.481481481	0.51851852	1.0000
RS3810027	10	5	12	27	0.592592593	0.40740741	1.0000
RS4456611	7	6	13	26	0.519230769	0.48076923	1.0000
RS4941185	10	2	15	27	0.648148148	0.35185185	1.0000
RS7230970	6	11	10	27	0.407407407	0.59259259	1.0000
RS7236090	4	11	11	26	0.365384615	0.63461538	1.0000
RS8083946	8	9	10	27	0.481481481	0.51851852	1.0000
RS899968	7	9	10	26	0.461538462	0.53846154	1.0000
RS949037	10	5	11	26	0.596153846	0.40384615	1.0000

Table 4-13: Preliminary Mixed Models Analyses for the Trails Making Tests

Preliminary Mixed Models Anaylsis BCL-2 versus Trails Making Tests

			Trails A			Trails B		
SNP	**n=**	**Genotype**	**coefff**	**P**	**P for Type-3 Test**	**coefff**	**P**	**P for Type-3 Test**
RS1026825	27	xx	4.5196	0.5166	0.805	4.9557	0.8011	0.7158
		yy	1.4964	0.8633		19.9951	0.4188	
		xy						
		yy & xy	-4.0879	0.522	0.522	0.7902	0.9655	0.9655
		xx						
RS12454712	27	xx	0.2609	0.9763	0.3136	6.681	0.7907	0.26
		yy	-9.4225	0.1585*		-26.96	0.1538*	
		xy						
		xx & xy	9.4965	0.1229*	0.1229*	28.6688	0.1013	0.1013*
		yy						
RS12968517	27	xx	-8.4604	0.1965*	0.2941	6.4201	0.7367	0.931
		yy	4.8798	0.6823		-1.6717	0.9626	
		xy						
		yy & xy	9.3073	0.1281*	0.1281*	-6.7412	0.7033	0.7033
		xx						
RS1381548	27	xx	4.1072	0.5896	0.0695*	-4.8366	0.8279	0.1167*
		yy	-13.49	0.0459*		-39.719	0.0443*	
		xy						
		xx & xy	14.6129	0.0225*	0.0225*	38.5489	0.037*	0.037*
		yy						
RS1481031	27	xx	-2.18	0.7344	0.024*	-11.881	0.5201	0.2376
		yy	-15.097	0.0841*		-42.662	0.0943*	
		xy						
		yy & xy	-1.9702	0.7551	0.7551	0.6783	0.9699	0.9699
		xx						
RS17756073	27	xx	-8.9559	0.1348*	0.0916*	-31.977	0.0669*	0.1129*
		yy	-33.488	0.0606*		-65.31	0.1747*	
		xy						
		yy & xy	6.7477	0.2669	0.2669	27.2651	0.1133*	0.1133*
		xx						
RS17759659	26	xx	10.5502	0.1262*	0.2924	8.6219	0.6686	0.846
		yy	9.8784	0.4188		18.544	0.6141	
		xy						
		yy & xy	-8.6282	0.1766*	0.1766*	-5.0944	0.7842	0.7842
		xx						
RS1801018	27	xx	7.7341	0.4243	0.1043*	23.7917	0.3925	0.1835*
		yy	14.8893	0.0371*		37.2176	0.0688*	
		xy						
		xx & xy	-12.239	0.0475*	0.0475*	-28.837	0.1008*	0.1008*
		yy						
RS1944419	27	xx	9.9139	0.2218	0.2974	18.9721	0.4196	0.5419
		yy	-4.5381	0.5361		20.3002	0.3516	
		xy						
		xx & xy	6.9173	0.338	0.338	-15.76	0.4496	0.4496
		yy						
RS3810027	27	xx	13.6042	0.0313*	0.0238*	7.9726	0.6731	0.2583
		yy	19.3832	0.0168*		39.4572	0.1052*	
		xy						
		yy & xy	-8.2504	0.1893*	0.1893*	3.3622	0.8536	0.8536
		xx						
*** p≤ 0.2**								

Table 4-13 continued

Preliminary Mixed Models Anaylsis BCL-2 versus Trails Making Tests								
			Trails A			Trails B		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS4456611	26	xx	5.5315	0.4607	0.4869	-9.0855	0.6845	0.9189
		yy	8.9745	0.2604		-3.7355	0.8739	
		xy						
		xx & xy	-7.0888	0.3388	0.3388	0.5397	0.9803	0.9803
		yy						
RS4941185	27	xx	13.1124	0.0322*	0.0373*	25.5936	0.1701*	0.3783
		yy	21.7224	0.0596*		15.2361	0.658	
		xy						
		yy & xy	-10.667	0.0838*	0.0838*	-23.889	0.1825*	0.1825*
		xx						
RS7230970	27	xx	8.6351	0.2944	0.5671	2.8771	0.9042	0.8987
		yy	3.9647	0.5725		-6.9887	0.7324	
		xy						
		xx & xy	-0.6542	0.9175	0.9175	8.0703	0.653	0.653
		yy						
RS7236090	26	xx	-21.263	0.0151*	0.0215*	-45.353	0.0854*	0.1592*
		yy	-14.675	0.0225*		-27.738	0.149*	
		xy						
		xx & xy	8.3842	0.1866*	0.1866*	14.9231	0.4147	0.4147
		yy						
RS8083946	27	xx	14.7858	0.0408*	0.0709*	26.6297	0.2031	0.1664*
		yy	-0.0049	0.9995		-13.914	0.4901	
		xy						
		xx & xy	6.6697	0.3083	0.3083	25.8721	0.1606*	0.1606*
		yy						
RS899968	26	xx	2.2068	0.7866	0.8722	-5.5186	0.8118	0.6348
		yy	-2.0584	0.7848		-20.019	0.356	
		xy						
		xx & xy	2.9521	0.6547	0.6547	17.7382	0.3511	0.3511
		yy						
RS949037	26	xx	-1.8485	0.7963	0.9568	-19.274	0.3494	0.6175
		yy	-2.0285	0.8213		-15.266	0.5493	
		xy						
		yy & xy	1.3037	0.8414	0.8414	14.6773	0.4337	0.4337
		xx						
Covariates								
Time (months)	27	3	3.6677	0.5706	0.6923	27.7681	0.0555*	0.1135*
		6	3.1438	0.4275		12.7622	0.1395*	
		12				.	.	
Age	27		-0.0776	0.7936	0.7936	0.823	0.3197	0.3197
GCS	27		9.9724	0.1314*	0.1314*	21.0076	0.2635	0.2635
Gender	27		-5.952	0.467	0.467	-3.2324	0.8871	0.8871
Race	27		-7.7874	0.4104	0.4104	-17.328	0.52	0.52
Hypothermic	27		26.1905	0.0191*	0.0191*	61.1685	0.0549*	0.0549*
Hypoxia	25		21.97	0.1535*	0.1535*	60.5626	0.1682*	0.1682*
Hypotensive	25		21.97	0.1535*	0.1535*	60.5626	0.1682*	0.1682*
Seizures	0				NA			NA
APOEε4	26		5.592	0.4444	0.4444	9.5593	0.6512	0.6512
* p≤ 0.2								

Table 4-14: Primary Mixed Models Analyses for Trails Making Tests and BCL-2 SNP's

Primary Mixed Models Analyses of Cognitive- Behavioral Outcomes: Trails Making Tests and BCL-2 SNP																
Model	Trails A								Trails B							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS12454712 (n=27)																
Genotype	xx	3.667	0.6936	0.4561	xx & xy	7.4566	0.2298	0.2298	xx	31.8107	0.2337	0.1668	xx & xy	26.9143	0.14	0.14
	yy	-6.6763	0.312		yy				yy	-20.3132	0.2745		yy			
	xy								xy							
Time (months)	3	3.9187	0.5409	0.7422		3.8272	0.548	0.7378	3	28.2084	0.0522	0.1218	3	28.4536	0.05*	0.1149
	6	2.5664	0.5156			2.6333	0.5025		6	11.514	0.1838		6	11.8489	0.1707	
	12								12				12			
Age		-0.01647	0.9567	0.9567		-0.0564	0.8406	0.8406		1.1506	0.1811	0.1811		0.8142	0.3187	0.3187
GCS		15.1863	0.0416*	0.0416*		14.3327	0.04*	0.04*		44.6549	0.0331*	0.0331*		37.8349	0.0599	0.0599
Gender		3.0716	0.7183	0.7183		2.4834	0.7627	0.7627		22.1635	0.3481	0.3481		16.9955	0.4685	0.4685
Race		0.7567	0.9391	0.9391		-0.5447	0.9526	0.9526		17.934	0.5246	0.5246		6.8439	0.7995	0.7995
Hypothermia		26.52	0.036*	0.036*		26.0353	0.0346*	0.0346*		63.7296	0.0692	0.0692		59.9851	0.0882	0.0882
RS12968517 (n=27)																
Genotype	xx	-15.6631	0.0305*	0.087	yy & xy	14.3516	0.0289*	0.0289*	xx	2.7219	0.9039	0.8951	yy & xy	-5.8154	0.7773	0.7773
	yy	-5.4007	0.619		xx				yy	-13.6506	0.708		xx			
	xy								xy							
Time (months)	3	5.4142	0.3966	0.6052		5.477	0.3888	0.5985	3	29.2066	0.048*	0.113	3	29.1621	0.0477*	0.1129
	6	3.1092	0.4289			3.1113	0.427		6	11.84	0.1758		6	11.7712	0.1774	
	12								12				12			
Age		-0.09896	0.7108	0.7108		-0.07557	0.7699	0.7699		0.7539	0.3948	0.3948		0.8115	0.343	0.343
GCS		18.2788	0.0119*	0.0119*		17.1832	0.0108*	0.0108*		33.5792	0.138	0.138		30.9802	0.1406	0.1406
Gender		9.6927	0.2565	0.2565		9.1529	0.2708	0.2708		11.0431	0.6839	0.6839		0.97425	0.7115	0.7115
Race		-5.9533	0.5012	0.5012		-6.1913	0.4756	0.4756		5.9296	0.8426	0.8426		5.4075	0.8529	0.8529
Hypothermia		26.1369	0.0218*	0.0218*		25.7196	0.0208*	0.0208*		76.8013	0.0417*	0.0417*		75.8718	0.039*	0.039*
* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001																

Table 4-14 continued

Primary Mixed Models Analyses of Cognitive- Behavioral Outcomes: Trails Making Tests and BCL-2 SNP																
Model	Trails A								Trails B							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS1381548 (n=27)																
Genotype	xx	0.1532	0.9842	0.1203	xx & xy	12.9308	0.0366*	0.0366*	xx	-7.24	0.7513	0.1195	xx & xy	37.5431	0.0391*	0.0391*
	yy	-12.8583	0.0515		yy				yy	-39.2619	0.044*		yy			
	xy								xy							
Time (months)	3	6.3216	0.3291	0.5413	3	6.2883	0.329	0.5356	3	0.325211	0.0286*	0.0733	3	32.6531	0.0273*	0.069
	6	3.2988	0.4061		6	3.3353	0.3964		6	12.5058	0.1536		6	12.751	0.1429	
	12				12				12				12			
Age		0.02737	0.9205	0.9205		0.02702	0.9182	0.9182		1.0229	0.2117	0.2117		1.0702	0.1756	0.1756
GCS		12.8173	0.0529	0.0529		12.8165	0.0438*	0.0438*		33.6835	0.0809	0.0809		32.6457	0.0788	0.0788
Gender		-1.3161	0.8698	0.8698		-1.2906	0.8682	0.8682		3.158	0.8909	0.8909		4.2545	0.8487	0.8487
Race		-2.7622	0.7546	0.7546		-2.751	0.7456	0.7456		1.7135	0.9481	0.9481		0.2885	0.9909	0.9909
Hypothermia		24.6427	0.0338*	0.0338*		24.5304	0.0291*	0.0291*		61.7036	0.0681	0.0681		60.4372	0.0655	0.0655
RS1481031 (n=27)																
Genotype	xx	-1.7675	0.7934	0.2611	yy & xy	2.4441	0.7274	0.7274	xx	-20.9682	0.295	0.2192	yy & xy	23.0362	0.2612	0.2612
	yy	-18.8485	0.1139		xx				yy	-46.015	0.185		xx			
	xy								xy							
Time (months)	3	4.6394	0.4674	0.7167	3	4.2165	0.5112	0.7211	3	29.0819	0.0471*	0.1132	3	28.4989	0.0507	0.1149
	6	2.3144	0.5547		6	2.6218	0.5057		6	11.4957	0.1864		6	12.0174	0.1664	
	12				12				12				12			
Age		0.02717	0.9245	0.9245		-0.04162	0.8877	0.8877		1.1198	0.1839	0.1839		0.9805	0.2497	0.2497
GCS		9.1083	0.2195	0.2195		13.6126	0.0631	0.0631		28.594	0.1842	0.1842		38.9161	0.0641	0.0641
Gender		-6.6272	0.5178	0.5178		2.4292	0.7844	0.7844		0.7965	0.9781	0.9781		22.0559	0.3818	0.3818
Race		-14.7801	0.2928	0.2928		0.3684	0.9726	0.9726		-16.8972	0.6783	0.6783		19.9491	0.5226	0.5226
Hypothermia		17.0883	0.2615	0.2615		31.9764	0.0179*	0.0179*		55.899	0.2096	0.2096		90.6725	0.0203*	0.0203*

* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001

Table 4-14 continued

Primary Mixed Models Analyses of Cognitive- Behavioral Outcomes: Trails Making Tests and BCL-2 SNP																
Model	Trails A								Trails B							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS17756073 (n=27)																
Genotype	xx	-8.347	0.164	0.3471	yy & xy	7.4562	0.1958	0.1958	xx	-33.7229	0.0532	0.1456	yy & xy	32.2942	0.0516	0.0516
	yy	-15.5602	0.5007		xx				yy	-24.4641	0.7041		xx			
	xy								xy							
Time (months)	3	3.4323	0.594	0.7615	3	3.7909	0.5524	0.7324	3	27.6693	0.0581	0.1286	3	27.849	0.0551	0.1227
	6	2.6153	0.5095		6	2.713	0.491		6	11.8794	0.1728		6	11.9242	0.1696	
	12				12				12				12			
Age		0.007088	0.9802	0.9802		0.01269	0.9642	0.9642		1.0894	0.1827	0.1827		1.0984	0.1705	0.1705
GCS		13.6389	0.0478*	0.0478*		13.2431	0.0507	0.0507		34.5537	0.0736	0.0736		33.9206	0.0714	0.0714
Gender		1.5482	0.861	0.861		-0.4497	0.9566	0.9566		7.412	0.7617	0.7617		4.2598	0.8502	0.8502
Race		-1.8591	0.8396	0.8396		-2.3175	0.7987	0.7987		0.2351	0.9929	0.9929		-0.4245	0.9868	0.9868
Hypothermia		23.6748	0.101	0.101		29.0384	0.0152*	0.0152*		61.5636	0.1415	0.1415		70.9545	0.0319*	0.0319*
RS17759659 (n=26)																
Genotype	xx	8.62	0.2276	0.4623	yy & xy	-7.8583	0.2206	0.2206	xx	-0.5805	0.9786	0.9973	yy & xy	-0.0406	0.9983	0.9983
	yy	3.2913	0.788		xx				yy	-2.7462	0.9425		xx			
	xy								xy							
Time (months)	3	5.0747	0.4439	0.6831	3	5.0962	0.4389	0.675	3	29.2598	0.0541	0.1347	3	29.0301	0.0551	0.1369
	6	2.7275	0.5086		6	2.7764	0.4994		6	11.1573	0.2234		6	11.0901	0.2253	
	12				12				12				12			
Age		-0.06529	0.8345	0.8345		-0.05132	0.865	0.865		0.831	0.3863	0.3863		0.08228	0.3729	0.3729
GCS		10.8412	0.1597	0.1597		11.3663	0.1191	0.1191		32.4669	0.1682	0.1682		32.0363	0.1484	0.1484
Gender		2.975	0.7423	0.7423		2.6613	0.7613	0.7613		11.382	0.6654	0.6654		12.0664	0.644	0.644
Race		2.2726	0.8824	0.8224		2.4074	0.8072	0.8072		3.3398	0.9152	0.9152		3.2939	0.9139	0.9139
Hypothermia		30.7984	0.0165*	0.0165*		31.1314	0.0126*	0.0126*		74.3693	0.054	0.054		74.1193	0.0469*	0.0469*
* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001																

Table 4-14 continued

Primary Mixed Models Analyses of Cognitive- Behavioral Outcomes: Trails Making Tests and BCL-2 SNP																
Model	Trails A								Trails B							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS1801018 (n=27)																
Genotype	xx	-5.0222	0.6645	0.5763	xx & xy	-6.4113	0.3369	0.3369	xx	-8.4272	0.8067	0.7414	xx & xy	-14.458	0.4594	0.4594
	yy	4.3639	0.6		yy				yy	11.0095	0.6537		yy			
	xy								xy							
Time (months)	3	5.409	0.4044	0.6466	3	5.4518	0.3992	0.6334	3	30.8112	0.0383*	0.0941	3	30.8082	0.0378*	0.0925
	6	2.6762	0.4953		6	2.7882	0.4763		6	12.0421	0.1681		6	12.0924	0.1651	
	12				12				12				12			
Age		-0.1168	0.6944	0.6944		-0.1072	0.712	0.712		0.686	0.4353	0.4353		0.7096	0.4063	0.4063
GCS		13.0442	0.0885	0.0885		11.8486	0.0895	0.0895		32.2102	0.1484	0.1484		30.3329	0.1369	0.1369
Gender		4.2316	0.6532	0.6532		2.6088	0.7566	0.7566		18.3606	0.5084	0.5084		15.4089	0.5274	0.5274
Race		1.5324	0.8779	0.8779		0.7863	0.9348	0.9348		9.6999	0.746	0.746		8.4983	0.7679	0.7679
Hypothermia		29.528	0.047*	0.047*		26.5284	0.0366*	0.0366*		70.954	0.1053	0.1053		65.849	0.0764	0.0764
RS3810027 (n=28)																
Genotype	xx	14.7678	0.0196*	0.032*	yy & xy	-9.1309	0.1304	0.1304	xx	6.9443	0.7356	0.6448	yy & xy	1.3776	0.9406	0.9406
	yy	18.566	0.0351*		xx				yy	27.002	0.3566		xx			
	xy								xy							
Time (months)	3	3.8674	0.5428	0.7307	3	3.1303	0.624	0.8061	3	30.4952	0.0399*	0.0965	3	29.5679	0.0466*	0.1066
	6	2.6891	0.4946		6	2.273	0.5642		6	12.1766	0.1649		6	11.8855	0.1734	
	12				12				12				12			
Age		-0.2317	0.3817	0.3817		-0.04229	0.8771	0.8771		0.5211	0.565	0.565		0.7989	0.3524	0.3524
GCS		5.3	0.4196	0.4196		12.1681	0.0669	0.0669		22.6062	0.3235	0.3235		32.5197	0.1136	0.1136
Gender		8.2332	0.2881	0.2881		3.5851	0.6583	0.6583		18.8668	0.465	0.465		12.3254	0.6181	0.6181
Race		-2.2696	0.7899	0.7899		2.1649	0.8137	0.8137		-3.9488	0.8956	0.8956		2.7617	0.9246	0.9246
Hypothermia		29.7727	0.0079**	0.0079**		33.1874	0.0064**	0.0064**		68.3584	0.0643	0.0643		73.7587	0.0439*	0.0439*
* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001																

Table 4-14 continued

Primary Mixed Models Analyses of Cognitive- Behavioral Outcomes: Trails Making Tests and BCL-2 SNP																
Model	**Trails A**								**Trails B**							
Variables		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**
RS4941185 (n=27)																
Genotype	xx	10.2107	0.1141	0.1747	yy & xy	-7.8852	0.2103	0.2103	xx	17.1999	0.3866	0.6806	yy & xy	-16.15	0.3881	0.3881
	yy	16.4091	0.1671		xx				yy	7.1988	0.8424		xx			
	xy								xy							
Time (months)	3	4.9014	0.4405	0.7029	3	4.5355	0.4773	0.6962	3	30.007	0.0417*	0.1017	3	29.7865	0.0423*	0.102
	6	2.2232	0.5707		6	2.6794	0.4956		6	11.822	0.1765		6	11.8961	0.1717	
	12				12				12				12			
Age		-0.03237	0.9076	0.9076		-0.09768	0.7282	0.7282		0.7603	0.3882	0.3882		0.07294	0.3883	0.3883
GCS		7.3429	0.3001	0.3001		10.0654	0.1524	0.1524		25.6996	0.2487	0.2487		26.9254	0.1987	0.1987
Gender		0.969	0.9036	0.9036		1.1646	0.8865	0.8865		12.0077	0.6237	0.6237		12.0581	0.6145	0.6145
Race		-1.2454	0.8902	0.8902		0.08205	0.9929	0.9929		5.8733	0.8386	0.8386		6.4886	0.8167	0.8167
Hypothermia		27.4807	0.0199*	0.0199*		29.6733	0.0136*	0.0136*		73.1311	0.0467*	0.0467*		74.0561	0.0378*	0.0378*
RS7236090 (n=26)																
Genotype	xx	-19.0883	0.0396*	0.0577	xx & xy	8.7143	0.2446	0.2446	xx	-24.4786	0.4162	0.6766	xx & xy	7.3982	0.7452	0.7452
	yy	-16.5922	0.0387*		yy				yy	-17.1129	0.5097		yy			
	xy								xy							
Time (months)	3	5.7374	0.4075	0.5452	3	4.2003	0.5437	0.6581	3	27.2757	0.0822	0.1411	3	26.2848	0.0918	0.1535
	6	3.7673	0.3474		6	3.3423	0.4056		6	13.0496	0.1423		6	12.8968	0.1463	
	12				12				12				12			
Age		-0.2845	0.3193	0.3193		-0.06836	0.8134	0.8134		0.5472	0.5634	0.5634		0.8109	0.3616	0.3616
GCS		5.8035	0.4275	0.4275		9.0268	0.2546	0.2546		25.2404	0.3114	0.3114		29.5223	0.2241	0.2241
Gender		-0.7977	0.9178	0.9178		-0.7137	0.9324	0.9324		9.7398	0.702	0.702		10.1955	0.6864	0.6864
Race		-13.0203	0.2299	0.2299		-8.3621	0.4698	0.4698		-7.9336	0.8293	0.8293		-1.9891	0.9556	0.9556
Hypothermia		17.9347	0.1277	0.1277		28.3169	0.0223*	0.0223*		59.0178	0.1472	0.1472		71.7483	0.0585	0.0585
RS8083946 (n=27)																
Genotype	xx	16.6332	0.0285*	0.0835	xx & xy	-1.172	0.8682	0.8682	xx	29.6515	0.2122	0.4384	xx & xy	4.7974	0.8185	0.8185
	yy	9.7958	0.1854		yy				yy	10.6299	0.6556		yy			
	xy								xy							
Time (months)	3	5.2131	0.426	0.6605	3	4.252	0.5103	0.7273	3	30.9382	0.0379	0.0944	3	29.782	0.0436	0.1044
	6	2.7372	0.4943		6	2.5609	0.5164		6	11.8775	0.1751		6	11.9251	0.1718	
	12				12				12				12			
Age		-0.1347	0.6077	0.6077		-0.05476	0.8515	0.8515		0.6241	0.4645	0.4645		0.7757	0.3695	0.3695
GCS		9.8973	0.1211	0.1211		13.0922	0.0657	0.0657		26.2499	0.2025	0.2025		31.7487	0.1249	0.1249
Gender		10.1414	0.2343	0.2343		1.728	0.8406	0.8406		25.4296	0.3439	0.3439		11.531	0.6418	0.6418
Race		-4.2471	0.6178	0.6178		-1.6106	0.8665	0.8665		-0.3901	0.9889	0.9889		4.208	0.8826	0.8826
Hypothermia		32.1908	0.0107*	0.0107*		31.2224	0.023*	0.023*		73.7601	0.0612	0.0612		70.318	0.0771	0.0771
*** p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001**																

4.2.2 Specific Aim 2

2. Specific Aims 2: Compare the relationship between BCL-2 genotype and biological/ clinical data.

RQ2. Is there a relationship between BCL-2 genotype and biological/ clinical data (bcl-2 protein, neurometabolites [lactate, pyruvate, LP ratio], and CBF [right hemisphere, left hemisphere, and global] from patients who have sustained a severe TBI?

4.2.2.1 Bcl-2 Protein Concentrations

Sub-sample description Bcl-2 Protein

The mean age of the overall sample of 42 subjects was 33.5 years old (range 18-69; SD± 13.9). The sample was primarily Caucasian (n=37; 88.1%) and male (n=33; 78.6%). Slightly over half of this sub-sample had admission GCS of 3-5 (n=22; 52.4%). None of the subjects in this cohort received a hypothermia intervention. In the overall subsample, the presence of sustained hypoxia (n=12; 28.6%), hypotension (n=1; 2.4%), documented pre-admission seizures (n=9; 21.4%), and the occurrence of APOE ε4 (n=17; 41.5%) were considered. Refer to table 4-27 for sample description. All of the SNP's genotyped for the protein sub-sample met the HWE criteria for genotype representation in a population except RS949037 (X2=3.879121; P=0.04889). Refer to table 4-27 for genotype frequencies for each SNP and HWE calculations.

Bcl-2 protein concentrations (U/mL) were analyzed at days 1-6 post injury. Descriptive statistics for the Bcl-2 protein concentrations are included in table 4-27. For day 3 (range maximum 74.81 U/mL) and day 4 (range maximum 55.16 U/mL) post injury there is 1 extreme value on each of those days by different subjects (See figure 4-11). These extreme levels were

within normal limits for the ELISA kit and as discussed in the background and literature section there is evidence that Bcl-2 protein levels can peak as late as day four after neurotrauma (Lee et al, 2004; Xiong et al, 2001). Therefore, because the values were extreme to this data set analyses are shown with and without these extreme values or "outliers" (See figures 4-11 & 4-12).

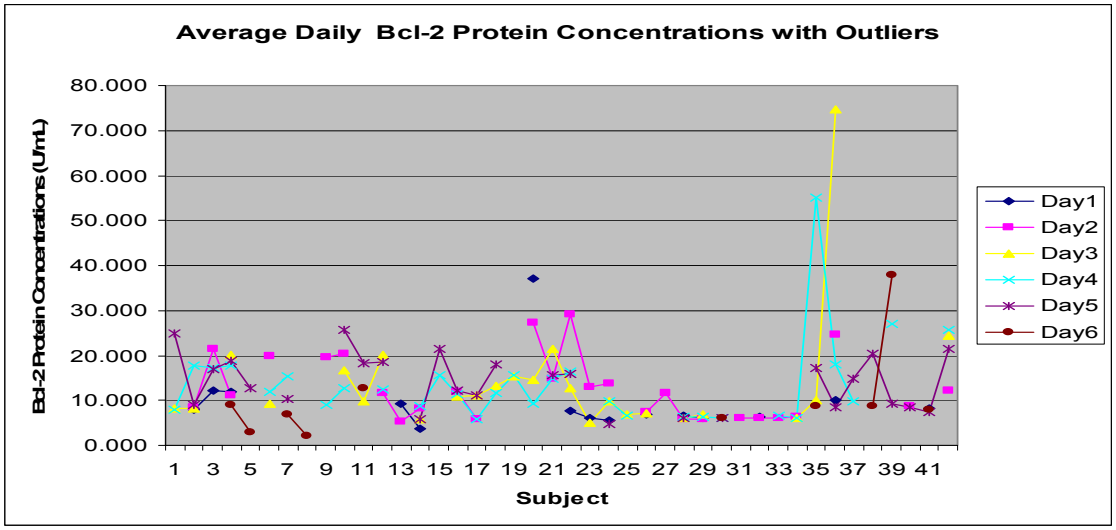


Figure 4-11: Average Daily Bcl-2 Protein Concentrations WITH Outliers

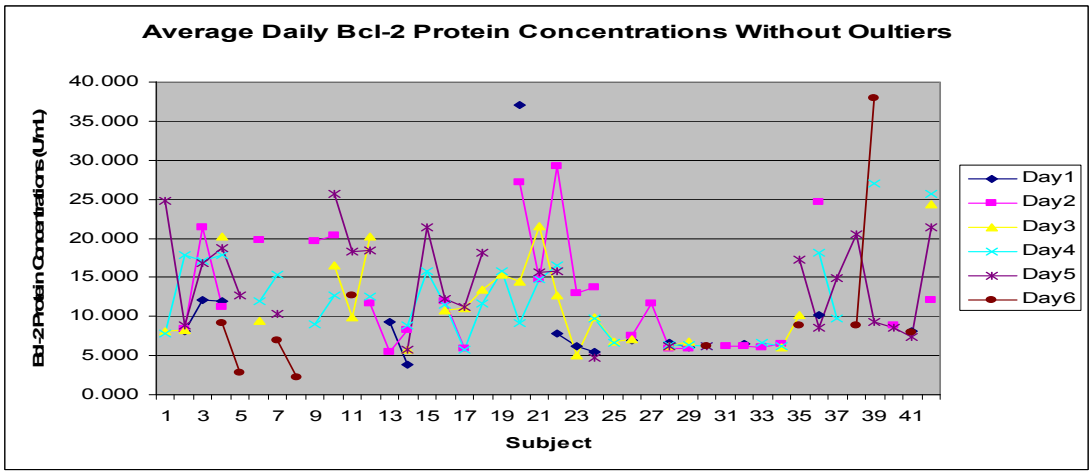


Figure 4-12: Average Daily Bcl-2 Protein Concentrations WITHOUT Outliers

Preliminary Mixed Models Analysis for Bcl-2 Protein Levels

In the preliminary mixed models analyses, each SNP was analyzed individually for a relationship with Bcl-2 protein (with and without outliers) over time. Each of the covariates was analyzed for potential relationship with Bcl-2 protein (with and without outliers). By doing this the SNP's and covariates of interest in building the larger model were ascertained. The criterion used was a P for Type 3 tests of ≤0.2. In addition, covariates that were deemed prudent through the evidence in the literature (age, gender, race, and GCS) were added to the primary full model. Hypothermia was not used in the model as none of the subjects received a hypothermia intervention. (Refer to tables 4-28 and 4-29).

The preliminary mixed model analysis for Bcl-2 Protein with outliers indicated that the SNP's and covariates of interest, meeting the criterion are: RS1026825 (genotypes P=0.1956; dichotomized genotype P=0.0975); RS12454712 (genotypes P=0.0105; dichotomized genotype P=0.0245); RS 1481031 (genotypes P=0.1782; dichotomized genotype P=0.175); RS4456611 (genotypes P=0.1613; dichotomized genotype P=0.0586); RS7236090 (genotypes P=0.2312; dichotomized genotype P=0.0843); RS899968 (genotypes P=0.1946; dichotomized genotype P=0.5241); gender (P= 0.1587); race (P=0.0266); hypoxia (P=0.0378); and seizures (P= 0.0558). Time (days) (P=0.6211), age (P= 0.2622), and GCS (P=0.7642) were not indicated in the preliminary analysis, however, they were controlled for in the full model. (Refer to tables 4-28 and 4-29).

The preliminary mixed model analysis for Bcl-2 Protein without outliers indicated that the SNP's and covariates of interest, meeting the criterion are: RS1026825 (genotypes P=0.2292; dichotomized genotype P=0.0899); RS12454712 (genotypes P=0.0016; dichotomized genotype P=0.0023); RS 1481031 (genotypes P=0.0708; dichotomized genotype P=0.0549); RS17756073

(genotypes P=0.0763; dichotomized genotype P=0.2168); RS4456611 (genotypes P=0.3106; dichotomized genotype P=0.123); RS7236090 (genotypes P=0.0503; dichotomized genotype P=0.0212); gender (P= 0.1626); race (P=0.1481); hypoxia (P=0.0302); and seizures (P= 0.0885). Time (days) (P=0.3939), age (P= 0.4817), and GCS (P=0.5821) were not indicated in the preliminary analysis, however, they were controlled for in the full model. (Refer to tables 4-28 and 4-29).

Primary Mixed Models Analyses for Bcl-2 Protein

Bcl-2 Protein without outliers: In analyzing the 6 BCL-2 SNP's (RS1026825, RS12454712, RS1481031; RS4456611; RS7236090; and RS899968) of interest in relation to the Bcl-2 protein concentrations (with outlier data) none SNP's had significant or marginally significant findings. None of the covariates included in the model were significant. (Refer to tables 4-30 to 4-32).

Bcl-2 Protein without outliers: In analyzing the 6 BCL-2 SNP's of interest (RS1026825, RS12454712, RS1481031, RS17756073, RS4456611, and RS7236090) in relation to the Bcl-2 protein levels (without outliers) 4 of the SNP's had either marginally significant or significant relationships with the Bcl-2 protein concentrations (without outliers). (Refer to tables 4-30 to 4-32).

The mixed model with SNP RS12454712: genotype YY was marginally (P= 0.0714) with overall genotype (P for type 3 F tests =0.1686). YY genotype is associated with decrease Bcl-2 protein concentrations. This trend was marginally significant evident in the dichotomized analysis (P=0.0646). In contrast, subjects with 0-1 copies of the Y allele had significantly higher bcl-2 protein concentrations. Time of sample from injury was found to be significant (genotypes

P=0.0036; dichotomized genotype P=0.0031), Bcl-2 protein concentrations spiked on days 2, 4, & 5 compared to day 6. (Refer to table 4-30).

The mixed models analysis with RS1481031 genotype XX (P= 0.018) with marginal significance overall (P for type 3 F tests =0.0547) was associated with lower blc-2 protein concentrations. This trend was evident in the dichotomized analysis (P=0.0599). In contrast, 0-1 X alleles was associated with higher bcl-2 concentrations. Time was found to been significant (genotypes P=0.0029; dichotomized genotype P=0.0023). Bcl-2 protein concentrations spiked on days 2, 4, & 5 compared to day 6. Gender was found to be marginally significant (genotypes P=0.0578; dichotomized genotype P=0.0906), with females having lower bcl-2 protein concentrations. Seizure was found to be significant (genotypes P=0.0452; dichotomized genotype P=0.0315). History of a pre-admission seizure was related to having lower bcl-2 protein concentrations. (Refer to table 4-31).

The mixed models analysis with RS17756073 genotype XX (P= 0.0103) and YY (P=0.0084) with overall genotype significant (P for type 3 F tests= 0.0055). XX and YY genotypes are associated with a decrease Bcl-2 protein levels compared to subjects who were heterozygous (XY). When the subjects were dichotomized, 0-1 X alleles, there was an associated with a trend in higher bcl-2 levels (P=0.083). Time was found to been significant (genotypes P=0.0018; dichotomized genotype P=0.0032). Bcl-2 protein levels spiked on days 2, 4, & 5 compared to day 6. Gender was found to be significant (genotypes P=0.049; dichotomized genotype P=0.06), with females having lower bcl-2 protein concentrations. (Refer to table 4-31).

The mixed models analysis with RS7236090 genotype XX (P= 0.0028) and YY (P=0.0475) with overall genotype significant (P for type 3 F tests =0.0056). XX and YY genotypes are associated with decreased bcl-2 protein concentrations compared to subjects who

were heterozygous (XY). In contrast, subjects with 0-1 X alleles had significantly higher bcl-2 concentrations in the dichotomized analysis (P=0.0112). Time was found to been significant (genotypes P=0.009; dichotomized genotype P=0.0058). Bcl-2 protein concentrations spiked on day 5 compared to day 6. Gender was found to be significant (genotypes P=0.0042; dichotomized genotype P=0.0121), with females having lower bcl-2 protein concentrations. Seizure was found to be marginally significant (genotypes P=0.092; dichotomized genotype P=0.0802). History of a pre-admission seizure was related to having lower bcl-2 protein concentrations. (Refer to table 4-32).

The mixed models analyses indicated that the following SNP's were not significant: RS1026825 (genotypes P=0.6688; dichotomized genotype P=0.5182); RS4456611 (genotypes P=0.7884; dichotomized genotype P=0.7136). Like the SNP's in the primary analyses that were marginally/ significant, the models for the SNP's that were not significant continued to report trends that the covariates of time, gender, and seizures on pre admission remained of interest with marginal or statistically significant relationships. Among all of the models the covariates of age, GCS, race, and hypoxia were not indicated as marginally or statistically significant. (Refer to tables 4-30 to 4-32).

Table 4-15: BCL-2 Genotypes vs. Bcl-2 Protein Concentrations Descriptive Data

BCL-2 Genotypes Versus Bcl-2 Protein Concentrations: Descriptive Data

Variables	n=	Mean	Std. Deviation	Range
Age	42	33.5	± 13.9	18-69
			Frequency (%)	Frequency % of Unknown
GCS	42	score 3 to 5	22 (52.4%)	0
		score 6 to 8	20 (47.6%)	
Gender (male)	42		33 (78.6%)	0
Race (Caucasian)	42		37 (88.1%)	0
Hypothermic	42		0 (0%)	0
Hypoxia	37		12 (28.6%)	5 (11.9%)
Hypotensive	42		1 (2.4%)	0
Seizures	38		9 (21.4%)	4 (9.52%)
APOEε4	41		17 (41.5%)	1 (2.38%)

		Frequency (%) of Unknown	With the Outliers			Without the Outliers		
Bcl-2 Protein Levels (U/mL)	n=		Mean	Std. Deviation	Range	Mean	Std. Deviation	Range
Day 1	15	27(64.29%)	9.76	± 7.91	3.84-37.12	9.76	± 7.91	3.84-37.12
Day 2	27	15(35.71%)	12.71	± 7.10	5.42-29.17	12.71	± 7.10	5.42-29.17
Day 3	25	17 (40.48%)	14.2	± 13.74	5.039-74.81	11.68	± 5.53	5.04-24.34
Day 4	30	12 (28.57%)	13.84	± 9.57	5.77-55.16	12.42	± 5.64	5.77-27.06
Day 5	27	15(35.71%)	14.07	± 6.08	4.76-25.64	14.07	± 6.08	4.76-25.64
Day 6	10	32 (76.19%)	10.34	± 10.15	2.19-37.88	10.34	± 10.15	2.19-37.88

Hardy-Weinberg Equilibrium

SNP	n=	XX	YY	XY	X	Y	HWE	X^2	P
RS1026825	41	11	9	21	0.524390244	0.475609756	1	.	.
RS12454712	42	10	17	15	0.416666667	0.583333333	1	.	.
RS12968517	41	15	7	19	0.597560976	0.402439024	1	.	.
RS1381548	42	9	16	17	0.416666667	0.583333333	1	.	.
RS1481031	41	18	6	17	0.646341463	0.353658537	1	.	.
RS17756073	41	22	2	17	0.743902439	0.256097561	1	.	.
RS17759659	40	18	3	19	0.6875	0.3125	1	.	.
RS1801018	42	6	21	15	0.321428571	0.678571429	1	.	.
RS1944419	41	9	9	23	0.5	0.5	1	.	.
RS3810027	42	16	9	17	0.583333333	0.416666667	1	.	.
RS4456611	41	9	8	24	0.512195122	0.487804878	1	.	.
RS4941185	42	14	7	21	0.583333333	0.416666667	1	.	.
RS7230970	42	6	15	21	0.392857143	0.607142857	1	.	.
RS7236090	40	12	10	18	0.525	0.475	1	.	.
RS8083946	42	13	14	15	0.488095238	0.511904762	1	.	.
RS899968	40	6	14	20	0.4	0.6	1	.	.
RS949037	40	13	13	14	0.5	0.525	1.050625	3.879121	0.04889*

*** X^2 p≤ 0.05**

Table 4-16: Preliminary Mixed Models Analyses of Bcl-2 Protein Concentrations

Preliminary Mixed Models Analysis of Bcl-2 Protein								
			With Outliers			Without Outliers		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS1026825	41	xx	-4.39	0.0729*	0.1956*	-3.3724	0.0916*	0.2292
		yy	-1.81	0.4637		-0.621	0.7611	
		xy						
		yy & xy	3.8005	0.0975*	0.0975*	3.1644	0.0899*	0.0899*
		xx	.	.	.	.	.	.
RS12454712	42	xx	-4.76	0.0458*	0.0105*	-3.5379	0.057*	0.0016*
		yy	-6.21	0.0037*		-6.1622	0.0004*	
		xy						
		xx & xy	-4.4405	0.0245*	0.0245*	4.8188	0.0023*	0.0023*
		yy	.	.	.	.	.	.
RS12968517	41	xx	0.416	0.8472	0.3323	0.2418	0.8927	0.4469
		yy	-3.83	0.1845*		-2.6976	0.2534	
		xy						
		yy & xy	-1.3419	0.5184	0.5184	-0.9173	0.5914	0.5914
		xx	.	.	.	.	.	.
RS1381548	42	xx	-3.52	0.1669*	0.3163	-1.5628	0.457	0.3794
		yy	0.03168	0.9884		1.4087	0.4341	
		xy						
		xx & xy	-1.2071	0.5544	0.5544	-1.9428	0.239	0.239
		yy	.	.	.	.	.	.
RS1481031	41	xx	-3.7108	0.0863*	0.1782*	-3.9397	0.0252*	0.0708*
		yy	-3.6858	0.2032		-3.0363	0.1972*	
		xy						
		yy & xy	2.7475	0.175*	0.175*	3.1526	0.0549*	0.0549*
		xx	.			.		
RS17756073	41	xx	-1.6183	0.4248	0.2945	-2.7071	0.094*	0.0763*
		yy	-7.4686	0.1382*		-7.5283	0.059*	
		xy						
		yy & xy	0.9293	0.6428	0.6428	1.9954	0.2168	0.2168
		xx	.			.		
RS17759659	40	xx	1.2531	0.5556	0.832	0.8589	0.6242	0.8795
		yy	1.1864	0.7837		0.8302	0.8114	
		xy						
		yy & xy	-1.1089	0.5854	0.5854	-0.7536	0.6527	0.6527
		xx						
RS1801018	42	xx	-1.4772	0.676	0.877	-0.9099	0.7397	0.4777
		yy	0.2586	0.9033		1.66	0.3398	
		xy						
		xx & xy	-0.5815	0.7671	0.7671	-1.8828	0.2375	0.2375
		yy	.			.		
RS1944419	41	xx	-2.5145	0.3275	0.3848	-1.3488	0.5224	0.5679
		yy	1.6703	0.5073		1.3254	0.526	
		xy						
		yy & xy	2.9994	0.2208	0.2208	1.7297	0.3887	0.3887
		xx	.			.		
		xx & xy	-2.3695	0.3307	0.3307	-1.6984	0.3959	0.3959
		yy	.			.		
RS3810027	42	xx	3.1514	0.1639*	0.3221	2.5882	0.1627*	0.3731
		yy	2.9288	0.2504		1.4223	0.4999	
		xy						
		yy & xy	-1.9446	0.3325	0.3325	-2.0379	0.2159	0.2159
		xx	.			.		

Table 4-16 continued

Preliminary Mixed Models Analysis of Bcl-2 Protein								
			With Outliers			Without Outliers		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS4456611	41	xx	-4.7406	0.0577*	0.1613*	-2.9723	0.1474*	0.3106
		yy	-0.9053	0.7171		0.1827	0.9303	
		xy						
		yy & xy	4.5329	0.0586*	0.0586*	3.0261	0.123*	0.123*
		xx	.			.		
RS4941185	42	xx	-1.1329	0.6065	0.8725	0.02389	0.9895	0.8906
		yy	-0.6322	0.827		1.0792	0.6466	
		xy						
		yy & xy	0.9835	0.6352	0.6352	0.2324	0.8915	0.8915
		xx	.			.		
RS7230970	42	xx	-0.0753	0.9792	0.5177	1.3803	0.5713	0.7857
		yy	-2.3479	0.2738		-0.3609	0.8393	
		xy						
		xx & xy	2.3529	0.2437	0.2437	0.6826	0.6843	0.6843
		yy	.			.		
RS7236090	40	xx	-3.9205	0.1099*	0.2312	-4.7304	0.0152*	0.0503*
		yy	-0.1206	0.9621		-1.7211	0.3847	
		xy						
		yy & xy	3.8863	0.0843*	0.0843*	4.124	0.0212*	0.0212*
		xx	.			.		
RS8083946	42	xx	2.9841	0.2094	0.4055	1.8123	0.3595	0.5974
		yy	2.5477	0.297		1.649	0.4093	
		xy						
		xx & xy	-0.973	0.6448	0.6448	-0.7345	0.6705	0.6705
		yy	.			.		
RS899968	40	xx	-5.1738	0.0919*	0.1946*	-3.7782	0.1293	0.2978
		yy	0.2363	0.913		-0.3002	0.8671	
		xy						
		xx & xy	-1.3507	0.5241	0.5241	-0.5452	0.7554	0.7554
		yy	.			.		
RS949037	40	xx	-1.4638	0.5469	0.5959	-1.2654	0.5344	0.8142
		yy	1.1755	0.6436		-0.3412	0.8711	
		xy						
		yy & xy	1.9651	0.3652	0.3652	1.1139	0.5322	0.5322
		xx	.			.		
		xx & xy	-1.8852	0.4034	0.4034	-0.2727	0.8827	0.8827
		yy	.			.		
Covariates								
Time (Days)	42	1	0.1901	0.9589	0.6211	0.296	0.9029	0.3939
		2	3.0666	0.3639		2.7943	0.2136	
		3	4.2136	0.2139		1.3459	0.5476	
		4	3.6772	0.2637		1.6467	0.4493	
		5	3.7846	0.2485		3.3156	0.1187*	
		6	.			.		
Age	42		-0.0809	0.2622	0.2622	-0.0418	0.4817	0.4817
GCS	42		-0.5906	0.7642	0.7642	-0.8952	0.5821	0.5821
Gender	42		-3.509	0.1587*	0.1587*	-2.8289	0.1626*	0.1626*
Race	42		-6.1515	0.0266*	0.0266*	-3.4737	0.1481*	0.1481*
Hypothermic	0			n/a			n/a	
Hypoxia	37		4.47	0.0378*	0.0378*	3.4754	0.0302*	0.0302*
Hypotensive	42		-3.7415	0.5107	0.5107	-4.5433	0.3539	0.3539
Seizures	41		4.2432	0.0558*	0.0558*	3.0976	0.0885*	0.0885*
APOE4	41		-1.5947	0.4276	0.4276	0.1293	0.9385	0.9385
* p≤ 0.2								

Table 4-17: Primary Mixed Models Analyses of BCL-2 SNP's and Bcl-2 Protein Concentrations

Primary Mixed Models Analyses of BCL-2 SNP and Bcl-2 Protein																
Variables	**With Outliers**								**Without Outliers**							
		coefff	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**
RS1026825 (n=36)																
Genotype	xx	-1.6789	0.5598	0.6589	yy & xy	0.9364	0.7322	0.7322	xx	-1.607	0.4276	0.6688	yy & xy	1.2422	0.5182	0.5182
	yy	-2.3143	0.3999		xx				yy	-1.21	0.5362		xx			
	xy	.							xy							
Time (Day)	1	0.1793	0.9626	0.1792	1	0.6546	0.8624	0.1226	1	0.6911	0.7362	0.0045**	1	0.8393	0.6796	0.0033**
	2	4.0786	0.2668		2	4.742	0.1866		2	4.2033	0.0348*		2	4.4096	0.0245*	
	3	6.058	0.0967		3	6.7029	0.0604		3	3.5609	0.0682		3	3.759	0.0507	
	4	5.7239	0.1063		4	6.4117	0.0633		4	4.018	0.0362*		4	4.23	0.0249*	
	5	5.6224	0.0973		5	3.1103	0.0674		5	5.6441	0.002**		5	5.8006	0.0013**	
	6	.	.		6				6				6			
Age		-0.1233	0.1874	0.1874		-0.1108	0.2288	0.2288		-0.0608	0.3614	0.3614		-0.0521	0.4184	0.4184
GCS		0.375	0.8588	0.8588		0.7116	0.7315	0.7315		-1.1278	0.4538	0.4538		-1.01	0.4952	0.4952
Gender		-4.2878	0.1416	0.1416		-3.1662	0.2207	0.2207		-3.6923	0.0662	0.0662		-3.1511	0.0074**	0.0074**
Race		-2.511	0.4188	0.4188		-3.3393	0.2595	0.2595		-0.5057	0.8248	0.8248		-0.9542	0.6574	0.6574
Hypoxia		-1.7269	0.5892	0.5892		-0.5714	0.8431	0.8431		-0.6814	0.7712	0.7712		-0.0815	0.9692	0.9692
Seizure		5.3779	0.094	0.094		4.5036	0.1361	0.1361		4.3373	0.0599	0.0599		3.8861	0.071	0.071
RS12454712 (n=37)																
Genotype	xx	-2.1739	0.4082	0.4722	xx & xy	1.8061	0.3675	0.3675	xx	-0.793	0.6591	0.1686	xx & xy	2.6104	0.0646	0.0646
	yy	-2.7359	0.2366		yy				yy	-2.9539	0.0714		yy			
	xy	.							xy							
Time (Day)	1	0.2906	0.9382	0.1579	1	0.70104	0.8482	0.1429	1	0.8232	0.684	0.0036**	1	0.9039	0.6522	0.0031**
	2	4.4481	0.2079		2	4.8919	0.161		2	4.5849	0.0178*		2	4.6761	0.0147*	
	3	6.0456	0.0845		3	6.3974	0.0658		3	3.5611	0.0605		3	3.6306	0.0541	
	4	5.8957	0.0835		4	6.2383	0.0649		4	4.1002	0.028*		4	4.1769	0.0242*	
	5	5.6761	0.0874		5	6.0125	0.068		5	5.6913	0.0016**		5	5.7653	0.0013**	
	6	.	.		6				6				6			
Age		-0.0823	0.3237	0.3237		-0.1071	0.1715	0.1715		-0.0452	0.4305	0.4305		-0.0533	0.3217	0.3217
GCS		0.2057	0.9194	0.9194		0.1072	0.9579	0.9579		-1.585	0.263	0.263		-1.6248	0.2446	0.2446
Gender		-2.279	0.3861	0.3861		-2.5564	0.327	0.327		-2.2933	0.191	0.191		-2.3862	0.1662	0.1662
Race		-4.1002	0.1587	0.1587		-3.7782	0.1885	0.1885		-1.4435	0.4802	0.4802		-1.3357	0.5049	0.5049
Hypoxia		0.4455	0.8779	0.8779		-0.1712	0.9512	0.9512		0.661	0.7464	0.7464		0.4223	0.8285	0.8285
Seizure		3.5495	0.2432	0.2432		4.0712	0.1721	0.1721		3.0503	0.1476	0.1476		3.2469	0.1116	0.1116

*** p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001**

Table 4-17 continued

Primary Mixed Models Analyses of BCL-2 SNP and Bcl-2 Protein																
Variables	**With Outliers**								**Without Outliers**							
		coefff	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**
RS1481031 (n=37)																
Genotype	xx	-2.9331	0.1655	0.2658	yy & xy	1.4853	0.4281	0.4281	xx	-3.502	0.018*	0.0547	yy & xy	2.4875	0.0599	0.0599
	yy	-3.9702	0.1579		xx				yy	-2.9332	0.1305		xx			
	xy								xy							
Time (Day)	1	0.8132	0.8261	0.1912	1	0.6952	0.8515	0.1424	1	0.91	0.6485	0.0029*	1	0.8819	0.6597	0.0023**
	2	4.9004	0.1587		2	5.0433	0.1487		2	4.7438	0.0128*		2	4.818	0.0119*	
	3	6.0326	0.0521		3	6.3381	0.0686		3	3.4289	0.0677		3	3.547	0.0596	
	4	5.979	0.0761		4	6.2134	0.0662		4	4.0291	0.0289*		4	4.1429	0.0252*	
	5	5.9126	0.0719		5	6.0839	0.0648		5	5.7427	0.0013**		5	5.8146	0.0011**	
	6	.			6				6				6			
Age		-0.0536	0.528	0.528		-0.103	0.1989	0.1989		-0.0091	0.8737	0.8737		-0.0422	0.4432	0.4432
GCS		-0.3942	0.8461	0.8461		0.2054	0.9191	0.9191		-1.9819	0.1548	0.1548		-1.5636	0.2603	0.2603
Gender		-3.4476	0.1727	0.1727		-2.9672	0.2429	0.2429		-3.1664	0.0578	0.0578		-2.8498	0.0906	0.0906
Race		-4.5933	0.1189	0.1189		-3.3807	0.2353	0.2353		-1.6864	0.4089	0.4089		-0.6927	0.7261	0.7261
Hypoxia		0.8186	0.7736	0.7736		-0.1135	0.9679	0.9679		1.1577	0.5541	0.5541		0.5503	0.779	0.779
Seizure		4.3064	0.1371	0.1371		4.8107	0.1014	0.1014		3.979	0.0452	0.0452		4.3559	0.0315	0.0315
RS17756073 (n=36)																
Genotype	xx	-2.107	0.267	0.2149	yy & xy	1.4255	0.4558	0.4558	xx	-3.145	0.0103*	0.0055**	yy & xy	2.2924	0.083	0.083
	yy	-6.7406	0.1178		xx				yy	-7.3466	0.0084**		xx			
	xy								xy							
Time (Day)	1	0.9869	0.7962	0.1356	1	0.6572		0.1401	1	1.066	0.6013	0.0018**	1	0.9595	0.6418	0.0032**
	2	5.2944	0.1391		2	4.8335			2	5.0847	0.0087**		2	4.6471	0.0175*	
	3	6.8892	0.052		3	6.6062			3	4.1061	0.0309*		3	3.7923	0.0478*	
	4	6.6347	0.0549		4	6.4212			4	4.6425	0.0131*		4	4.3462	0.0216*	
	5	6.3803	0.0575		5	6.2551			5	6.2637	0.0006**		5	6.0393	0.0009**	
	6				6				6				6			
Age		-0.1023	0.1771	0.1771		-0.1113	0.1565	0.1565		-0.0464	0.3125	0.3125		-0.0587	0.2712	0.2712
GCS		0.4793	0.816	0.816		1.1396	0.5851	0.5851		-0.8615	0.4876	0.4876		-0.259	0.8533	0.8533
Gender		-2.9102	0.2425	0.2425		-3.0312	0.237	0.237		-2.9444	0.049*	0.049*		-3.1699	0.06	0.06
Race		-3.7946	0.1758	0.1758		-3.8692	0.1838	0.1838		-1.249	0.4653	0.4653		-1.3015	0.5142	0.5142
Hypoxia		0.3702	0.8941	0.8941		-0.3841	0.8927	0.8927		0.94	0.579	0.579		0.2505	0.8979	0.8979
Seizure		3.7478	0.2019	0.2019		4.0127	0.1862	0.1862		2.8577	0.1087	0.1087		3.2176	0.1151	0.1151

*** p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001**

Table 4-17 continued

Primary Mixed Models Analyses of BCL-2 SNP and Bcl-2 Protein																
Variables	**With Outliers**								**Without Outliers**							
		coefff	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**		**coefff**	**P**	**P for Type-3 Test**
RS4456611 (n=41)																
Genotype	xx	-2.0729	0.4274	0.724	yy & xy	2.0448	0.4184	0.4184	xx	-0.4286	0.8137	0.7884	yy & xy	0.6465	0.7136	0.7136
	yy	-0.1588	0.9468		xx				yy	0.9865	0.5607		xx			
	xy								xy							
Time (Day)	1	0.9478	0.8051	0.1174	1	0.93	0.8071	0.1146	1	1.0821	0.5983	0.0018**	1	0.9812	0.6312	0.0018**
	2	5.7335	0.1106		2	5.7087	0.1098		2	5.3028	0.0068**		2	5.2329	0.0073**	
	3	6.9939	0.0509		3	6.9788	0.0499		3	3.898	0.0423*		3	3.8343	0.045*	
	4	6.7372	0.0528		4	6.7363	0.0511		4	4.4514	0.0184*		4	4.3763	0.0199*	
	5	6.5549	0.0515		5	6.5517	0.0505		5	6.0566	0.0008**		5	6.006	0.0009*8	
	6				6				6				6			
Age		-0.1065	0.1943	0.1943		-0.1071	0.1836	0.1836		-0.5727	0.331	0.331		-0.0552	0.3419	0.3419
GCS		0.7081	0.7531	0.7531		0.777	0.7134	0.7134		-1.0297	0.5191	0.5191		-1.2843	0.3975	0.3975
Gender		-3.0167	0.2439	0.2439		-3.025	0.2365	0.2365		-3.307	0.0624	0.0624		-3.2929	0.0607	0.0607
Race		-3.2858	0.2632	0.2632		-3.2858	0.2535	0.2535		-0.6033	0.7758	0.7758		-0.7157	0.7314	0.7314
Hypoxia		-0.352	0.9061	0.9061		-0.4162	0.8852	0.8852		0.3078	0.8871	0.8871		0.5191	0.8055	0.8055
Seizure		3.8698	0.2193	0.2193		3.8854	0.2095	0.2095		4.0182	0.0697	0.0697		3.9134	0.0726	0.0726
RS7236090 (n=35)																
Genotype	xx	-1.942	0.4007	0.5976	yy & xy	1.5658	0.4809	0.4809	xx	-4.3665	0.0028**	0.0056**	yy & xy	3.6926	0.0112*	0.0112*
	yy	-2.1102	0.4641		xx				yy	-3.4194	0.0475*		xx			
	xy								xy							
Time (Day)	1	0.31	0.9398	0.2047	1	0.0722	0.9859	0.1695	1	-0.1839	0.9309	0.009**	1	-0.3006	0.8886	0.0058**
	2	4.5722	0.2405		2	4.5212	0.2448		2	3.5139	0.0831		2	3.5968	0.0802	
	3	6.361	0.0986		3	6.3055	0.101		3	2.6627	0.1812		3	2.7299	0.1759	
	4	6.0823	0.1032		4	6.1744	0.0972		4	3.2731	0.0932		4	3.4517	0.0806	
	5	5.7184	0.1133		5	5.7721	0.1095		5	4.9731	0.0079**		5	5.0982	0.0071**	
	6				6				6				6			
Age		-0.1573	0.0804	0.0804		-0.1672	0.058	0.058		-0.0762	0.1428	0.1428		-0.0929	0.0867	0.0867
GCS		0.3645	0.8636	0.8636		0.2344	0.9106	0.9106		-1.7279	0.168	0.168		-1.9335	0.1405	0.1405
Gender		-3.8284	0.1514	0.1514		-3.568	0.1716	0.1716		-4.5549	0.0042**	0.0042**		-4.0466	0.0121*	0.0121*
Race		-4.1093	0.2159	0.2159		-3.0649	0.2949	0.2949		-1.7183	0.3773	0.3773		-0.0157	0.9932	0.9932
Hypoxia		-0.9479	0.7455	0.7455		-1.2946	0.6497	0.6497		-0.301	0.8609	0.8609		-0.8177	0.6482	0.6482
Seizure		4.2082	0.1663	0.1663		4.3672	0.1455	0.1455		2.9982	0.092	0.092		3.2648	0.0802	0.0802
RS899968 (n=35)																
Genotype	xx	-2.6135	0.4656	0.7618	yy	-0.0828	0.9702	0.9702	xx	-3.0574	0.2314	0.4714	yy	0.326	0.8353	0.8353
	yy	-0.2078	0.9272		xx & xy				yy	-0.6656	0.6738		xx & xy			
	xy								xy							
Time (Day)	1	0.7587	0.8432	0.1231	1	0.6607	0.863	0.1316	1	0.8949	0.66	0.0025**	1	0.8541	0.675	0.0028**
	2	5.3129	0.1437		2	5.1989	0.1515		2	4.8663	0.0129*		2	4.8266	0.0137*	
	3	7.0775	0.0497*		3	6.8926	0.055		3	3.962	0.0387*		3	3.8668	0.0436*	
	4	6.6669	0.0582		4	6.5432	0.0623		4	4.3461	0.0213*		4	4.276	0.0235*	
	5	6.3688	0.0594		5	6.2512	0.0637		5	5.9399	0.001**		5	5.8708	0.0011**	
	6				6				6				6			
Age		-0.095	0.3203	0.3203		-0.1197	0.179	0.179		-0.0306	0.6498	0.6498		-0.0608	0.3388	0.3388
GCS		0.3684	0.8702	0.8702		0.5327	0.811	0.811		-1.7125	0.276	0.276		-1.5582	0.3228	0.3228
Gender		-3.2708	0.2394	0.2394		-3.1831	0.2479	0.2479		-3.5405	0.0606	0.0606		-3.4341	0.0698	0.0698
Race		-3.1743	0.2928	0.2928		-3.2172	0.2816	0.2816		-0.7596	0.7215	0.7215		-0.8268	0.7004	0.7004
Hypoxia		-0.6255	0.8368	0.8368		-0.2397	0.9354	0.9354		0.0172	0.9937	0.9937		0.5548	0.796	0.796
Seizure		4.9639	0.1116	0.1116		4.6642	0.1265	0.1265		4.6295	0.0363*	0.0363*		4.1397	0.0562	0.0562

*** p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001**

4.2.2.2 Neurometabolites

Sub-sample Description Neurometabolites

The mean age of the overall sample of 36 subjects was 31.4 years old (range 17-71; SD± 13.9). The sample was all Caucasian and primarily male (n=27; 75%). Over half of the sub-sample had admission GCS of 6-8 (n=21; 58.3%). Of the subjects enrolled in the study, 15 (41.7%) received therapeutic hypothermia. In the sub-sample the presence of sustained hypoxia (n=6; 16.67%), hypotension (n=5; 13.89%), documented pre-admission seizures (n=1; 2.78%), and the occurrence of APOE ε4 (n=8; 22.22%) were covariates of consideration. Refer to table 4-33 for sample description. All of the SNP's genotyped for the neurometabolite sub-sample met the HWE criteria for genotype representation in a population except RS4941185 (X2=15.277345; P=<.0001) and RS899968 (X2=11.26643; P=<.0001). Refer to table 4-33 for genotype frequencies for each SNP and HWE calculations.

Lactate, pyruvate and lactate pyruvate ratio (LP) levels were analyzed at days 1-5 post injury. Descriptive statistics for the lactate and pyruvate levels and LP ratios for each of the 5 days are included in table 4-33.

Preliminary Mixed Models Analysis for Neurometabolites

In the preliminary mixed models analyses, each SNP was analyzed individually for a relationship with lactate, pyruvate, and LP concentrations over time. Each of the covariates was analyzed for potential relationship with lactate, pyruvate, and LP concentrations over time. By doing this the SNP's and covariates of interest in building the larger model were ascertained. The criterion used was a P for Type 3 tests of ≤0.2 in addition to covariates that are deemed prudent

through the evidence in the literature (age, gender, GCS, and hypothermia). Race was not used in the model as all of the subjects were Caucasian. (Refer to tables 4-34 to 4-37).

Lactate

The preliminary mixed model analyses for lactate indicated that the SNP's and covariates of interest, meeting the criterion were: RS17759659 (genotypes P=0.2042; dichotomized genotype P=0.0742); RS1801018 (genotypes P=0.1385; dichotomized genotype P=0.0473); RS1944419 (genotypes P=0.3196; [preliminary models were run for both homozygous wild type and variant because of equal sample size] XX dichotomized genotype P=0.1285; YY dichotomized genotype P=0.4567); RS3810027 (genotypes P=0.1781; dichotomized genotype P=0.0905); RS7236090 (genotypes P=0.1776; dichotomized genotype P=0.2821); RS8083946 (genotypes P=0.2161; dichotomized genotype P=0.1124); time (days) (P=0.0107); GCS (P=0.1429); gender (P= 0.0448). Age (P= 0.5091) and hypothermia (P=.6106) were not indicated in the preliminary analysis, however, they were controlled for in the primary full model. (Refer to tables 4-34 to 4-37).

Pyruvate

The preliminary mixed model analysis for pyruvate indicated that the SNP's and covariates of interest, meeting the criterion were: RS17759659 (genotypes P=0.0079; dichotomized genotype P=0.0019); RS1801018 (genotypes P=0.0595; dichotomized genotype P=0.0192); RS3810027 (genotypes P=0.1022; dichotomized genotype P=0.169); RS4941185 (genotypes P=0.0727; dichotomized genotype P=0.0211); RS7236090 (genotypes P=0.2753; dichotomized genotype P=0.1062); RS949037 (genotypes P=0.0581; dichotomized genotype P=0.0207); and time (days) (P=0.0008). Age (P= 0.7648), GCS (P=0.6887), gender (P= 0.4182),

and hypothermia (P=0.3964) were not indicated in the preliminary analysis, however, they were controlled for in the primary full model. (Refer to tables 4-34 to 4-37).

Lactate: Pyruvate Ratio

The preliminary mixed model analysis for LP indicated that the SNP's and covariates of interest, meeting the criterion were: RS1026825 (genotypes P=0.2824; dichotomized genotype P=0.1089); RS17759659 (genotypes P=0.0183; dichotomized genotype P=0.0048); RS1801018 (genotypes P=0.0255; dichotomized genotype P=0.0075); RS1944419 (genotypes P=0.2738; [preliminary models were run for both homozygous wild type and variant because of equal sample size] XX dichotomized genotype P=0.1055; YY dichotomized genotype P=0.4335); RS3810027 (genotypes P=0.3672; dichotomized genotype P=0.1694); RS4941185 (genotypes P=0.1247; dichotomized genotype P=0.7522); RS7236090 (genotypes P=0.138; dichotomized genotype P=0.3901); RS8083946 (genotypes P=0.0281; dichotomized genotype P=0.1461); RS949037 (genotypes P=0.3937; dichotomized genotype P=0.1739); time (days) (P=0.006); GCS (P=0.1107); gender (P= 0.0543). Age (P= 0.6126) and hypothermia (P=.7937) were not indicated in the preliminary analysis, however, they were controlled for in the primary full model. (Refer to tables 4-34 to 4-37).

Primary Mixed Models Analyses for Neurometabolites

Lactate

In analyzing the 6 BCL-2 SNP's (RS17759659, RS1801018, RS1944419, RS3810027, RS7236090, and RS8083946) of interest in relation to the lactate levels none SNP's had significant findings. SNP's (RS3810027 and RS8083946) were marginally significant. Time and

gender were consistently marginally or statistically significant in relation to lactate levels among the mixed models analyzed for these SNP's. (Refer to tables 4-38 to 4-42).

The mixed model with SNP RS3810027: genotype XX was marginally significant (P= 0.0521) with overall genotype (P for type 3 F tests =0.01396). XX genotype is associated with a decrease in lactate concentrations. In contrast subjects with 0-1 X alleles have a trend of higher lactate concentrations in the dichotomized analysis (P=0.0791). Time of sample from injury was found to be significant (genotypes P=0.0084; dichotomized genotype P=0.0088), lactate concentrations are significantly higher days 1 and 2 post injury in relation to day 5. (Refer to table 4-40).

The mixed model with SNP RS8083946: genotype YY was significant (P= 0.037) with overall genotype (P for type 3 F tests =0.0725). YY genotype is associated with a decrease in lactate concentrations. This trend was not significant in the dichotomized analysis (P=0.1021). Time of sample from injury was found to be significant (genotypes P=0.0124; dichotomized genotype P=0.0103), lactate concentrations are significantly higher days 1 and 2 post injury in relation to day 5. Gender was found to be significant (genotypes P=0.026; dichotomized genotype P=0.0727) and was associated with higher lactate levels in females. (Refer to table 4-41).

Pyruvate

In analyzing the 6 BCL-2 SNP's (RS17759659, RS1801018, RS3810027, RS4941185, RS7236090, and RS949037) of interest in relations to the pyruvate concentrations 4 SNP's (RS17759659, RS1801018, RS4941185, and RS949037) had significant findings. Time was the only consistently significant in relation to pyruvate concentrations among the mixed models analyzed for these SNP's. (Refer to tables 4-38 to 4-42).

The mixed model with SNP RS17759659: genotype YY was significant (P= 0.0016) with overall genotype (P for type 3 F tests =0.0049). YY genotype is associated with a decrease in pyruvate concentrations. In contrast subjects with 0-1 Y alleles have significantly higher pyruvate concentrations in the dichotomized analysis (P=0.0011). Time of sample from injury was found to be significant (genotypes P=0.0011; dichotomized genotype P=0.0011), only day 1 was significantly higher that day 5. (Refer to table 4-38).

The mixed model with SNP RS1801018: genotype XX was significant (P= 0.0231) with overall genotype (P for type 3 F tests =0.00594). XX genotype is associated with a decrease in pyruvate concentrations. In contrast, subjects with 0-1 copies of the X allele have significantly higher pyruvate concentrations in the dichotomized analysis (P=0.0175). Time of sample from injury was found to be significant (genotypes P=0.0022; dichotomized genotype P=0.0021), only day 1 was significantly higher that day 5. (Refer to table 4-39).

The mixed model with SNP RS4941185: genotype XX was marginally significant (P= 0.0651) with overall genotype (P for type 3 F tests =0.1303). XX genotype is associated with an increase in pyruvate concentrations. In contrast, subjects with 0-1 X alleles have significantly higher pyruvate concentration in the dichotomized analysis (P=0.0416). Time of sample from injury was found to be significant (genotypes P=0.001; dichotomized genotype P=0.001), only day 1 was significantly higher that day 5. (Refer to table 4-40).

The mixed model with SNP RS949037: genotype YY was significant (P= 0.0063) with overall genotype (P for type 3 F tests =0.0136). YY genotype is associated with a decrease in pyruvate concentrations. In contrast, subjects with 0-1 Y alleles had significantly higher pyruvate concentrations in the dichotomized analysis (P=0.0032). Time of sample from injury was found

to be significant (genotypes P=0.0027; dichotomized genotype P=0.0027), only day 1 was significantly higher that day 5. (Refer to table 4-42).

Lactate: Pyruvate (LP) Ratio

In analyzing the 9 BCL-2 SNP's (RS1026825, RS17759659, RS1801018, RS1944419, RS3810027, RS4941185, RS7236090, RS8083946, and RS949037) of interest in relations to the LP ratios, 2 SNP's (RS17759659 and RS1801018) had significant findings and 1 SNP was marginally significant (RS8083946). Time was the only consistently significant in relation to LP ratios among the mixed models analyzed for these SNP's. (Refer to tables 4-38 to 4-42).

The mixed model with SNP RS17759659: genotype YY was significant (P= 0.01) with overall genotype (P for type 3 F tests =0.0287). YY genotypes were associated with an increase in LP ratios. In contrast, subjects with 0-1 Y alleles had significantly lower LP ratios as indicated in the dichotomized analysis (P=0.0079). Time of sample from injury was found to be significant (genotypes P=0.00133; dichotomized genotype P=0.0033), days 1 and 2 were significantly higher that day 5. (Refer to table 4-38).

The mixed model with SNP RS1801018: genotype XX was significant (P= 0.0342) with overall genotype (P for type 3 F tests =0.0661). XX genotypes were associated with an increase in LP ratios. In contrast, subjects with 0-1 X alleles had significantly lower LP ratios as indicated in the dichotomized analysis (P=0.024). Time of sample from injury was found to be significant (genotypes P=0.0087; dichotomized genotype P=0.009), days 1 and 2 were significantly higher that day 5. (Refer to table 4-39).

The mixed model with SNP RS8083946: genotype YY was significant (P= 0.0388) and genotype XX was marginally significant (P= 0.0783) with overall genotype (P for type 3 F tests =0.064). XX and YY genotypes were associated with a decrease in LP ratios. The dichotomized

analysis (YY compared to XX and XY) was not significant (P=0.1235). Time of sample from injury was found to be significant (genotypes P=0.007; dichotomized genotype P=0.0057), days 1 and 2 were significantly higher that day 5. Gender was statistically significant (genotypes P= 0.038; dichotomized genotypes P=0.1156), compared to males, females had higher LP ratios. (Refer to table 4-41).

Table 4-18: BCL-2 Genotypes vs. Neurometabolite Descriptive Data

BCL-2 Genotypes Versus Neurometabolite Descriptive Data

Varaibles	n=	Mean	Std. Deviation	Range
Age	36	31.14	13.9	17-71
			Frequency (%)	Frequency (%) of Unknown
GCS	36	scores 3 to 5	15(41.7%)	0
		scores 6 to 8	21(58.3%)	0
Gender (male)	36		27(75%)	0
Race (Caucasian)	36		all caucasian	0
Hypothermic	36		15(41.7%)	0
Hypoxia	15		6(16.7%)	21 (58.33%)
Hypotensive	15		5(13.9%)	21 (58.33%)
Seizures	17		1(2.78%)	19 (52.78%)
APOEε4	36		8(22.2%)	0

Neurometabolites	n=	Mean	Std. Deviation	Range	Frequency (%) of Unknown
Lactacte Concentration (µmol)					
Day 1	30	12751.2	21410.34	1228.57- 1206600.79	3(8.33%)
Day 2	33	10084.91	20316.16	1737.895-120799.91	6 (16.67%)
Day 3	33	5061.11	2591.21	1836.46-13186.45	6 (16.67%)
Day 4	29	4470.85	19104.22	1868.55-9026.36	7 (19.44%)
Day 5	23	4342.79	2263.85	1534.66-10100.6	13 (36.11%)
Pyruvate Concentration (µmol)					
Day 1	29	228.84	92.19	83.06-429.63	4 (11.11%)
Day 2	33	204.19	71.36	93.20-359.58	6 (16.67%)
Day 3	33	193.04	62.5	84.3-361.5	6 (16.67%)
Day 4	29	204.95	61.14	104.07-328.89	7 (19.44%)
Day 5	23	193.1	67.91	98.62-374.34	13 (36.11%)
Lactate Pyruvate (LP) Ratio					
Day 1	29	50.73	54.91	11.89-303.9	4 (11.11%)
Day 2	33	48.5	84.32	13.84-501.74	6 (16.67%)
Day 3	33	27.7	14.36	15.51-71.85	6 (16.67%)
Day 4	29	21.89	6.62	8.99-34.48	7 (19.44%)
Day 5	23	22.27	7.93	9.14-45.36	13 (36.11%)

Hardy-Weinberg Equilibrium

SNP	n=	XX	YY	XY	X	Y	HWE	X^2	P
RS1026825	36	11	9	16	0.527777778	0.4722222	1		
RS12454712	36	5	17	14	0.333333333	0.6666667	1		
RS12968517	36	18	6	12	0.666666667	0.3333333	1		
RS1381548	36	3	10	23	0.402777778	0.5972222	1		
RS1481031	36	19	4	13	0.708333333	0.2916667	1		
RS17756073	36	16	4	16	0.666666667	0.3333333	1		
RS17759659	35	6	7	22	0.485714286	0.5142857	1		
RS1801018	35	6	5	24	0.514285714	0.4857143	1		
RS1944419	34	12	10	12	0.529411765	0.4705882	1		
RS3810027	35	18	3	14	0.714285714	0.2857143	1		
RS4456611	36	8	5	23	0.541666667	0.4583333	1		
RS4941185	35	12	8	15	0.414285714	0.3	0.510204082	15.277345	<.0001
RS7230970	35	6	10	19	0.442857143	0.5571429	1		
RS7236090	36	10	9	17	0.513888889	0.4861111	1		
RS8083946	36	4	19	13	0.291666667	0.7083333	1		
RS899968	36	7	12	7	0.291666667	0.4305556	0.521604938	11.26643	<.0001
RS949037	35	4	14	17	0.357142857	0.6428571	1		

*** X^2 p≤ 0.05**

Table 4-19: Preliminary Mixed Models Analyses of BCL-2 vs Neurometabolites

Preliminary Mixed Models Analysis of BCL-2 vs Neurometabolites											
			Lactate			Pyruvate			Lactate:Pyruvate Ratio		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS1026825	36	xx	4195.96	0.2456	0.4704	-32.6433	0.1831*	0.4061	18.638	0.1371*	0.2824
		yy	271.58	0.9447		-14.104	0.5887		0.5235	0.969	
		xy									
		yy & xy	-4116.6	0.2161	0.2161	27.7345	0.2163	0.2163	-18.5	0.1089*	0.1089*
		xx									
RS12454712	36	xx	-3528.5	0.4863	0.5313	-27.6095	0.4116	0.6849	-11.86	0.506	0.6446
		yy	-3569.4	0.2861		-126904	0.5766		-9.993	0.3954	
		xy									
		xx & xy	2742.67	0.3736	0.3736	5.7549	0.7833	0.7833	7.1742	0.5074	0.5074
		yy									
RS12968517	36	xx	1392.19	0.6898	0.6479	-4.9823	0.8337	0.8955	4.0849	0.7377	0.6251
		yy	-2593.7	0.5723		-14.7417	0.6411		-10.47	0.5143	
		xy									
		yy & xy	-2300.5	0.4555	0.4555	-0.01439	0.9995	0.9995	-7.776	0.4706	0.4706
		xx									
RS1381548	36	xx	-1498.9	0.8166	0.6891	0.4193	0.9917	0.6745	-3.776	0.8675	0.7952
		yy	-3019.6	0.3948		-20.6643	0.3869		-8.321	0.5033	
		xy									
		xx & xy	2897.41	0.403	0.403	20.7356	0.3715	0.3715	8.0002	0.5097	0.5097
		yy									
RS1481031	36	xx	-4189.6	0.2074	0.4023	-5.2076	0.8198	0.9641	-12.38	0.289	0.4843
		yy	-4717.1	0.3743		-7.855	0.829		-16.91	0.3654	
		xy									
		yy & xy	3131.11	0.3094	0.3094	3.4249	0.8701	0.8701	8.5776	0.4281	0.4281
		xx									
* p≤ 0.2											

Table 4-19 continued

Preliminary Mixed Models Analysis of BCL-2 vs Neurometabolites											
			Lactate			Pyruvate			Lactate:Pyruvate Ratio		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS17756073	36	xx	3191.72	0.3382	0.5863	10.6194	0.6346	0.6596	9.0445	0.4375	0.5853
		yy	3391.75	0.55007		-20.1993	0.5621		15.848	0.3699	
		xy									
		yy & xy	-2446.8	0.4313	0.4313	-14.8255	0.4796	0.4796	-5.554	0.6109	0.6109
		xx									
RS17759659	35	xx	-335.54	0.9409	0.2042	-3.1097	0.9049	0.0079*	-1.896	0.8975	0.0183*
		yy	6689.86	0.0876*		-76.3575	0.0025*		35.223	0.0069*	
		xy									
		xx & xy	-6752.8	0.0742*	0.0742*	75.7433	0.0019*	0.0019*	-35.57	0.0048*	0.0048*
		yy									
RS1801018	35	xx	7814.96	0.0568*	0.1385*	-61.233	0.0271*	0.0595*	35.557	0.0106*	0.0255*
		yy	-823.32	0.8679		13.1922	0.6636		-5.943	0.7189	
		xy									
		yy & xy	-7931.3	0.0473*	0.0473*	63.2985	0.0192*	0.0192*	-36.37	0.0075*	0.0075*
		xx									
RS1944419	34	xx	5154.19	0.1914*	0.3196	-15.5471	0.5476	0.7973	19.048	0.1621*	0.2738
		yy	-6.1375	0.9988		-1.0016	0.9693		0.2034	0.9882	
		xy									
		yy & xy	-5159.3	0.1285*	0.1285*	15.0531	0.4987	0.4987	-18.95	0.1055*	0.1055*
		xx									
		xx & xy	2626.87	0.4567	0.4567	-6.6137	0.7689	0.7689	9.5461	0.4335	0.4335
		yy									
RS3810027	35	xx	-6157.4	0.0661*	0.1781*	-39.9896	0.0663*	0.1022*	-16.65	0.1599*	0.3672
		yy	-4413	0.429		-61.8993	0.1031*		-7.554	0.7039	
		xy									
		yy & xy	5332.65	0.0905*	0.0905*	28.8401	0.169*	0.169*	15.239	0.1694*	0.1694*
		xx									
* p≤ 0.2											

Table 4-19 continued

Preliminary Mixed Models Analysis of BCL-2 vs Neurometabolites												
			Lactate			Pyruvate			Lactate:Pyruvate Ratio			
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	
RS4456611	36	xx	-3353.7	0.3819	0.6724	-32.1672	0.2098	0.3662	-7.778	0.5643	0.7924	
		yy	-280.69	0.9503		-27.6985	0.3626		3.489	0.8253		
		xy										
		yy & xy	3309.04	0.3693	0.3693	27.1041	0.2763	0.2763	8.4793	0.5137	0.5137	
		xx										
RS4941185	35	xx	2359.65	0.5031	0.2027	51.3918	0.0309*	0.0727*	5.8862	0.6275	0.1247*	
		yy	7206.88	0.076*		4.3265	0.867		28.153	0.0453*		
		xy										
		yy & xy	107.17	0.9746	0.9746	-49.9683	0.0211*	0.0211*	3.7163	0.7522	0.7522	
		xx										
RS7230970	35	xx	-2715.1	0.5403	0.6349	-5.7876	0.847	0.6096	-12.79	0.4054	0.4918	
		yy	-3242.5	0.3865		22.012	0.3809		-13.59	0.2967		
		xy										
		xx & xy	2608.44	0.4633	0.4633	-23.3713	0.3253	0.3253	10.609	0.3931	0.3931	
		yy										
RS7236090	36	xx	-5606.7	0.1201*	0.1776*	-37.6731	0.1273*	0.2753	-18.24	0.1439*	0.138*	
		yy	-5747.4	0.1311*		-2.6619	0.9165		-23.81	0.0743*		
		xy										
		yy & xy	3657.3	0.2821	0.2821	36.7929	0.1062*	0.1062*	10.252	0.3901	0.3901	
		xx										
RS8083946	36	xx	-3979.5	0.453	0.2161	37.1854	0.3033	0.4971	-18.85	0.3089	0.0281*	
		yy	-5713.5	0.0837*		-3.5528	0.874		-19.6	0.0888*		
		xy										
		xx & xy	4879.86	0.1124*	0.1124*	11.9886	0.5671	0.5671	15.648	0.1461*	0.1461*	
		yy										
* p≤ 0.2												

Table 4-19 continued

Preliminary Mixed Models Analysis of BCL-2 vs Neurometabolites												
			Lactate			Pyruvate			Lactate:Pyruvate Ratio			
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	
RS899968	36	xx	-2570	0.5468	0.75	3.4057	0.9055	0.9905	-8.845	0.5523	0.7123	
		yy	-2213.7	0.5317		-0.5919	0.9804		-9.003	0.467		
		xy										
		xx & xy	1519.08	0.647	0.647	1.5553	0.9443	0.9443	6.5937	0.5693	0.5693	
		yy										
RS949037	35	xx	-44.545	0.9936	0.6988	20.7994	0.5227	0.0581*	-3.567	0.8523	0.3937	
		yy	2753.72	0.4198		-43.2223	0.0419*		14.652	0.2137		
		xy										
		xx & xy	-2766.9	0.3937	0.3937	46.9819	0.0207*	0.0207*	-15.25	0.1739*	0.1739*	
		yy										
Covariates												
Time (Days)	36	1	9286.03	0.0058*	0.0107*	47.1822	0.0002*	0.0008*	30.865	0.0081*	0.006*	
		2	6638	0.0424*		15.18	0.2077		29.304	0.0098*		
		3	1517.87	0.6387		3.553	0.7659		8.1207	0.4668		
		4	628.01	0.8488		11.9472	0.3238		1.6195	0.8866		
		5	.	.		.	.		.	.		
Age	36		-73.195	0.5091	0.5091	0.2241	0.7648	0.7648	-0.198	0.6126	0.6126	
GCS	36		-4538.6	0.1429*	0.1429*	-8.4914	0.6887	0.6887	-17.23	0.1107*	0.1107*	
Gender	36		6931.03	0.0448*	0.0448*	19.1683	0.4182	0.4182	23.434	0.0543*	0.0543*	
Race	17				N/A			NA			NA	
Hypothermic	36		1576.42	0.6106	0.6106	17.5545	0.3964	0.3964	2.8457	0.7937	0.7937	
Hypoxia	15		7660.51	0.2729	0.2729	-6.8801	0.8228	0.8228	29.357	0.2249	0.2249	
Hypotensive	15		2814.74	0.7134	0.7134	1.0373	0.9747	0.9747	5.6277	0.8329	0.8329	
Seizures	17		3304.32	0.8173	0.8173	2.7005	0.9648	0.9648	12.67	0.7989	0.7989	
APOEε4	36		919.92	0.8035	0.8035	-27.7319	0.257	0.257	4.2516	0.7429	0.7429	
* p≤ 0.2												

Table 4-20: Primary Mixed Models Analyses of BCL-2 and Neurometabolites

Primary Mixed Models Analyses of BCL-2 SNP and Neurometabolites																							
		Lactate							Pyruvate							Lactate:Pyruvate Ratio							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS1026825 (n=36)																							
Genotype	xx	5217.1	0.1752	0.3583	yy & xy	-4906	0.1526	0.1526	xx	-35.12	0.2235	0.4692	yy & xy	27.898	0.2777	0.2777	xx	21.49	0.1131	0.2397	yy & xy	-20.46	0.0907	0.0907
	yy	790.58	0.8393		xx				yy	-16.76	0.5548		xx				yy	2.5986	0.849		xx			
	xy								xy								xy							
Time (Day)	1	8698	0.101	0.0211*	1	8694.6	0.01*	0.0206*	1	47.73	0.0002**	0.0007**	1	47.468	0.0002**	0.0008**	1	28.643	0.0145*	0.0121*	1	28.645	0.0143*	0.0118*
	2	6285.9	0.0562		2	6262.2	0.0568		2	15.433	0.2011		2	15.429	0.2013		2	28.354	0.0129*		2	28.276	0.013*	
	3	1289.2	0.6921		3	1270.5	0.6959		3	3.8646	0.7465		3	3.8529	0.7472		3	7.511	0.5031		3	7.4504	0.506	
	4	649.92	0.8448		4	638.62	0.8473		4	11.997	0.3224		4	12.026	0.3213		4	1.865	0.8704		4	1.8226	0.8731	
	5	.			5				5				5				5				5			
Age		-91.91	0.4069	0.4069		-95.8	0.3743	0.3743		-0.065	0.9371	0.9371		0.0064	0.9937	0.9937		-0.197	0.6122	0.6122		-0.209	0.58	0.58
GCS		-1129	0.7356	0.7356		-1263	0.6957	0.6957		-14.93	0.551	0.551		-12.21	0.6155	0.6155		-5.725	0.6252	0.6252		-6.165	0.5862	0.5862
Gender		7918.6	0.04*	0.04*		7798.8	0.0368*	0.0368*		9.2205	0.7364	0.7364		11.472	0.6691	0.6691		25.829	0.0547	0.0547		25.415	0.051	0.051
Hypothermia		3928.9	0.2279	0.2279		3912.8	0.2226	0.2226		12.786	0.5946	0.5946		13.115	0.5807	0.5807		10.379	0.3604	0.3604		10.334	0.3552	0.3552
RS17759659 (n=35)																							
Genotype	xx	-303.8	0.9479	0.2442	xx & xy	-6262	0.0925	0.0925	xx	2.9274	0.9154	0.0049**	xx & xy	83.042	0.0011**	0.0011**	xx	-1.536	0.9209	0.0287*	xx & xy	-34	0.0079**	0.0079**
	yy	6225.6	0.1031		yy				yy	-82.62	0.0016**		yy				yy	33.81	0.01*		yy			
	xy								xy								xy							
Time (Day)	1	8983.1	0.0087**	0.0181*	1	8954.3	0.0088*	0.0182	1	47.52.95	0.0002**	0.0011**	1	47.47	0.0002**	0.0011**	1	28.244	0.0167*	0.0133*	1	28.126	0.017*	0.0133*
	2	6535.4	0.05*		2	6513.4	0.0504		2	15.63	0.201		2	15.61	0.2014		2	28.376	0.0136*		2	28.29	0.0138*	
	3	1456	0.6586		3	1437.2	0.6622		3	3.5748	0.7678		3	3.5575	0.7688		3	7.5917	0.5025		3	7.5147	0.5062	
	4	635.94	0.8491		4	621.54	0.8523		4	12.614	0.303		4	12.652	0.3013		4	1.3464	0.9067		4	1.2759	0.9114	
	5				5				5				5				5				5			
Age		-136.7	0.2328	0.2328		-135.6	0.2225	0.2225		0.2118	0.7663	0.7663		0.2007	0.7718	0.7718		-0.357	0.3452	0.3452		-0.352	0.3385	0.3385
GCS		-1919	0.563	0.563		-1966	0.5359	0.5359		-21.39	0.3035	0.3035		-20.82	0.2933	0.2933		-5.841	0.5953	0.5953		-6.091	0.563	0.563
Gender		7073.4	0.0633	0.0633		7101.3	0.0585	0.0585		28.315	0.2253	0.2253		28.455	0.2154	0.2154		19.581	0.1174	0.1174		19.674	0.1104	0.1104
Hypothermia		2875.8	0.3829	0.3829		2838.9	0.3655	0.3655		10.3	0.6154	0.6154		10.94	0.5733	0.5733		8.1402	0.4555	0.4555		7.9148	0.4458	0.4458
* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001																								

Table 4-20 continued

Primary Mixed Models Analyses of BCL-2 SNP and Neurometabolites																							
		Lactate							Pyruvate							Lactate:Pyruvate Ratio							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS1801018 (n=35)																							
Genotype	xx	6116.5	0.1384	0.2475	yy & xy	-6450	0.11	0.11	xx	-68.25	0.0231*	0.0594	yy & xy	69.342	0.0175*	0.0175*	xx	30.13	0.0342*	0.0661	yy & xy	-31.45	0.024*	0.024*
	yy	-2582	0.6194		xx				yy	7.2754	0.8285		xx				yy	-10.18	0.5634		xx			
	xy								xy								xy							
Time (Day)	1	9195	0.0086**	0.0164*	1	9145.3	0.0088**	0.017*	1	44.955	0.0007**	0.0022**	1	44.933	0.0007**	0.0021**	1	30.465	0.0117*	0.0087**	1	30.253	0.0122*	0.009**
	2	6549.6	0.0545		2	6566.2	0.0535		2	13.024	0.2978		2	12.958	0.2999		2	29.445	0.0124*		2	29.533	0.012*	
	3	1349.1	0.6888		3	1406.1	0.6757		3	1.9068	0.8778		3	1.8025	0.8843		3	7.7469	0.5037		3	7.9954	0.4893	
	4	569.48	0.8683		4	563.07	0.8697		4	10.653	0.3971		4	10.661	0.3965		4	1.4155	0.9046		4	1.395	0.9059	
	5				5				5				5				5				5			
Age		-106.7	0.3487	0.3487		-95.76	0.3854	0.3854		-0.045	0.9542	0.9542		-0.074	0.9231	0.9231		-0.241	0.5307	0.5307		-0.198	0.5954	0.5954
GCS		-1785	0.5884	0.5884		-1832	0.5745	0.5745		-14.88	0.5131	0.5131		-14.77	0.5096	0.5096		-7.399	0.5074	0.5074		-7.597	0.4918	0.4918
Gender		6176.2	0.0956	0.0956		6220.5	0.0898	0.0898		20.917	0.4063	0.4063		21.102	0.3948	0.3948		18.621	0.135	0.135		18.78	0.1279	0.1279
Hypothermia		3899.4	0.251	0.251		3356.7	0.2883	0.2883		13.331	0.5711	0.5711		15.149	0.4853	0.4853		10.578	0.3549	0.3549		8.4299	0.4283	0.4283
RS1944419 (n=34)																							
Genotype	xx	3047.5	0.4534	0.7342	yy & xy	-2795	0.4363	0.4363	xx	-26.6	0.3446	0.523	yy & xy	28.549	0.2559	0.2559	xx	12.545	0.3808	0.6245	yy & xy	-12.34	0.3293	0.3293
	yy	568.3	0.8879		xx				yy	4.4747	0.8688		xx				yy	0.4458	0.975		xx			
	xy								xy								xy							
Time (Day)	1	8970.8	0.012*	0.0281*	1	8955.2	0.012	0.0279	1	43.936	0.0008**	0.0025**	1	43.916	0.0008**	0.0026**	1	29.175	0.0183*	0.0168*	1	29.091	0.0184*	0.0165*
	2	6605.9	0.0577		2	6616	0.0565		2	10.416	0.4007		2	10.483	0.3972		2	29.754	0.0136*		2	29.694	0.0134*	
	3	1514.7	0.6592		3	1518.4	0.6578		3	1.3666	0.9114		3	1.4831	0.909		3	8.3933	0.4782		3	8.3409	0.48	
	4	773.5	0.8241		4	774.69	0.8237		4	9.5771	0.4399		4	9.6042	0.4385		4	2.2926	0.8483		4	2.2661	0.8498	
	5				5				5				5				5				5			
Age		-88.59	0.5069	0.5069		-84.64	0.5104	0.5104		-0.802	0.3808	0.3808		-0.78	0.3806	0.3806		-0.019	0.9681	0.9681		-0.015	0.9735	0.9735
GCS		-2077	0.5635	0.5635		-2041	0.5635	0.5635		-22.51	0.3603	0.3603		-22.25	0.3565	0.3565		-6.397	0.6125	0.6125		-6.346	0.6089	0.6089
Gender		6912.1	0.1146	0.1146		6943	0.1073	0.1073		20.175	0.4903	0.4903		20.002	0.4858	0.4858		20.878	0.172	0.172		21.001	0.1626	0.1326
Hypothermia		2774.2	0.4216	0.4216		2836.7	0.4026	0.4026		7.2392	0.7575	0.7575		7.4667	0.7455	0.7455		8.351	0.4906	0.4906		8.4709	0.476	0.476

* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001

Table 4-20 continued

Primary Mixed Models Analyses of BCL-2 SNP and Neurometabolites																							
		Lactate								Pyruvate								Lactate:Pyruvate Ratio					
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS3810027 (n=35)																							
Genotype	xx	-6628	0.0521	0.1396	yy & xy	5682.9	0.0791	0.0791	xx	-36.81	0.1184	0.1445	yy & xy	25.388	0.2678	0.2678	xx	-19.57	0.1121	0.2792	yy & xy	17.8	0.1248	0.1248
	yy	-5035	0.3417		xx				yy	-63.83	0.1058		xx				yy	-9.367	0.6267		xx			
	xy								xy								xy							
Time (Day)	1	9997.8	0.004**	0.0084**	1	9963.8	0.0041**	0.0088**	1	48.922	0.0002**	0.0007**	1	48.843	0.0002**	0.0007**	1	32.639	0.0066**	0.0056**	1	32.551	0.0067**	0.0057**
	2	7231.7	0.0316*		2	7249.2	0.0311*		2	16.65	0.1769		2	16.668	0.1765		2	31.409	0.0072**		2	31.41	0.0071**	
	3	2109	0.5287		3	2125.3	0.5254		3	4.6059	0.7086		3	4.6555	0.7056		3	10.367	0.37		3	10.375	0.3693	
	4	1019.2	0.7645		4	1058.5	0.7556		4	12.602	0.3132		4	12.714	0.3089		4	3.2056	0.7847		4	3.2632	0.7808	
	5				5				5				5				5				5			
Age		-117.3	0.2955	0.2955		-114.5	0.307	0.307		-0.266	0.7406	0.7406		-0.244	0.7683	0.7683		-0.25	0.5406	0.5406		-0.245	0.5425	0.5425
GCS		-4123	0.2299	0.2299		-4075	0.2351	0.2351		-1.215	0.9594	0.9594		-0.724	0.9765	0.9765		-17.72	0.1594	0.1594		-17.63	0.1561	0.1561
Gender		5585.9	0.1454	0.1454		5510.2	0.1503	0.1503		21.702	0.4132	0.4132		20.37	0.4553	0.4553		16.315	0.2401	0.2401		16.212	0.237	0.237
Hypothermia		-2205	0.5085	0.5085		-2103	0.5279	0.5279		-25.73	0.2793	0.2793		-24.87	0.3095	0.3095		-2.376	0.8848	0.8848		-2.253	0.8508	0.8508
RS4941185 (n=35)																							
Genotype	xx	1768.3	0.6333	0.2817	yy & xy	882.37	0.7959	0.7959	xx	49.973	0.0651	0.1303	yy & xy	-48.84	0.0416*	0.0416*	xx	2.539	0.845	0.1919	yy & xy	7.5273	0.5332	0.5332
	yy	6446.5	0.1193		xx				yy	2.9604	0.918		xx				yy	24.595	0.0909		xx			
	xy								xy								xy							
Time (Day)	1	8760.3	0.0102*	0.0217*	1	9018.9	0.0081**	0.017*	1	47.216	0.0002**	0.001*	1	47.166	0.0003**	0.001**	1	28.9	0.0143**	0.0127*	1	29.834	0.0115*	0.0097**
	2	6351.8	0.0572		2	6600.3	0.048*		2	16.308	0.183		2	16.317	0.1826		2	28.531	0.0136**		2	29.444	0.0109*	
	3	1311	0.6912		3	1423.7	0.6659		3	3.8055	0.7535		3	3.7929	0.7542		3	7.5203	0.5083		3	7.93	0.4854	
	4	587.31	0.8616		4	697.66	0.8356		4	11.499	0.3499		4	11.516	0.3492		4	1.5742	0.892		4	1.9906	0.8636	
	5				5				5				5				5				5			
Age		-121.2	0.303	0.303		-95.71	0.4259	0.4253		-0.33	0.6952	0.6952		-0.32	0.6965	0.6965		-0.273	0.5068	0.5068		-0.177	0.6752	0.6752
GCS		-1298	0.6986	0.6986		-2811	0.3998	0.3998		2.0504	0.931	0.931		1.443	0.9485	0.9485		-7.447	0.5273	0.5273		-13.2	0.2643	0.2643
Gender		6780.6	0.0684	0.0684		6686.7	0.0809	0.0809		13.537	0.5982	0.5982		13.673	0.5879	0.5879		21.477	0.0978	0.0978		21.196	0.1148	0.1148
Hypothermia		2578.9	0.425	0.425		2855.9	0.3921	0.3921		14.229	0.5354	0.5354		14.412	0.522	0.522		5.1504	0.6482	0.6482		6.1971	0.5969	0.5969
* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001																								

Table 4-20 continued

Primary Mixed Models Analyses of BCL-2 SNP and Neurometabolites																							
		Lactate							Pyruvate							Lactate:Pyruvate Ratio							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS7236090 (n=36)																							
Genotype	xx	-5725	0.1445	0.1771	yy & xy	2233.6	0.5179	0.5179	xx	-48.61	0.0957	0.2356	yy & xy	39.446	0.1113	0.1113	xx	-17.27	0.2128	0.1829	yy & xy	4.522	0.7135	0.7135
	yy	-7883	0.0835		xx				yy	-20.85	0.5268		xx				yy	-29.06	0.0732		xx			
	xy								xy								xy							
Time (Day)	1	9110.2	0.0069**	0.0143*	1	9060.7	0.0073**	0.0151*	1	47.366	0.0002**	0.0008**	1	47.238	0.0002**	0.0009**	1	30.161	0.0099**	0.006**	1	30.125	0.01*	0.0082*
	2	6515.5	0.0472*		2	6540.7	0.0466*		2	15.375	0.2029		2	15.32	0.2046		2	29.272	0.0101*		2	29.335	0.01*	
	3	1552.7	0.6323		3	1514.8	0.6409		3	3.9257	0.7427		3	3.8301	0.7488		3	8.5089	0.4471		3	8.3472	0.456	
	4	664.87	0.8408		4	729.35	0.8256		4	11.846	0.3288		4	11.867	0.328		4	2.0143	0.8598		4	2.2158	0.8459	
	5				5				5				5				5				5			
Age		-98.93	0.3594	0.3594		-94.48	0.3987	0.3987		0.1163	0.8847	0.8847		0.1137	0.886	0.886		-0.231	0.5459	0.5459		-0.213	0.5936	0.5936
GCS		976.46	0.789	0.789		-2518	0.4315	0.4315		10.89	0.6854	0.6854		1.7283	0.9384	0.9384		0.8703	0.9464	0.9464		-11.94	0.2979	0.2979
Gender		7358.5	0.0499*	0.0499*		6189.1	0.1029	0.1029		13.333	0.6186	0.6186		10.274	0.6928	0.6928		24.023	0.0703	0.0703		19.69	0.1441	0.1441
Hypothermia		5772.3	0.1016	0.1016		3027.3	0.3497	0.3497		31.753	0.2169	0.2169		24.681	0.2778	0.2778		15.99	0.1974	0.1974		5.9375	0.6054	0.6054
RS8083946 (n=36)																							
Genotype	xx	-8501	0.1092	0.0725	xx & xy	4873.3	0.1021	0.1021	xx	32.504	0.4209	0.6488	xx & xy	10.092	0.6471	0.6471	xx	-33.08	0.0783	0.064	xx & xy	16.322	0.1235	0.1235
	yy	-6531	0.037*		yy				yy	-2.889	0.9037		yy				yy	-22.87	0.0388*		yy			
	xy								xy								xy							
Time (Day)	1	9346.3	0.0058**	0.0124*	1	9576.5	0.0047**	0.0103*	1	47.734	0.0002**	0.0008**	1	47.47	0.0002**	0.0008**	1	30.755	0.0087**	0.007**	1	31.672	0.007**	0.0057**
	2	6876.3	0.0369*		2	7015.7	0.0334*		2	15.634	0.1962		2	15.483	0.2005		2	30.4	0.0078**		2	30.911	0.0069**	
	3	1752.2	0.5896		3	1888.1	0.5613		3	4.0574	0.7346		3	3.9063	0.7441		3	9.1144	0.4159		3	9.61	0.3915	
	4	951.49	0.7735		4	1004.1	0.7614		4	12.111	0.3181		4	12.061	0.3201		4	2.902	0.7989		4	3.0923	0.7862	
	5				5				5				5				5				5			
Age		-135.5	0.2109	0.2109		-97.73	0.3659	0.3659		0.1855	0.828	0.828		0.0258	0.975	0.975		-0.37	0.3325	0.3325		-0.221	0.5654	0.5654
GCS		-2745	0.3627	0.3627		-3148	0.309	0.309		-4.322	0.8529	0.8529		-3.424	0.8824	0.8824		-12.23	0.2524	0.2524		-13.74	0.2143	0.2143
Gender		8365.8	0.026*	0.026*		6510.4	0.0727	0.0727		10.646	0.7046	0.7046		17.383	0.5156	0.5156		27.38	0.038*	0.038*		20.193	0.1156	0.1156
Hypothermia		2765.2	0.3701	0.3701		1964.5	0.5295	0.5295		15.079	0.5303	0.5303		18.323	0.4373	0.4373		5.9074	0.5861	0.5861		2.7819	0.802	0.802

* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001

Table 4-20 continued

Primary Mixed Models Analyses of BCL-2 SNP and Neurometabolites																							
		Lactate								Pyruvate								Lactate:Pyruvate Ratio					
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS949037 (n=35)																							
Genotype	xx	-1398	0.8153	0.9391	xx & xy	-961.2	0.7877	0.7877	xx	-1.777	0.9601	0.0136*	xx & xy	69.146	0.0032*	0.0032*	xx	-3.471	0.8668	0.6294	xx & xy	-11.88	0.3388	0.3388
	yy	647.37	0.8665		yy				yy	-69.57	0.0063**		yy				yy	11.078	0.4081		yy			
	xy								xy								xy							
Time (Day)	1	8850.3	0.0118*	0.026*	1	8832.4	0.0118*	0.0261*	1	43.212	0.0008**	0.0027**	1	43.154	0.0008*	0.0027*	1	29.375	0.0159*	0.0134*	1	29.298	0.016*	0.0135*
	2	6437.6	0.0599		2	6422.8	0.0601		2	10.613	0.3848		2	10.606	0.3848		2	29.455	0.0136*		2	29.182	0.0137*	
	3	1376.3	0.6843		3	1378.7	0.6833		3	2.413	0.8422		3	2.4279	0.8411		3	7.6166	0.514		3	7.5952	0.5145	
	4	726.39	0.8331		4	718.68	0.8347		4	9.789	0.4259		4	9.8049	0.425		4	2.1124	0.8589		4	2.0789	0.861	
	5				5				5				5				5				5			
Age		-82.66	0.5586	0.5586		-72.64	0.5845	0.5845		-1.288	0.1428	0.1428		-1.276	0.121	0.121		0.0367	0.9401	0.9401		0.0624	0.8921	0.8921
GCS		-2271	0.5345	0.5345		-2183	0.543	0.543		-34.92	0.1227	0.1227		-34.76	0.1144	0.1144		-6.138	0.6273	0.6273		-5.898	0.6343	0.6343
Gender		6976.7	0.0969	0.0969		7116.8	0.0842	0.0842		4.424	0.8579	0.8579		4.6683	0.8463	0.8463		24.762	0.0887	0.0887		25.14	0.0778**	0.0778**
Hypothermia		3224.7	0.3623	0.3623		3035.8	0.3679	0.3679		4.6455	0.8278	0.8278		4.4082	0.8282	0.8282		9.228	0.4505	0.4505		8.7878	0.4503	0.4503

* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001

4.2.2.3 Cerebral Blood Flow

Sub-sample Description Cerebral Blood Flow

The mean age of the sub-sample of 17 subjects was 31.94 years old (range 16-65; SD± 17.594). The sample was all Caucasian and primarily male (n=13; 76.5%). Over half of the sub-sample had an admission GCS of 3-5 (n=9; 52.9%). Of the subjects enrolled in the study, a portion received therapeutic hypothermia (n=8; 41.2%). In the overall sub-sample the presence of sustained hypoxia (n=4; 23.53%), hypotension (n=4; 23.53%), hypoxia (n=4; 23.53%), documented pre-admission seizures (n=0) and the occurrence of APOEε4 (n=4; 23.53%), were considered. Refer to table 4-43 for sample description. All of the SNP's genotyped for the CBF sub-sample met the HWE criteria for genotype representation in a population. Refer to table 4-43 for genotype frequencies for each SNP and HWE calculations.

Right hemisphere, left hemisphere, and global CBF were analyzed over days 1-5 post injury. CBF peaked overall on day 1 across the three regions analyzed. Descriptive statistics for the CBF are included in table 4-43.

Preliminary Mixed Models Analysis for CBF

In the preliminary mixed models analyses, each SNP was analyzed individually for a relationship with right hemisphere, left hemisphere, and global CBF over time. Each of the covariates was analyzed for potential relationship with right hemisphere, left hemisphere, and global CBF over time. By doing this the SNP's and covariates of interest in building the larger model were ascertained. The criterion used was a P for Type 3 tests of ≤0.2 in addition to covariates that are deemed prudent through the evidence in the literature (age, gender, GCS, and

hypothermia). Race was not included in the model as all of the subjects were Caucasian. (Refer to tables 4-44 to 4-47).

Right Hemisphere

The preliminary mixed model analyses for right hemisphere indicated that there were 13 SNP's and 2 covariates of interest, meeting the criterion were: RS1026825 (genotypes P=0.014; [preliminary models were run for both homozygous variant and wild type because of equal sample size] XX dichotomized genotype P=0.3736; YY dichotomized genotype P=0.0307); RS12454712 (genotypes P=0.3432; dichotomized genotype P=0.0132); RS1381548 (genotypes P=0.1529; [preliminary models were run for both homozygous wild type and variant because of equal sample size] XX dichotomized genotype P=0.1484; YY dichotomized genotype P=0.0538); RS1481031 (genotypes P=0.017; dichotomized genotype P=0.004); RS17756073 (genotypes P=0.0436; dichotomized genotype P=0.0172); RS1801018 (genotypes P=0.016; dichotomized genotype P=0.0332); RS3810027 (genotypes P=0.0542; dichotomized genotype P=0.8445); RS4456611 (genotypes P=0.1067; [preliminary models were run for both homozygous wild type and variant because of equal sample size] XX dichotomized genotype P=0.1003; YY dichotomized genotype P=0.0428); RS4941185 (genotypes P=0.161; dichotomized genotype P=0.0552); RS7236090 (genotypes P=0.1314; dichotomized genotype P=0.2243); RS8083946 (genotypes P=0.0444; dichotomized genotype P=0.1117); RS899968 (genotypes P=0.0099; dichotomized genotype P=0.004); RS949037 (genotypes P=0.0406; dichotomized genotype P=0.4167); time (day) (P=0.0788); and gender (P= 0.0406). Age (P= 0.3142), GCS (P=0.3733), and hypothermia (P=.7908) were not indicated in the preliminary analysis, however, they were controlled for in the full model. (Refer to tables 4-44 to 4-47).

Left Hemisphere

The preliminary mixed model analyses for left hemisphere indicated that there were 13 SNP's and 4 covariates of interest, meeting the criterion were: RS1026825 (genotypes P=0.0193; [preliminary models were run for both homozygous variant and wild type because of equal sample size] XX dichotomized genotype P=0.327; YY dichotomized genotype P=0.0362); RS12968517 (genotypes P=0.1629; dichotomized genotype P=0.0703); RS1381548 (genotypes P=0.1992; [preliminary models were run for both homozygous variant and wild type because of equal sample size] XX dichotomized genotype P=0.1249; YY dichotomized genotype P=0.0792); RS1481031 (genotypes P=0.0378; dichotomized genotype P=0.0089); RS17756073 (genotypes P=0.0354; dichotomized genotype P=0.0153); RS1801018 (genotypes P=0.0384; dichotomized genotype P=0.0325); RS3810027 (genotypes P=0.1197; dichotomized genotype P=0.5906); RS4456611 (genotypes P=0.1833; [preliminary models were run for both homozygous variant and wild type because of equal sample size] XX dichotomized genotype P=0.1233; YY dichotomized genotype P=0.0879); RS4941185 (genotypes P=0.2766; dichotomized genotype P=0.1039); RS7236090 (genotypes P=0.1457; dichotomized genotype P=0.173); RS8083946 (genotypes P=0.0609; dichotomized genotype P=0.1049); RS899968 (genotypes P=0.0568; dichotomized genotype P=0.0273); RS949037 (genotypes P=0.1161; dichotomized genotype P=0.6107); time (day) (P=0.1381); age (P= 0.1435); GCS (P=0.1981); and gender (P= 0.0352). Hypothermia (P=0.9048) was not indicated in the preliminary analysis, however, it was controlled for in the full model. (Refer to tables 4-44 to 4-47).

Global

The preliminary mixed model analyses for Global indicated that there were 14 SNP's and 2 covariates of interest, meeting the criterion were: RS1026825 (genotypes P=0.0183; [preliminary models were run for both homozygous variant and wild type because of equal sample size] XX dichotomized genotype P=0.3657; YY dichotomized genotype P=0.0381); RS12454712 (genotypes P=0.4041; dichotomized genotype P=0.185); RS12968517 (genotypes P=0.3197; dichotomized genotype P=0.1666); RS1381548 (genotypes P=0.1614; [preliminary models were run for both homozygous variant and wild type because of equal sample size] XX dichotomized genotype P=0.1309; YY dichotomized genotype P=0.0587); RS1481031 (genotypes P=0.0214; dichotomized genotype P=0.0049); RS17756073 (genotypes P=0.0333; dichotomized genotype P=0.012); RS1801018 (genotypes P=0.0198; dichotomized genotype P=0.0244); RS3810027 (genotypes P=0.0715; dichotomized genotype P=0.7653); RS4456611 (genotypes P=0.1079; [preliminary models were run for both homozygous variant and wild type because of equal sample size] XX dichotomized genotype P=0.1008; YY dichotomized genotype P=0.044); RS4941185 (genotypes P=0.204; dichotomized genotype P=0.0727); RS7236090 (genotypes P=0.1384; dichotomized genotype P=0.2008); RS8083946 (genotypes P=0.0545; dichotomized genotype P=0.1278); RS899968 (genotypes P=0.0198; dichotomized genotype P=0.0084); RS949037 (genotypes P=0.0737; dichotomized genotype P=0.4698); time (day) (P=0.1092); and gender (P= 0.0327). Age (P= 0.2507), GCS (P=0.3032), and hypothermia (P=0.8968) were not indicated in the preliminary analysis, however, they were controlled for in the full model. (Refer to tables 4-44 to 4-47).

Primary Mixed Models Analyses for CBF

Right Hemisphere CBF

In analyzing the 13 BCL-2 SNP's (RS1026825, RS12454712, RS1381548, RS1481031, RS17756073, RS1801018, RS3810027, RS4456611, RS4941185, RS7236090, RS8083946, RS899968, and RS949037) of interest from the preliminary analyses, in relation to the right hemisphere CBF, 10 SNP's had significant findings (RS1026825, RS1381548, RS1481031, RS17756073, RS1801018, RS4456611, RS4941185, RS7236090, RS899968, and RS949037). Time was consistently statistically significant and gender was implicated as significant or trending for 2 SNP's (RS1801018 and RS4941185) in relation to right hemisphere CBF among the mixed models analyzed for these SNP's. (Refer to tables 4-48 to 4-54)

The mixed model with SNP RS1026825: genotype YY was significant (P= 0.0117) with overall genotype (P for type 3 F tests =0.0135). YY genotype is associated with a decrease in right hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.024). In contrast, subjects with 0-1 Y alleles had significantly higher right hemisphere CBF. Time of test from injury was found to be significant (genotypes P=0.0171; dichotomized genotype P=0.0254), compared to CBF on day 5, day 1 CBF was significantly higher and CBF on day 4 was significantly lower than day 4. (Refer to table 4-48).

The mixed model with SNP RS1381548: genotype YY was significant (P= 0.0477) with overall genotype (P for type 3 F tests =0.0681). YY genotype is associated with a decrease right hemisphere CBF. This trend was significant in the YY dichotomized analysis (P=0.0216). In contrast, subjects with 0-1 Y alleles had significantly higher CBF. Time of test from injury was found to be marginally significant (genotypes P=0.0869; YY dichotomized genotype P=0.0556), with day 4 CBF trending to lower CBF's. (Refer to table 4-49).

The mixed model with SNP RS1481031: genotype XX was significant (P= 0.0071) with overall genotype (P for type 3 F tests =0.0174). XX genotype is associated with a decrease in right hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0046). In contrast, subjects with 0-1 X alleles had significantly higher CBF. Time of test from injury was found to be significant (genotypes P=0.0108; dichotomized genotype P=0.0051), day 1 was associated with significantly higher and day 4 was associated with significantly lower CBF compared to day 5. (Refer to table 4-50).

The mixed model with SNP RS17756073: genotype XX (P= 0.1421) with overall genotype (P for type 3 F tests =0.0322). XX genotype is associated with an increase in right hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0116). In contrast, subjects with 0-1 X alleles had significantly lower CBF. Time of test from injury was found to be significant (genotypes P=0.024; dichotomized genotype P=0.0194); CBF on day 1 was significantly higher compared to day 5. (Refer to table 4-50).

The mixed model with SNP RS1801018: genotypes XX (P= 0.0002) and YY (P= 0.0007) were both significant with overall genotype (P for type 3 F tests =0.0021). XX and YY genotypes were associated with a decrease in right hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0391). In contrast, subjects with 0-1 copies of the X allele had a significant increase in CBF. Time of test from injury was found to be significant (genotypes P=0.0014; dichotomized genotype P=0.0279); day 1-3 were associated with significantly higher CBF compared to CBF on day 5. Gender was found to be significant in the genotype model (genotypes P=0.009; dichotomized genotype P=0.3626) with males having a decrease in right hemisphere CBF. (Refer to table 4-51).

The mixed model with SNP RS3810027: genotype XX (P= 0.0292) with overall genotype (P for type 3 F tests =0.0829). XX genotype is associated with an increase in right hemisphere CBF. This trend was not significant in the YY dichotomized analysis (P=0.79). Time of test from injury was found to be marginally significant (genotypes P=0.0153; YY dichotomized genotype P=0.1024); day 4 was associated with significantly lower CBF compared CBF on day 5. (Refer to table 4-51).

The mixed model with SNP RS4456611: genotype YY (P= 0.1356) with overall genotype (P for type 3 F tests =0.0927). YY genotype is associated with a decrease right hemisphere CBF. This trend was significant in the YY dichotomized analysis (P=0.0295). In contrast, subjects with 0-1 Y alleles had a significant increase in CBF. Time of test from injury was found to be marginally significant (genotypes P=0.0994; YY dichotomized genotype P=0.0838), day 1 was associated with significantly higher CBF. (Refer to table 4-52).

The mixed model with SNP RS4941185: genotype YY was significant (P= 0.0193) with overall genotype (P for type 3 F tests =0.0539). YY genotype is associated with an increase in right hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0132). In contrast, subjects with 0-1 Y alleles had lower CBF. Time of test from injury was found to be significant (genotypes P=0.0429; dichotomized genotype P=0.0294); day 1 was associated with significantly higher CBF and day 4 was associated with a trend in lower CBF when compared to day 5 CBF's. Gender was found to be marginally significant in the models (genotypes P=0.0753; dichotomized genotype P=0.0641) with males having a decrease in right hemisphere CBF. (Refer to table 4-52).

The mixed model with SNP RS7236090: genotype YY (P= 0.0273) with overall genotype (P for type 3 F tests =0.0306). YY genotype was associated with an increase in right

hemisphere CBF. This trend was not significant in the dichotomized analysis (P=0.1119). Time of test from injury was found to be marginally significant in the genotypes model (genotypes P=0.0626; dichotomized genotype P=0.1196); day 4 was associated with lower CBF. (Refer to table 4-53).

The mixed model with SNP RS8083946: genotype YY (P= 0.087) with overall genotype (P for type 3 F tests =0.0666). YY genotype is associated with an increase in right hemisphere CBF. This trend was not significant in the dichotomized analysis (P=0.1435). Time of test from injury was found to be marginally significant in the genotypes model (genotypes P=0.036; dichotomized genotype P=0.0634); day 1 associated with a higher trend in CBF and day 4 had significantly lower CBF when compared to day 5 CBF's. (Refer to table 4-53).

The mixed model with SNP RS899968: genotype YY (P= 0.0124) with overall genotype (P for type 3 F tests =0.0135). YY genotype is associated with a decrease in right hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0033). In contrast subjects with 0- Y alleles had significantly higher CBF. Time of test from injury was found to be significant (genotypes P=0.0316; dichotomized genotype P=0.0269), day 1 had significantly higher CBF. (Refer to table 4-54).

The mixed model with SNP RS949037: genotypes XX (P=0.0293) and YY (P= 0.0729) had significantly lower right hemisphere CBF when compared to heterozygous genotypes. The overall genotype was significant (P for type 3 F tests =0.048). This trend was not significant in the YY dichotomized analysis (P=0.2432). Time of test from injury was found to be marginally significant in the genotypes model (genotypes P=0.0707; dichotomized genotype P=0.1518), day 1 had a trend for higher CBF's. (Refer to table 5-54).

Left Hemisphere CBF

In analyzing the 13 BCL-2 SNP's (RS1026825, RS12968517, RS1381548, RS1481031, RS17756073, RS1801018, RS3810027, RS4456611, RS4941185, RS7236090, RS8083946, RS899968, and RS949037) of interest in relations to the left hemisphere CBF, 8 SNP's had significant findings (RS1026825, RS1381548, RS1481031, RS17756073, RS1801018, RS4941185, RS7236090, and RS899968). Time was consistently statistically significant in relation to left hemisphere CBF among the mixed models analyzed for these SNP's. (Refer to tables 4-48 to 4-54).

The mixed model with SNP RS1026825: genotype YY was significant (P= 0.0134) with overall genotype (P for type 3 F tests =0.0132). YY genotype was associated with a decrease in left hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0296). In contrast, subjects with 0-1 Y alleles had significantly higher CBF. Time of test from injury was found to be significant (genotypes P=0.0345; dichotomized genotype P=0.0515); day 1 had significantly higher CBF. (Refer to table 4-48).

The mixed model with SNP RS12968517: genotype YY was marginally significant (P= 0.0767) with overall genotype (P for type 3 F tests =0.1715). YY genotype was associated with an increase in left hemisphere CBF. This trend was significant in the YY dichotomized analysis (P=0.0768). In contrast, subjects with 0-1 Y alleles had significantly lower CBF. Time of test from injury was found to be significant (genotypes P=0.0643; YY dichotomized genotype P=0.0485); day 1 had significantly higher CBF. (Refer to table 4-49).

The mixed model with SNP RS1381548: genotype YY was significant (P= 0.0591) with overall genotype (P for type 3 F tests =0.0668). YY genotype was associated with a decrease left hemisphere CBF. This trend was significant in the YY dichotomized analysis (P=0.0187). In

contrast, subjects with 0-1 Y alleles had significantly higher CBF. Time of test from injury was found to be significant (genotypes P=0.0722; YY dichotomized genotype P=0.0351); day 2 and day 4 were associated with lower CBF when compared to day 5 CBF. Age was marginally significant (genotypes P=0.062; YY dichotomized genotype P=0.0624) with increasing age associated with decreased left hemisphere CBF. GCS was statistically significant (genotypes P=0.0844; YY dichotomized genotype P=0.0327); subjects with higher GCS on admission scores had higher CBF. Hypothermia was marginally significant (genotypes P=0.1856; YY dichotomized genotype P=0.081) with normothermic subjects having decreased left hemisphere CBF. (Refer to table 4-49).

The mixed model with SNP RS1481031: genotype XX was significant (P= 0.0165) with overall genotype (P for type 3 F tests =0.0417). XX genotype was associated with a decrease in left hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0105). In contrast, subjects with 0-1 X alleles had significantly higher CBF. Time of test from injury was found to be significant (genotypes P=0.0336; dichotomized genotype P=0.0162); day 1 was associated with higher CBF and day 4 was associated with lower CBF. (Refer to table 4-50).

The mixed model with SNP RS17756073: genotype XX (P= 0.143) with overall genotype (P for type 3 F tests =0.022). XX genotype was associated with an increase in left hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0086). In contrast, subjects with 0-1 X alleles had significantly lower CBF. Time of test from injury was found to be significant (genotypes P=0.0305; dichotomized genotype P=0.0232); day 1 was associated with higher CBF. (Refer to table 4-50).

The mixed model with SNP RS1801018: genotypes XX (P= 0.0021) and YY (P=0.0248) were significant with an overall genotype significance (P for type 3 F tests =0.0099). Both

genotypes were associated with decreasing left hemisphere CBF. This trend was significant in the XX dichotomized analysis (P=0.0356). In contrast, subjects with 0-1 X alleles had significantly higher CBF. Time of test from injury was found to be significant (genotypes P=0.0145; dichotomized genotype P=0.0536); days 1-3 had significantly higher left hemisphere CBF than on day 5. Gender was found to be marginally significant in the genotype model (genotypes P=0.0805; dichotomized genotype P=0.5185) with males having a decrease in left hemisphere CBF. (Refer to table 4-51).

The mixed model with SNP RS4456611: genotype YY (P= 0.1487) with overall genotype (P for type 3 F tests= 0.185). YY genotype is associated with a decrease left hemisphere CBF. This trend was marginally significant in the YY dichotomized analysis (P=0.0623). In contrast, subjects with 0-1 Y alleles had significantly higher CBF. Time of test from injury was found not to have any overall statistical significance. (Refer to table 4-52).

The mixed model with SNP RS4941185: genotype YY was significant (P= 0.0534) with overall genotype (P for type 3 F tests =0.0853). YY genotype was associated with an increase in left hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0221). In contrast, subjects with 0-1 Y alleles had significantly higher CBF. Time of test from injury was found to be marginally significant (genotypes P=0.0709; dichotomized genotype P=0.0775); day 1 was associated with a trend towards higher CBF. (Refer to table 4-52).

The mixed model with SNP RS7236090: genotype YY (P= 0.0272) with overall genotype (P for type 3 F tests =0.0224). YY genotype was associated with an increase in left hemisphere CBF while XX (P= 0.0848) had a trend towards lower CBF. In the XX dichotomized analysis (P=0.0549), subjects with 0-1 X alleles had a trend towards significantly

higher CBF. Time of test from injury was not found to have an overall significant trend. (Refer to table 4-53).

The mixed model with SNP RS899968: genotype YY (P= 0.0558) with overall genotype (P for type 3 F tests =0.0906). YY genotype was associated with a decrease in left hemisphere CBF. This trend was significant in the dichotomized analysis (P=0.0264). In contrast, subjects with 0-1 Y alleles had significantly higher CBF. Time of test from injury was not found to have an overall significant trend. (Refer to table 4-54).

The mixed model with SNP RS949037: genotype XX (P= 0.0535) with overall genotype (P for type 3 F tests =0.0898). XX genotype was associated with a decrease in left hemisphere CBF. Subjects with 0-1 Y alleles did not have a significantly higher CBF in the dichotomized analysis (P=0.2937). Time of test from injury was not found to have an overall significant trend. (Refer to table 5-54).

Global CBF

In analyzing the 14 BCL-2 SNP's (RS1026825, RS12454712, RS12968517, RS1381548, RS1481031, RS17756073, RS1801018, RS3810027, RS4456611, RS4941185, RS7236090, RS8083946, RS899968, and RS949037) of interest from the preliminary analyses, in relation to the global CBF, 10 SNP's had significant findings (RS1026825, RS1381548, RS1481031, RS17756073, RS1801018, RS4456611, RS4941185, RS7236090, RS899968, and rs949037). Time was consistently statistically significant and gender was occasionally marginally significant in relation to global CBF among the mixed models analyzed for these SNP's. (Refer to tables 4-48 to 4-54). Figure4-13 depicts the Average Global CBF for each of the statistically significant or statistically tending SNP's.

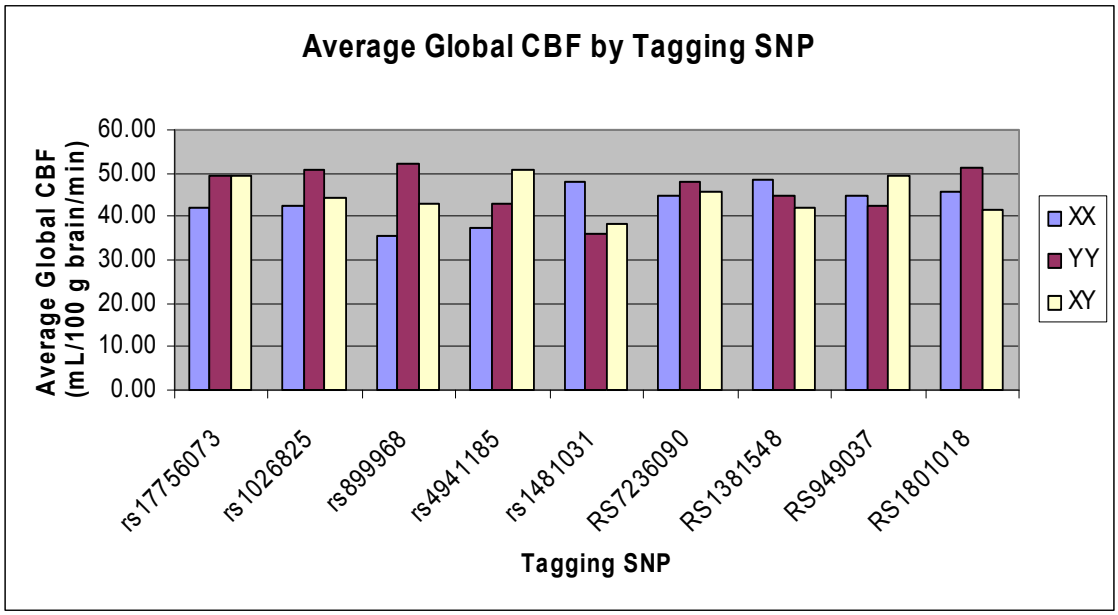


Figure 4-13: Average Global CBF by Tagging SNP

The mixed model with SNP RS1026825: genotypes XX (P=0.0651) and YY (P= 0.0139) were significant with an overall genotype (P for type 3 F tests =0.0156). Both genotypes were associated with a decrease in global CBF. In the YY dichotomized analysis, subjects with 0-1 Y alleles had significantly higher global CBF (P=0.0287). Time of test from injury was found to be significant (genotypes P=0.0273; dichotomized genotype P=0.0403); day 1 was associated with a trend towards higher CBF among all genotypes. (See figure 4-14 and table 4-48).

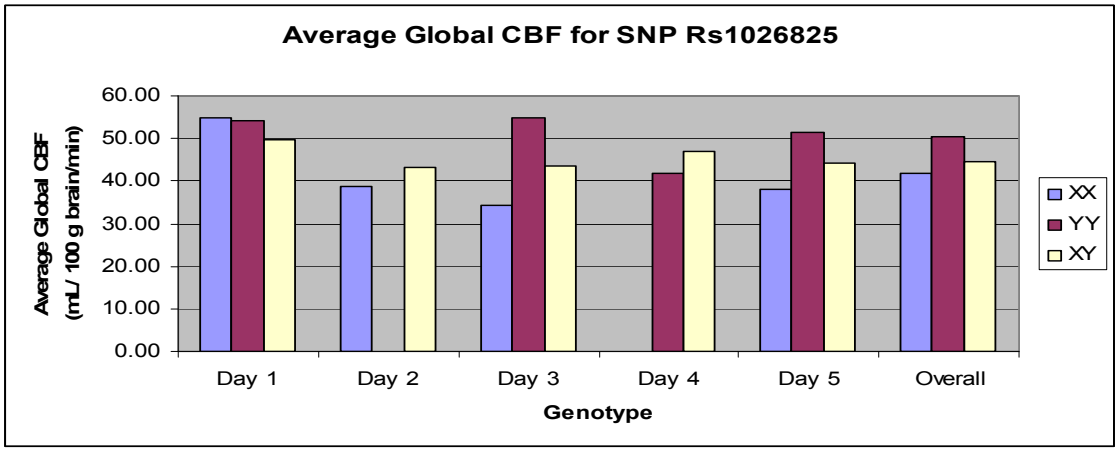


Figure 4-14: Average Global CBF for SNP rs1026825

The mixed model with SNP RS1381548: genotype YY was significant (P= 0.0475) with overall genotype (P for type 3 F tests =0.0604). YY genotype was associated with a decrease global CBF. This trend was significant in the YY dichotomized analysis (P=0.0177). In contrast, subjects with 0-1 Y alleles had significantly higher CBF. Time of test from injury was found to be significant (genotypes P=0.0777; YY dichotomized genotype P=0.0432); with days 2 (YY) and 4 (XY) associated with a trend towards lower CBF. (See figure 4-15 and table 4-49).

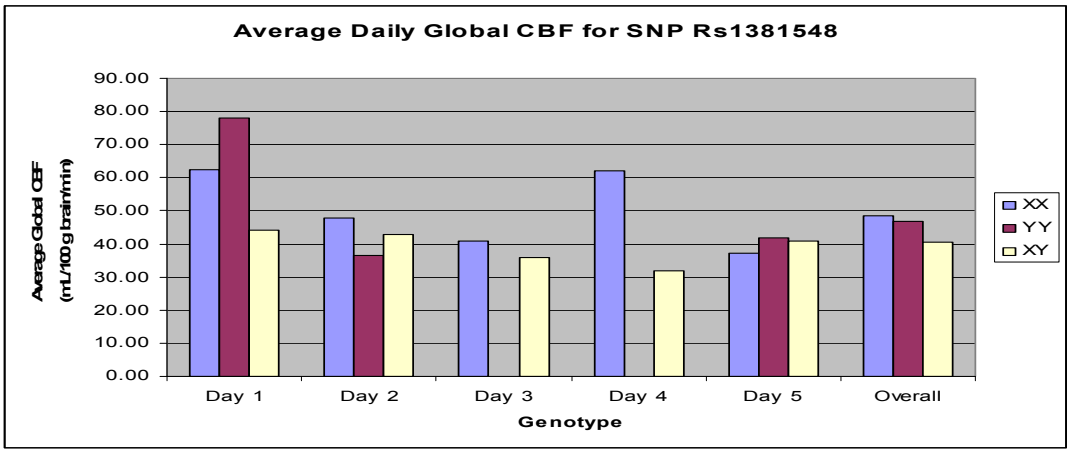


Figure 4-15: Average Daily Global CBF for SNP rs1381548

The mixed model with SNP RS1481031: genotype XX was significant (P= 0.0086) with overall genotype (P for type 3 F tests = 0.0218). XX genotype was associated with a decrease in global CBF. This trend was significant in the dichotomized analysis (P=0.0055). In contrast, subjects with 0-1 X alleles had significantly higher CBF. Time of test from injury was found to be significant (genotypes P=0.0177; dichotomized genotype P=0.0082); day 1 was associated with higher global CBF (genotype XX) and day 4 was associated with lower CBF (genotype XY). (See figure 4-16 and table 4-50).

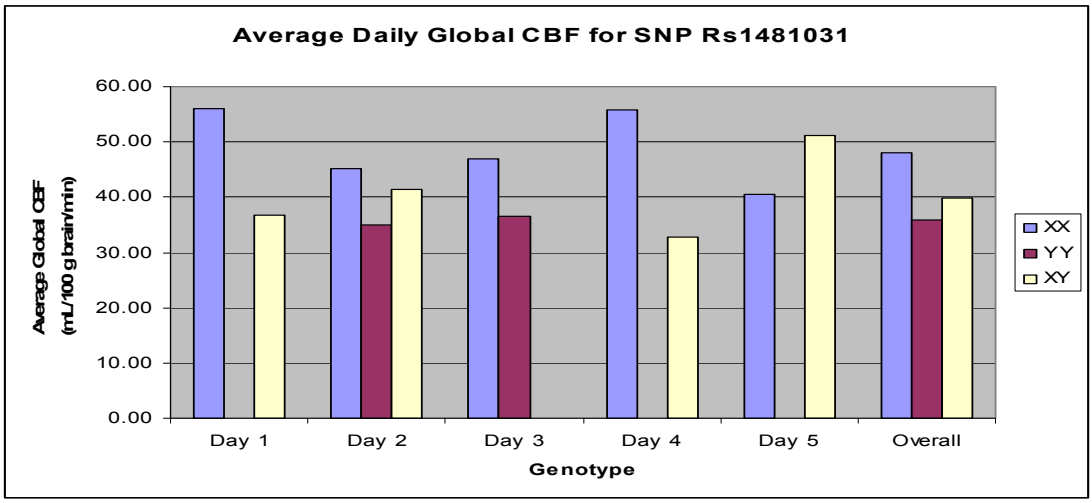


Figure 4-16: Average Daily Global CBF for rs1481031

The mixed model with SNP RS17756073: genotype XX (P= 0.1032) with overall genotype (P for type 3 F tests =0.0221). XX genotype was associated with an increase in global CBF. This trend was significant in the dichotomized analysis (P=0.0075). In contrast, subjects with 0-1 X alleles had significantly lower CBF. Time of test from injury was found to be significant (genotypes P=0.0266; dichotomized genotype P=0.0292); day 1 (YY) was associated with a trend towards higher CBF. (See Figure 4-17 and refer to table 4-50).

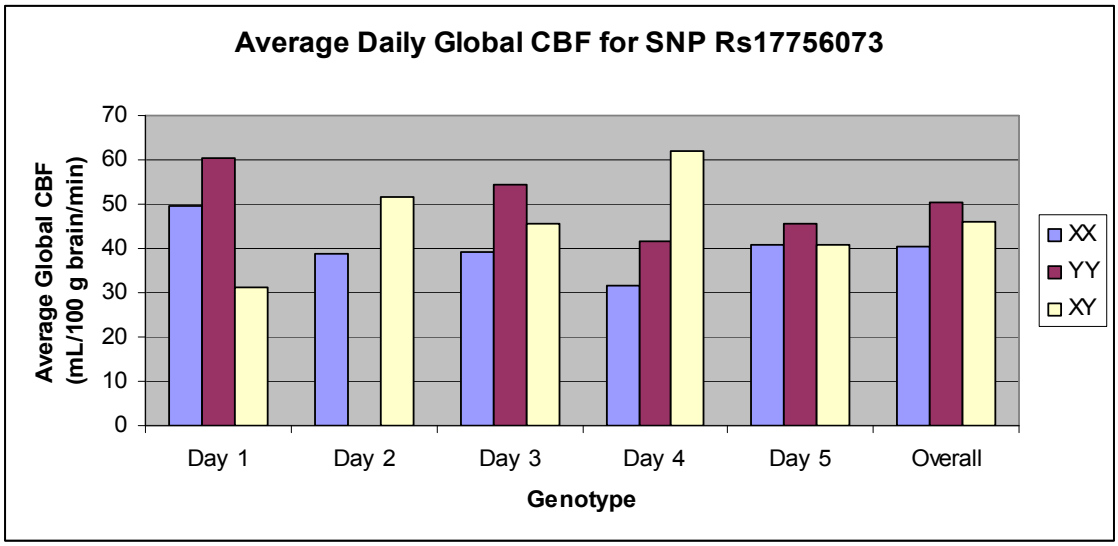


Figure 4-17: Average Daily Global CBF for SNP rs17756073

The mixed model with SNP RS1801018: genotypes XX (P= 0.0003) and YY (P=0.0031) were significant with an overall genotype (P for type 3 F tests =0.003). Both genotypes were associated with a decrease in global CBF. This trend was significant in the dichotomized analysis (P=0.0292). Subjects with 0-1 X alleles had significantly higher CBF. Time of test from injury was found to be significant (genotypes P=0.0035; dichotomized genotype P=0.0365); day 1(XX) and day 3 (XY) had significantly higher global CBF compared to day 5 CBF. (See Figure 4-18 and table 4-51). Gender was found to be significant in the genotype model (genotypes P=0.0166; dichotomized genotype P=0.3546) with males with XX and XY genotypes have a decrease in global CBF. [Note: Male XX n=4; YY n=4; XY n=5 and Females XX n=2 and XY n=2]. (See figure 4-19 and table 4-51).

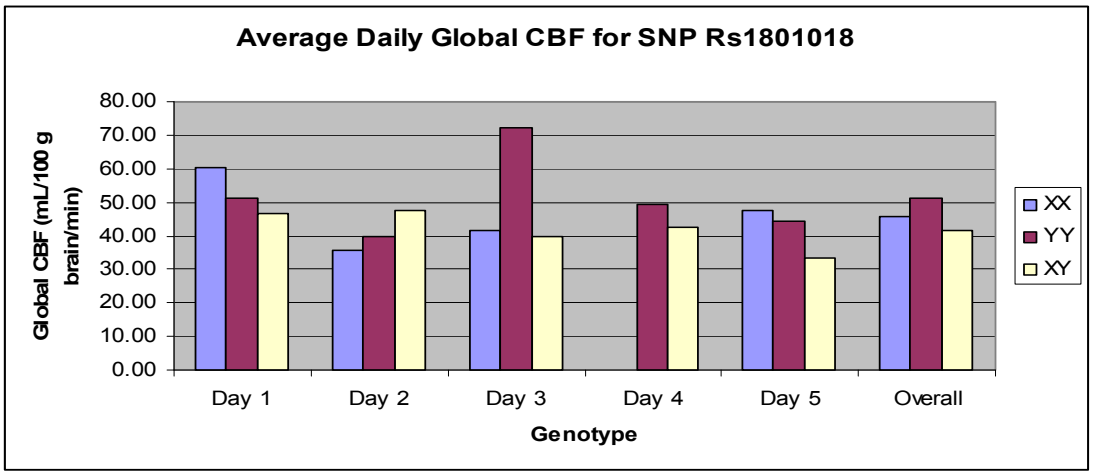


Figure 4-18:Average Daily Global CBF for SNP rs1801018

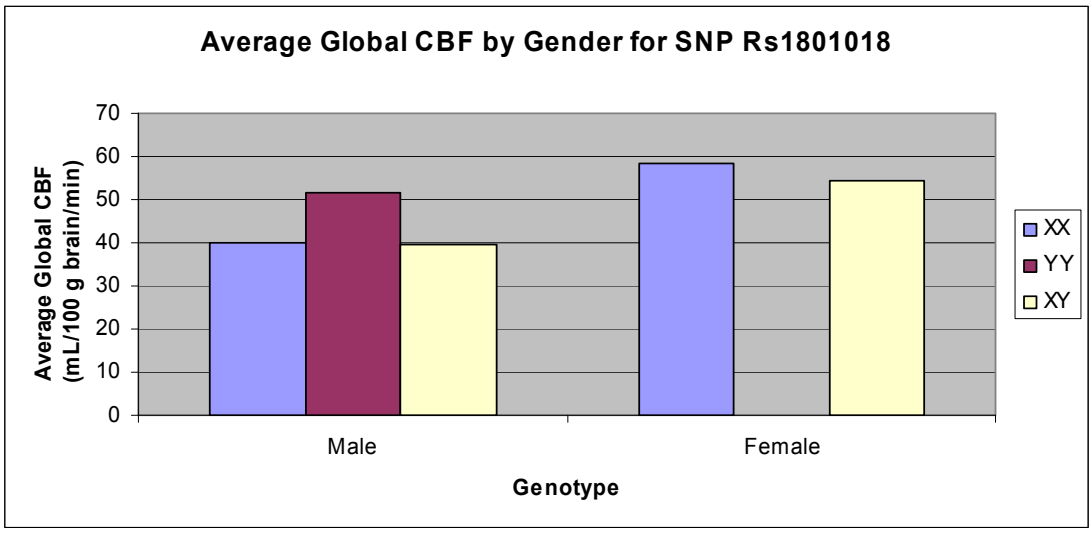


Figure 4-19: Average Global CBF by Gender for SNP rs1801018

The mixed model with SNP RS4456611: genotype YY (P= 0.1238) with overall marginal genotype significance (P for type 3 F tests =0.0983). YY genotype was associated with a decrease global CBF. This trend was significant in the YY dichotomized analysis (P=0.0306). In contrast, subjects with 0-1 Y alleles had significantly higher CBF. Time of test from injury was not found to be significant from day 5. (Refer to figure 4-20 and table 4-52).

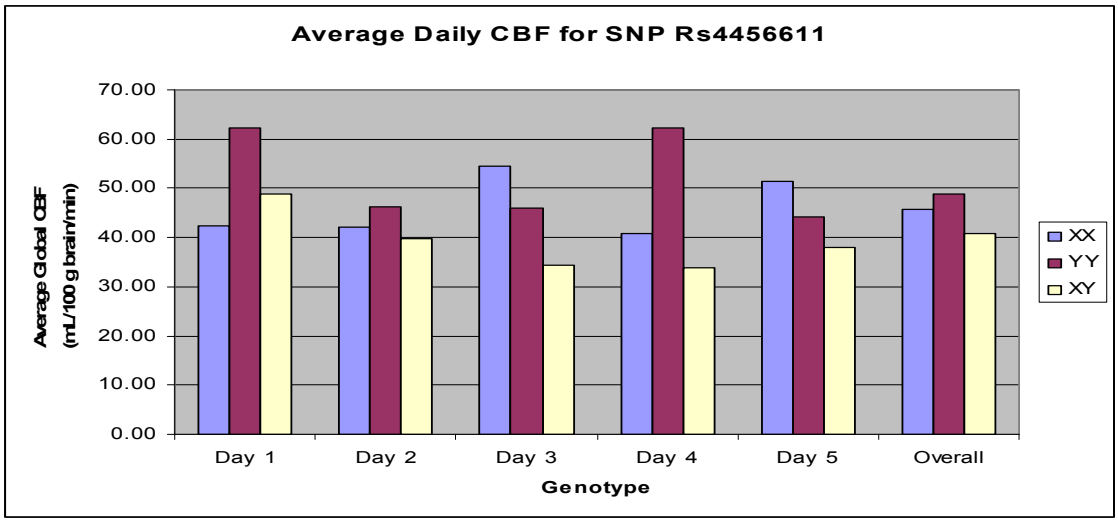


Figure 4-20: Average Daily CBF for SNP rs4456611

The mixed model with SNP RS4941185: genotype YY was significant (P= 0.0249) with overall genotype (P for type 3 F tests =0.0608). YY genotype was associated with an increase in global CBF. This trend was significant in the dichotomized analysis (P=0.0143). In contrast, subjects with 0-1 Y alleles had significantly lower CBF. Time of test from injury was found to be significant (genotypes P=0.0609; dichotomized genotype P=0.0483); day 1 (XY) had higher CBF's. (See figure 4-21 and table 4-52). Gender was found to be marginally significant (genotypes P=0.0763; dichotomized genotype P=0.0607) with males with XY genotype having decrease in global CBF. (See figure 4-22 and table 4-52).

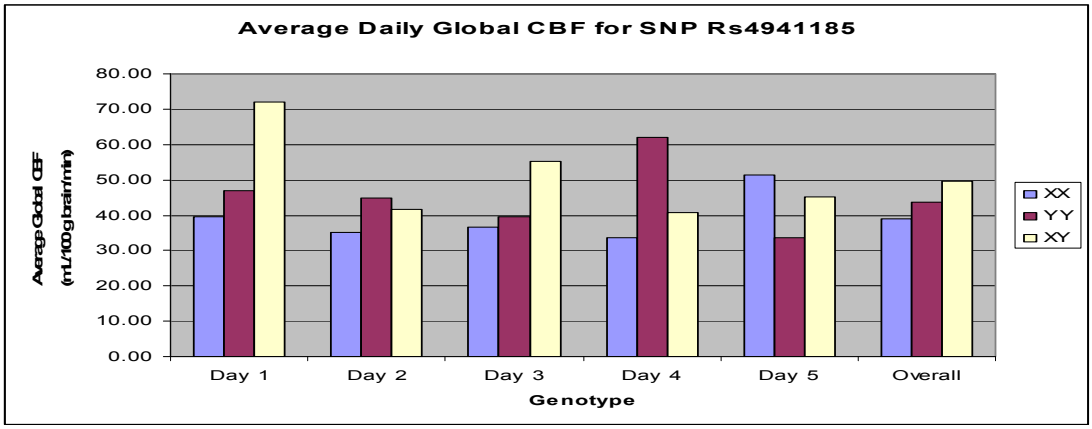


Figure 4-21: Average Daily Global CBF for SNP rs4941185

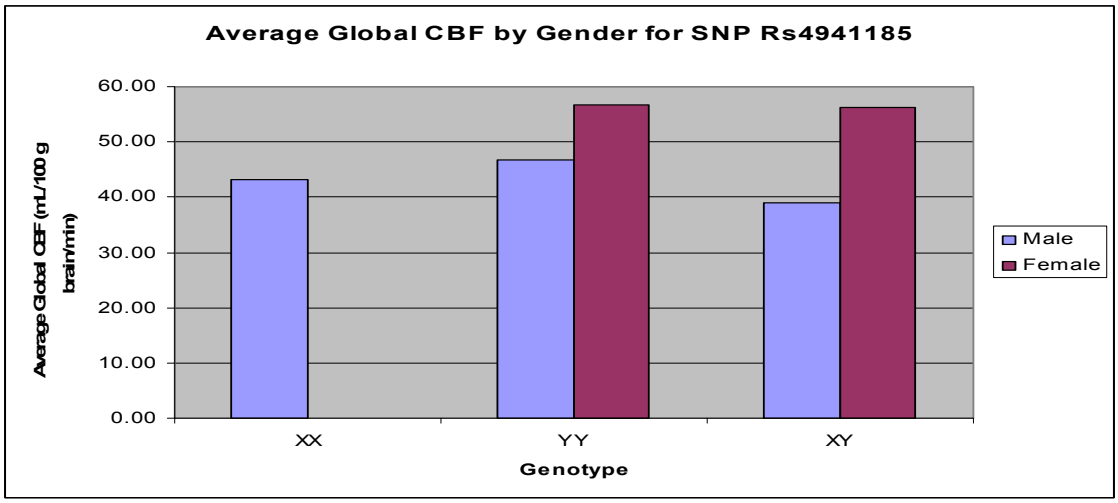


Figure 4-22: Average Global CBF by Gender for SNP rs4941185

The mixed model with SNP RS7236090: genotype YY (P= 0.0261) with overall genotype (P for type 3 F tests =0.0255). YY genotype was associated with an increase in global CBF. This trend was marginally significant in the XX dichotomized analysis (P=0.0797). Subjects with 0-1 X alleles had marginally significant higher CBF. Time of test from injury was found to be marginally significant in the genotypes model (genotypes P=0.08; dichotomized genotype P=0.1508); day 4 trending towards lower CBF. (Refer to table 4-53).

The mixed model with SNP RS899968: genotype YY (P= 0.0216) with overall genotype (P for type 3 F tests =0.0273). YY genotype was associated with a decrease in global CBF. This trend was significant in the dichotomized analysis (P=0.0068). In contrast, subjects with 0-1 Y alleles had significantly higher CBF. Time of test from injury was found to be marginally significant (genotypes P=0.0722; dichotomized genotype P=0.0617); day 1 trending toward higher global CBF for all genotypes. (See figure 4-23 and table 4-54).

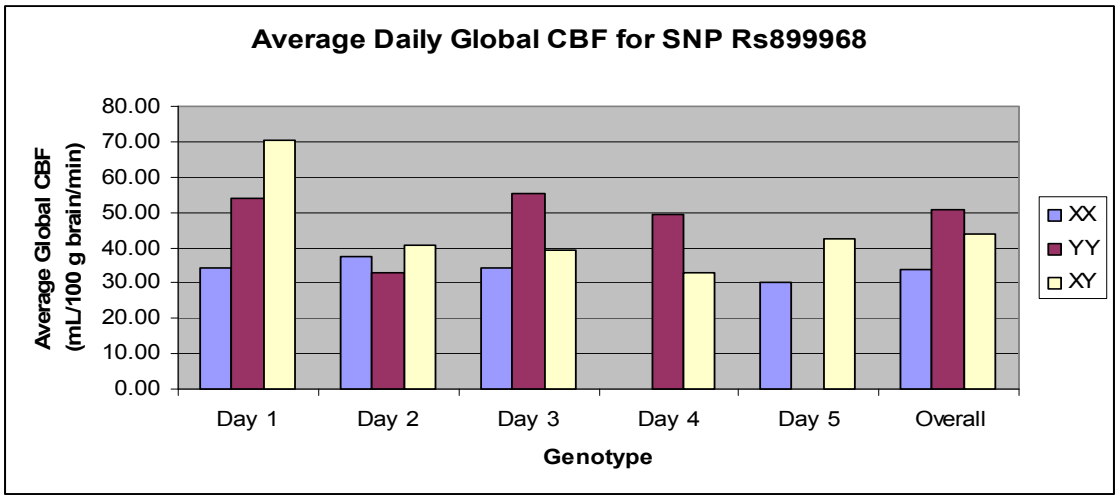


Figure 4-23: Average Daily Global CBF for SNP rs899968

The mixed model with SNP RS949037: genotypes XX (P= 0.0432) and YY (P= 0.078) were trending towards significance with overall genotype (P for type 3 F tests =0.068). Both genotypes were associated with a decrease in global CBF. The YY dichotomized analysis

(P=0.2528). Time of test from injury was found not to be significant for P for type 3 F tests. (Refer to table 4-54).

Table 4-21: BCL-2 Genotypes vs Cerebral Blood Flow Descriptive Data

BCL-2 Genotypes Versus Cerebral Blood Flow (CBF) Descriptive Data

Variable	n=	Mean	Std. Deviation	Range	
Age	17	31.94	17.594	16-65	
	n=		Frequency (%)	Frequency (%) of Unkown	
GCS	17	score 3 to 5	9(52.9%)	0	
		score 6 to 8	8(47.1%)		
Gender (male)	17		13(76.5%)	0	
Race (Caucasian)	17		all caucasian	0	
Hypothermic	17		7(41.2%)	0	
Hypoxia	8		4(23.53%)	9 (52.94%)	
Hypotensive	8		4(23.53%)	9 (52.94%)	
Seizures	8		0	9 (52.94%)	
APOEε4	17		4(23.5%)	0	
Cerebral Blood Flow (CBF) (mL/100 g brain/min)	**n=**	**Mean**	**Std. Deviation**	**Range**	**Frequency (%) of Unkown**
Right Hemisphere CBF					
Day 1	6	52.9967	18.13562	32.45-77.25	11(64.71%)
Day 2	8	40.3525	13.16545	26.55-63.78	9 (52.94%)
Day 3	7	45.0886	14.79306	33.68-73.67	10 (58.82%)
Day 4	4	43.9275	15.213	30.98-63.3	13 (76.47%)
Day 5	6	41.5567	8.01225	30.6-50.68	11(64.71%)
Left Hemisphere CBF					
Day 1	6	52.6767	19.08808	30.45-79.10	11(64.71%)
Day 2	8	45.2788	10.43846	34.98-64.43	9 (52.94%)
Day 3	7	45.81	13.60251	34.80-70.53	10 (58.82%)
Day 4	4	44.75	13.4268	32.7-60.9	13 (76.47%)
Day 5	6	42.9317	8.48712	29.83-51.93	11(64.71%)
Global CBF					
Day 1	6	52.7967	18.59752	31.33-78.15	11(64.71%)
Day 2	8	42.0838	11.5842	31.80-63.93	9 (52.94%)
Day 3	7	45.3886	14.24907	34.35-72.33	10 (58.82%)
Day 4	4	44.315	13.60251	31.8-62.1	13 (76.47%)
Day 5	6	42.28	8.33709	30.13-51.43	11(64.71%)

Hardy-Weinberg Equilibrium

SNP	n=	XX	YY	XY	X	Y	HWE
RS1026825	17	5	4	8	0.529411765	0.470588	1.0000
RS12454712	17	1	7	9	0.323529412	0.676471	1.0000
RS12968517	17	3	6	8	0.411764706	0.588235	1.0000
RS1381548	13	3	3	7	0.5	0.5	1.0000
RS1481031	17	10	1	6	0.764705882	0.235294	1.0000
RS17756073	17	9	4	4	0.647058824	0.352941	1.0000
RS17759659	16	4	3	9	0.53125	0.46875	1.0000
RS1801018	17	6	4	7	0.558823529	0.441176	1.0000
RS1944419	14	8	1	5	0.75	0.25	1.0000
RS3810027	16	5	6	5	0.46875	0.53125	1.0000
RS4456611	15	5	5	5	0.5	0.5	1.0000
RS4941185	17	4	5	8	0.470588235	0.529412	1.0000
RS7230970	15	2	6	7	0.366666667	0.633333	1.0000
RS7236090	15	8	1	6	0.733333333	0.266667	1.0000
RS8083946	17	5	4	8	0.529411765	0.470588	1.0000
RS899968	15	3	5	7	0.433333333	0.566667	1.0000
RS949037	17	3	8	6	0.352941176	0.647059	1.0000

Table 4-22: Preliminary Mixed Models Analyses for Cerebral Blood Flow

Preliminary Mixed Models Analysis of BCL-2 vs Cerebral Blood Flow (CBF)											
			Right Hemipshere			Left Hemisphere			Global		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS1026825	17	xx	-9.0706	0.0685*	0.014*	-8.6857	0.0772*	0.0193*	-8.868	0.0749*	0.0183*
		yy	-12.819	0.0108*		-12.0476	0.0141*		-12.27	0.0138*	
		xy									
		yy & xy xx	5.12	0.3736	0.3736	5.3601	0.327	0.327	5.076	0.3657	0.3657
		xx &xy yy	11.3571	0.0307*	0.0307*	10.6461	0.0362*	0.0362*	10.752	0.0381*	0.0381*
RS12454712	17	xx	5.4746	0.5291	0.3432	5.4593	0.517	0.428	6.1656	0.4742	0.4041
		yy	-5.6423	0.3651		-4.3607	0.4577		-4.332	0.4717	
		xy									
		xx &xy yy	8.3455	0.132*	0.132*	6.7399	0.2016	0.2016	7.1724	0.185*	0.185*
RS12968517	17	xx	4.8373	0.543	0.3953	4.7076	0.5176	0.1629*	4.8599	0.5256	0.3197
		yy	9.4376	0.1865*		12.5031	0.0603*		10.16	0.1407*	
		xy									
		xx &xy yy	-8.3711	0.2185	0.2185	-11.4696	0.0703*	0.0703*	-9.084	0.1666*	0.1666*
RS1381548	14	xx	4.9697	0.5776	0.1529*	6.9199	0.4192	0.1992*	5.9394	0.4943	0.1614*
		yy	-9.0538	0.1604*		-6.7807	0.2749		-8.185	0.1941*	
		xy									
		yy & xy xx	-11.742	0.1484*	0.1484*	-11.7325	0.1249*	0.1249*	-11.86	0.1309*	0.1309*
		xx &xy yy	10.9311	0.0538*	0.0538*	9.6739	0.0792*	0.0792*	10.537	0.0587*	0.0587*
RS1481031	17	xx	-14.323	0.0067*	0.017*	-12.6014	0.0151*	0.0378*	-13.78	0.0083*	0.0214*
		yy	5.0043	0.5565		3.7281	0.6697		4.3585	0.6104	
		xy									
		yy & xy xx	14.6698	0.004*	0.004*	13.0316	0.0089*	0.0089*	14.152	0.0049*	0.0049*
* p≤ 0.2											

Table 4-22 continued

Preliminary Mixed Models Analysis of BCL-2 vs Cerebral Blood Flow (CBF)											
			Right Hemipshere			Left Hemisphere			Global		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS17756073	17	xx	7.3972	0.2821	0.0436*	6.3622	0.3236	0.0354*	7.9418	0.2312	0.0333*
		yy	-5.3304	0.3923		-6.2189	0.2961		-5.04	0.403	
		xy									
		yy & xy	-11.727	0.0172*	0.0172*	-11.3905	0.0153*	0.0153*	-12.02	0.012*	0.012*
		xx									
RS17759659	16	xx	-8.0749	0.2947	0.4832	-5.6266	0.4362	0.5314	-6.397	0.391	0.556
		yy	-6.7559	0.3709		-7.294	0.3111		-6.619	0.3699	
		xy									
		yy & xy	5.5755	0.4351	0.4351	2.9304	0.6626	0.6626	3.9498	0.5674	0.5674
		xx									
RS1801018	17	xx	-19.525	0.0044*	0.016*	-16.7009	0.0116*	0.0384*	-18.6	0.0055*	0.0198*
		yy	-13.985	0.0508*		-9.8965	0.1504*		-11.8	0.0887*	
		xy									
		yy & xy	11.819	0.0332*	0.0332*	11.1768	0.0325*	0.0325*	12.024	0.0244*	0.0244*
		xx									
RS1944419	15	xx	5.6973	0.4292	0.5087	5.284	0.4407	0.6575	4.8928	0.4821	0.5966
		yy	10.0959	0.2687		6.6799	0.4406		8.4794	0.3375	
		xy									
		yy & xy	-1.8862	0.7655	0.7655	-2.8975	0.6256	0.6256	-1.788	0.7691	0.7691
		xx									
RS3810027	17	xx	21.5939	0.0176*	0.0542*	16.7122	0.0499*	0.1197*	19.609	0.025*	0.0715*
		yy	8.4871	0.1813*		4.7022	0.4374		7.1364	0.2461	
		xy									
		xx &xy	1.0642	0.8445	0.8445	2.7435	0.5906	0.5906	1.5644	0.7653	0.7653
		yy									
RS4456611	15	xx	3.2847	0.5947	0.1067*	4.272	0.4779	0.1833*	3.5598	0.5535	0.1079*
		yy	-7.5215	0.1942*		-5.2781	0.3501		-7.16	0.2064	
		xy									
		xx	-8.2888	0.1003*	0.1003*	-7.4861	0.1233*	0.1233*	-8.102	0.1008*	0.1008*
		yy & xy									
		xx &xy	9.3945	0.0428*	0.0428*	7.7593	0.0879*	0.0879*	9.1978	0.044*	0.044*
		yy									
* p≤ 0.2											

Table 4-22 continued

Preliminary Mixed Models Analysis of BCL-2 vs Cerebral Blood Flow (CBF)											
			Right Hemipshere			Left Hemisphere			Global		
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test
RS4941185	17	xx	1.5958	0.8111	0.161*	0.2561	0.9682	0.2766	1.1299	0.8624	0.204
		yy	16.3856	0.0587*		12.9805	0.1179*		14.891	0.0788*	
		xy									
		xx &xy	-15.943	0.0552*	0.0552*	-12.9401	0.1039*	0.1039*	-14.57	0.0727*	0.0727*
		yy									
RS7230970	15	xx	-3.181	0.7092	0.6307	2.7692	0.7346	0.6401	-1.751	0.8332	0.6167
		yy	4.8305	0.5209		6.3298	0.356		5.6081	0.4339	
		xy									
		xx &xy	-6.0003	0.3724	0.3724	-5.6019	0.3669	0.3669	-6.213	0.3333	0.3333
		yy									
RS7236090	17	xx	-4.6234	0.4529	0.1314*	-5.4305	0.3642	0.1457*	-4.935	0.4144	0.1384*
		yy	23.3969	0.1004*		20.4592	0.1347*		21.907	0.1143*	
		xy									
		yy & xy	7.7755	0.2243	0.2243	8.3728	0.173*	0.173*	7.997	0.2008	0.2008
		xx									
RS8083946	17	xx	-6.5011	0.3373	0.0444*	-6.2256	0.3193	0.0609*	-5.69	0.3788	0.0545*
		yy	10.3421	0.0722*		9.3136	0.1003*		10.178	0.0774*	
		xy									
		yy & xy	10.5603	0.1117*	0.1117*	9.8882	0.1049*	0.1049*	9.6382	0.1278*	0.1278*
		xx									
RS899968	16	xx	10.1619	0.2778	0.0099*	10.135	0.293	0.0568*	10.081	0.2837	0.0198*
		yy	-13.58	0.0147*		-9.9491	0.0831*		-12.32	0.0294*	
		xy									
		xx &xy	15.5102	0.004*	0.004*	12.1311	0.0273*	0.0273*	14.371	0.0084*	0.0084*
		yy									
* p≤ 0.2											

Table 4-22 continued

Preliminary Mixed Models Analysis of BCL-2 vs Cerebral Blood Flow (CBF)												
			Right Hemipshere			Left Hemisphere			Global			
SNP	n=	Genotype	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	coefff	P	P for Type-3 Test	
RS949037	16	xx	-15.491	0.0178*	0.0406*	-12.617	0.0473*	0.1161*	-13.85	0.0324*	0.0737*	
		yy	-7.9366	0.1263*		-5.8576	0.2539		-7.228	0.1655*		
		xy										
		xx &xy yy	4.492	0.4167	0.4167	2.6616	0.6107	0.6107	3.8902	0.4698	0.4698	
Covariates												
Time (Days)	17	1	12.8884	0.0578*	0.0788*	11.8996	0.0665*	0.1381*	12.419	0.0625*	0.1092*	
		2	0.9564	0.8783		3.8836	0.5203		1.9738	0.7483		
		3	5.5708	0.3984		4.7525	0.4531		5.3005	0.4142		
		4	-11.84	0.1975*		-8.9343	0.3048		-10.13	0.2571		
		5	.	.		.			.			
Age	17		-0.1767	0.3142	0.3142	-0.2326	0.1435*	0.1435*	-0.191	0.2507	0.2507	
GCS	17		-5.2122	0.3733	0.3733	-6.8174	0.1981*	0.1981*	-5.69	0.3032	0.3032	
Gender	17		-13.718	0.0406*	0.0406*	-12.9261	0.0352*	0.0352*	-13.54	0.0327*	0.0327*	
Race	17				NA			NA			NA	
Hypothermic	17		-1.6147	0.7908	0.7908	0.6692	0.9048	0.9048	-0.751	0.8968	0.8968	
Hypoxia	8		-2.7392	0.7794	0.7794	-7.0666	0.3688	0.3688	-3.967	0.6478	0.6478	
Hypotensive	8		-9.6525	0.2964	0.2964	-1.2524	0.8752	0.8752	-6.887	0.4104	0.4104	
Seizures	8				NA			NA			NA	
APOEε4	17		-8.7823	0.209	0.209	-7.2417	0.2588	0.2588	-8.322	0.2092	0.2092	
* p≤ 0.2												

Table 4-23: Primary Mixed Models of BCL-2 SNP and Cerebral Blood Flow

Primary Mixed Models Analyses of BCL-2 SNP and Cerebral Bloof Flow (CBF)																							
		Right Hemiphere							Left Hemiphere							Global							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS1026825 (n=17)																							
Genotype	xx	-9.7671	0.0648	0.0135*	xx & xy	12.76	0.024*	0.024*	xx	-10.148	0.0498*	0.0132*	xx & xy	11.811	0.0296*	0.0296*	xx	-9.743	0.0651	0.0156*	xx & xy	12.174	0.0287*	0.0287*
	yy	-13.486	0.0117*		yy				yy	-12.652	0.0134*		yy				yy	-13	0.0139*		yy			
	xy								xy								xy							
Time (Day)	1	12.955	0.0191*	0.0171*	1	14.951	0.0173*	0.0254*	1	12.419	0.0224*	0.0345*	1	14.056	0.0221*	0.0515	1	12.795	0.0217*	0.0273*	1	14.57	0.0203*	0.0403*
	2	2.2378	0.652		2	3.0604	0.5952		2	4.7601	0.3524		2	5.9903	0.3032		2	3.0559	0.5487		2	3.9748	0.495	
	3	5.0411	0.3182		3	6.3947	0.2792		3	4.7662	0.3525		3	6.065	0.3033		3	4.9482	0.3378		3	6.2994	0.2906	
	4	-6.1659	0.0444		4	-12.665	0.1415		4	-11.041	0.1447		4	-8.444	0.3069		4	-13.45	0.0872		4	-10.16	0.2304	
	5				5				5				5				5				5			
Age		-0.1293	0.6617	0.6617		-0.0005	0.9985	0.9985		-0.172	0.4966	0.4966		-0.0408	0.8641	0.8641		-0.127	0.6436	0.6436		0.0005	0.9985	0.9985
GCS		-1.2192	0.8751	0.8751		-1.5005	0.8367	0.8367		-3.3965	0.6062	0.6062		-3.8292	0.5529	0.5529		-1.853	0.7968	0.7968		-2.238	0.7424	0.7424
Gender		-11.402	0.2847	0.2847		-14.009	0.1693	0.1693		-8.6941	0.3347	0.3347		-10.923	0.2205	0.2205		-11.07	0.2654	0.2654		-13.47	0.159	0.159
Hypothermia		-1.2272	0.8814	0.8814		-1.3956	0.8547	0.8547		0.1493	0.9827	0.9827		-0.0329	0.996	0.996		-0.474	0.9502	0.9502		-0.657	0.926	0.926
RS12454712 (n=17)																							
Genotype	xx	1.409	0.8775	0.2228	xx & xy	10.999	0.0706	0.0706	xx	1.4661	0.8704	0.3701	xx & xy	8.4772	0.1442	0.1442	xx	2.1719	0.8119	0.3022	xx & xy	9.5863	0.1074	0.1074
	yy	-10.124	0.1581		yy				yy	-7.6914	0.2572		yy				yy	-8.409	0.2269		yy			
	xy								xy								xy							
Time (Day)	1	16.403	0.0304*	0.0402*	1	16.988	0.013*	0.0204*	1	14.733	0.0448*	0.0992	1	15.331	0.021*	0.0526	1	15.3	0.0414*	0.0717	1	16.168	0.0174*	0.0358*
	2	2.4563	0.7255		2	3.1125	0.6057		2	4.7723	0.4889		2	5.3142	0.3821		2	2.7909	0.6891		2	3.6683	0.547	
	3	9.4849	0.2236		3	10.011	0.1444		3	0.7991	0.295		3	8.509	0.2087		3	8.5084	0.273		3	9.2851	0.1764	
	4	-11.466	0.2371		4	-11.517	0.2039		4	-8.3809	0.3723		4	-8.2737	0.3497		4	-9.688	0.3121		4	-9.59	0.2864	
	5				5				5				5				5				5			
Age		-0.1299	0.6558	0.6558		-0.1344	0.6432	0.6432		-0.1654	0.5351	0.5351		-0.17	0.5197	0.5197		-0.123	0.6539	0.6539		-0.13	0.6363	0.6363
GCS		0.5784	0.9427	0.9427		0.388	0.9607	0.9607		-1.6274	0.8256	0.8256		-1.8212	0.7994	0.7994		0.001	0.9999	0.9999		-0.297	0.9683	0.9683
Gender		-11.335	0.2965	0.2965		-11.307	0.263	0.293		-8.1487	0.4073	0.4073		-8.1654	0.4002	0.4002		-10.77	0.2954	0.2954		-10.77	0.2917	0.2917
Hypothermia		-5.6186	0.5277	0.5277		-6.0105	0.4889	0.4889		-3.2438	0.6871	0.6871		-3.5933	0.6455	0.6455		-4.145	0.6202	0.6202		-4.678	0.5677	0.5677
* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001																								

Table 4-23 continued

Primary Mixed Models Analyses of BCL-2 SNP and Cerebral Bloof Flow (CBF)																							
		Right Hemiphere								Left Hemiphere								Global					
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS12968517 (n=14)																							
Genotype	xx	6.7391	0.4618	0.3288	xx & xy	-10.667	0.1825	0.1825	xx	6.0235	0.4719	0.1715	xx & xy	-13.15	0.0768	0.0768	xx	6.6407	0.451	0.2817	xx & xy	-11.11	0.1499	0.1499
	yy	10.857	0.1807		yy				yy	13.359	0.0767		yy				yy	11.279	0.149		yy			
	xy								xy								xy							
Time (Day)	1	15.409	0.0397*	0.0609	1	15.97	0.0286*	0.0495*	1	15.262	0.0252*	0.0643	1	15.787	0.0176*	0.0485*	1	15.104	0.0383*	0.0747	1	15.717	0.0268*	0.59
	2	0.9283	0.8852		2	1.2703	0.8398		2	4.4137	0.458		2	4.6813	0.4224		2	2.0649	0.7422		2	2.3977	0.6964	
	3	8.6854	0.228		3	7.6749	0.2654		3	8.6034	0.1916		3	7.7247	0.2219		3	8.6727	0.2167		3	7.6739	0.2539	
	4	-9.9912	0.3023		4	-10.992	0.2487		4	-6.9282	0.4306		4	-7.6508	0.376		4	-7.983	0.3924		4	-8.946	0.33	
	5				5				5				5				5				5			
Age		0.1322	0.6626	0.6626		-0.0683	0.8131	0.8131		-0.1447	0.598	0.598		-0.0872	0.7375	0.7375		-0.122	0.6709	0.6709		-0.059	0.8287	0.8287
GCS		1.2479	0.8804	0.8804		-0.8903	0.9092	0.9092		-1.4043	0.8517	0.8517		-3.3147	0.6388	0.6388		0.4959	0.9495	0.9495		-1.604	0.8279	0.8279
Gender		-10.841	0.3069	0.3069		-10.433	0.3236	0.3236		-8.1613	0.3885	0.3885		-7.8495	0.4034	0.4034		-10.59	0.2918	0.2918		-10.2	0.3079	0.3079
Hypothermia		-4.7061	0.5911	0.5911		-5.8422	0.503	0.503		-4.1892	0.5961	0.5961		-5.1946	0.506	0.506		-4.059	0.623	0.623		-5.194	0.527	0.527
RS1381548 (n=14)																							
Genotype	xx	-8.5362	0.5896	0.0681	yy & xy	-16.633	0.2271	0.2271	xx	-4.5079	0.761	0.0668	yy & xy	-17.822	0.1631	0.1631	xx	-6.688	0.6597	0.0604	yy & xy	-17.5	0.1887	0.1887
	yy	-18.865	0.0477*		xx				yy	-16.625	0.0591		xx				yy	-18.1	0.0475*		xx			
	xy								xy								xy							
Time (Day)	1	7.6135	0.4545	0.0869	1	21.266	0.056	0.1094	1	7.4631	0.4559	0.0722	1	22.166	0.0476*	0.1372	1	7.3014	0.4637	0.0777	1	21.477	0.0502	0.1154
	2	-20.224	0.2092		2	2.8551	0.8221		2	-20.628	0.1917		2	2.503	0.8371		2	-20.88	0.1877		2	2.4185	0.8454	
	3	-9.0809	0.4021		3	4.9787	0.6272		3	-11.135	0.2982		3	4.5717	0.6323		3	-10.28	0.3359		3	4.5209	0.6488	
	4	-23.102	0.0814		4	-8.2747	0.4973		4	-21.748	0.0853		4	-2.871	0.79		4	-22.56	0.0791		4	-5.954	0.6058	
	5				5				5				5				5				5			
Age		-0.4433	0.1103	0.1103		-0.1813	0.4929	0.4929		-0.4662	0.062	0.062		-0.184	0.4709	0.4709		-0.453	0.0835	0.0835		-0.178	0.4576	0.4576
GCS		16.506	0.1258	0.1258		0.8955	0.9165	0.9165		17.85	0.0844	0.0844		2.3065	0.7523	0.7523		17.392	0.0971	0.0971		1.7238	0.8276	0.8276
Gender		-4.418	0.6009	0.6009		-9.271	0.3158	0.3158		-6.7771	0.3643	0.3643		-12.385	0.2227	0.2227		-5.687	0.4712	0.4712		-10.91	0.2086	0.2086
Hypothermia		-16.308	0.1517	0.1517		-1.5605	0.8744	0.8744		-13.733	0.1856	0.1856		-1.0115	0.906	0.906		-14.96	0.1656	0.1656		-0.909	0.9215	0.9215
Genotype					xx & xy	15.738	0.0216*	0.0216*					xx & xy	14.951	0.0187*	0.0187*					xx & xy	15.633	0.0177*	0.0177*
					yy								yy								yy			
Time (Day)					1	10.057	0.2556	0.0556					1	8.8326	0.2936	0.0351					1	9.2786	0.2768	0.0432
					2	-14.511	0.1458						2	-17.316	0.0646						2	-16.22	0.0921	
					3	-6.0541	0.4762						3	-9.3607	0.2418						3	-7.779	0.3421	
					4	-20.299	0.0652						4	-20.187	0.0489*						4	-20.28	0.0547	
					5								5								5			
Age						-0.4202	0.1167	0.1167						-0.4508	0.0624	0.0624						-0.433	0.0865	0.0865
GCS						12.899	0.1045	0.1045						15.79	0.0327*	0.0327*						14.467	0.0571	0.0571
Gender						-5.555	0.4894	0.4894						-7.2801	0.3035	0.3035						-6.541	0.3827	0.3827
Hypothermia						-11.444	0.1067	0.1067						-11.103	0.081*	0.081*						-11.12	0.0938	0.0938

* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001

Table 4-23 continued

Primary Mixed Models Analyses of BCL-2 SNP and Cerebral Bloof Flow (CBF)																							
	Right Hemiphere								Left Hemiphere								Global							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS1481031 (n=17)																							
Genotype	xx	-15.062	0.0071*	0.0174*	yy & xy	15.415	0.0046**	0.0046**	xx	-13.248	0.0165*	0.0417*	yy & xy	13.625	0.0105*	0.0105*	xx	-14.56	0.0086**	0.0218*	yy & xy	14.914	0.0055*	0.0055*
	yy	6.1165	0.4833		xx				yy	4.2996	0.6325		xx				yy	5.3558	0.5416		xx			
	xy								xy								xy							
Time (Day)	1	12.149	0.0287*	0.0108*	1	13.519	0.0105*	0.0051**	1	12.052	0.0361*	0.0336*	1	13.004	0.0151*	0.0162*	1	12.126	0.0304*	0.0177*	1	13.315	0.0117*	0.0082*
	2	-0.3726	0.9396		2	0.7217	0.8775		2	2.6223	0.6105		2	3.3546	0.488		2	0.7762	0.8758		2	1.7202	0.7145	
	3	6.0339	0.2783		3	7.6806	0.1378		3	5.6428	0.3283		3	6.7631	0.1958		3	5.9645	0.2886		3	7.3872	0.1529	
	4	-19.085	0.0222*		4	-17.749	0.0262*		4	-14.617	0.0739		4	-13.871	0.0753		4	-16.84	0.0394*		4	-15.77	0.0437*	
	5				5				5				5				5				5			
Age		-0.0017	0.9957	0.9957		-0.0014	0.9963	0.9963		-0.0568	0.8506	0.8506		-0.0568	0.8478	0.8478		-0.003	0.9928	0.9928		-0.003	0.9926	0.9926
GCS		1.5737	0.8528	0.8528		0.8762	0.9152	0.9152		-0.722	0.9293	0.9293		-1.1921	0.8805	0.8805		0.8673	0.9159	0.9159		0.2628	0.9737	0.9737
Gender		-12.638	0.2696	0.2696		-12.279	0.2711	0.2711		-9.373	0.3892	0.3892		-9.1004	0.3934	0.3934		-12.11	0.2751	0.2751		-11.78	0.2771	0.2771
Hypothermia		-1.6677	0.8531	0.8531		-1.4639	0.8675	0.8675		-0.1148	0.9894	0.9894		0.0349	0.9967	0.9967		-0.823	0.9247	0.9247		-0.644	0.9397	0.9397
RS17756073 (n=17)																							
Genotype	xx	11.564	0.1421	0.0322*	yy & xy	-15.51	0.0116*	0.0116*	xx	10.69	0.143	0.022*	yy & xy	-15.234	0.0086**	0.0086**	xx	12.306	0.1032	0.0221*	yy & xy	-15.99	0.0075**	0.0075**
	yy	-4.7446	0.4649		xx				yy	-5.6842	0.3484		xx				yy	-4.558	0.4621		xx			
	xy								xy								xy							
Time (Day)	1	14.731	0.0209*	0.024*	1	14.281	0.0213*	0.0194*	1	14.004	0.0197*	0.0305*	1	13.432	0.0208*	0.0232*	1	14.439	0.0197*	0.0266*	1	13.984	0.0198*	0.0209*
	2	2.0343	0.7239		2	1.2132	0.8273		2	5.5454	0.3177		2	4.4675	0.4014		2	3.2236	0.565		2	2.4233	0.6529	
	3	4.3816	0.4804		3	2.9519	0.6098		3	4.3275	0.4634		3	2.4691	0.6516		3	4.1842	0.4873		3	2.7563	0.6222	
	4	-15.02	0.0969		4	-16.692	0.0569		4	-11.579	0.1661		4	-13.897	0.0872		4	-13.28	0.1241		4	-15.03	0.072	
	5				5				5				5				5				5			
Age		-0.1253	0.6122	0.6122		-0.1648	0.4952	0.4952		-0.1516	0.4912	0.4912		-0.2011	0.3638	0.3638		-0.122	0.5926	0.5926		-0.161	0.4737	0.4737
GCS		-6.7727	0.3474	0.3474		-7.0533	0.3236	0.3236		-8.9624	0.1723	0.1723		-9.1131	0.1704	0.1704		-7.805	0.2477	0.2477		-7.985	0.2351	0.2351
Gender		-6.1598	0.5041	0.5041		-4.477	0.6124	0.6124		-3.6614	0.6548	0.6548		-1.6042	0.8417	0.8417		-5.553	0.5158	0.5158		-3.931	0.6327	0.6327
Hypothermia		-10.534	0.1876	0.1876		-11.506	0.1437	0.1437		-8.8527	0.2136	0.2136		-9.888	0.1667	0.1667		-10.16	0.1722	0.1722		-11.03	0.1335	0.1335

* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001

Table 4-23 continued

Primary Mixed Models Analyses of BCL-2 SNP and Cerebral Bloof Flow (CBF)																							
	Right Hemiphere								Left Hemiphere								Global							
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS1801018 (n=17)																							
Genotype	xx	-25.13	0.0002**	0.0021**	yy & xy	11.881	0.0391*	0.0391*	xx	-20.433	0.0021**	0.0099**	yy & xy	11.301	0.0356*	0.0356*	xx	-23.6	0.0003**	0.003**	yy & xy	12.028	0.0292*	0.0292*
	yy	-24.942	0.0007**		xx				yy	-16.502	0.0248*		xx				yy	-21.27	0.0031**		xx			
	xy								xy								xy							
Time (Day)	1	24.683	0.0012**	0.0014**	1	17.65	0.0149*	0.0279*	1	21.021	0.0045**	0.0145*	1	16.203	0.0189*	0.0536	1	22.951	0.002**	0.0035**	1	17.081	0.0157*	0.0365*
	2	10.769	0.0769		2	4.3307	0.5135		2	13.419	0.0435*		2	7.7795	0.2268		2	11.568	0.0619		2	5.6419	0.3843	
	3	21.879	0.0022**		3	13.281	0.0799		3	18.091	0.0121*		3	12.607	0.0803		3	20.653	0.0034**		3	13.416	0.0688	
	4	-7.9328	0.3003		4	-8.0596	0.3725		4	-5.7309	0.471		4	-5.6443	0.5073		4	-6.697	0.3818		4	-6.208	0.4747	
	5				5				5				5				5				5			
Age		-0.0155	0.9099	0.9099		-0.1367	0.5579	0.5579		-0.0777	0.6503	0.6503		-0.1673	0.4306	0.4306		-0.024	0.8678	0.8678		-0.128	0.5524	0.5524
GCS		-0.2711	0.9454	0.9454		-1.8615	0.7727	0.7727		-2.8806	0.553	0.553		-4.124	0.4846	0.4846		-1.307	0.7515	0.7515		-2.701	0.6521	0.6521
Gender		-21.545	0.009**	0.009**		-7.9267	0.3626	0.3626		-13.948	0.0805	0.0805		-5.0565	0.5185	0.5185		-18.95	0.0166*	0.0166*		-7.497	0.3546	0.3546
Hypothermia		-2.9653	0.468	0.468		-4.5851	0.4992	0.4992		-1.9443	0.6911	0.6911		-3.0782	0.6121	0.6121		-2.526	0.5465	0.5465		-3.855	0.5368	0.5368
RS3810027 (n=16)																							
Genotype	xx	27.011	0.0292*	0.0829	xx & xy	1.5781	0.79	0.79	xx	19.467	0.0959	0.1986	xx & xy	3.3574	0.5425	0.5425	xx	24.225	0.0443*	0.1156	xx & xy	1.9944	0.7272	0.7272
	yy	11.644	0.1586		yy				yy	6.454	0.4151		yy				yy	9.9607	0.2183		yy			
	xy								xy								xy							
Time (Day)	1	7.8273	0.184	0.0153*	1	13.228	0.068	0.1024	1	8.7941	0.1483	0.0419*	1	12.13	0.0744	0.1515	1	8.2395	0.1682	0.0257*	1	12.756	0.0729	0.1359
	2	-3.0083	0.5975		2	-0.2916	0.9659		2	0.7928	0.892		2	2.9091	0.854		2	-1.644	0.7752		2	0.7861	0.9068	
	3	-1.557	0.7994		3	5.5817	0.4329		3	-0.0293	0.9963		3	5.2703	0.4347		3	-0.973	0.8756		3	5.5788	0.4262	
	4	-30.487	0.0086**		4	-12.284	0.2366		4	-22.739	0.0388*		4	-9.8926	0.3074		4	-26.76	0.0176*		4	-10.31	0.3057	
	5				5				5				5				5				5			
Age		0.1282	0.7182	0.7182		-0.1671	0.5702	0.5702		-0.0143	0.9645	0.9645		-0.2255	0.3969	0.3969		0.0962	0.7745	0.7745		-0.166	0.5477	0.5477
GCS		-6.1452	0.5515	0.5515		-0.8567	0.9227	0.9227		-6.9701	0.454	0.454		-3.2173	0.6851	0.6851		-6.368	0.5125	0.5125		-1.726	0.8351	0.8351
Gender		2.0117	0.8865	0.8865		-6.6568	0.5913	0.5913		3.801	0.7638	0.7638		-2.5702	0.8151	0.8151		1.9719	0.8816	0.8816		-5.911	0.6106	0.6106
Hypothermia		1.3544	0.8947	0.8947		-3.6406	0.6811	0.6811		0.7854	0.9313	0.9313		-2.8043	0.7212	0.7212		1.4056	0.8834	0.8834		-3.084	0.7091	0.7091

* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001

Table 4-23 continued

Primary Mixed Models Analyses of BCL-2 SNP and Cerebral Bloof Flow (CBF)																							
		Right Hemiphere								Left Hemiphere								Global					
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS4456611 (n=15)																							
Genotype	xx	1.3249	0.8582	0.0927	yy & xy	-9.4863	0.1023	0.1023	xx	-1.0033	0.8891	0.185	yy & xy	-6.0274	0.2829	0.2829	xx	0.6734	0.9253	0.0983	yy & xy	-8.512	0.1346	0.1346
	yy	-9.9092	0.1356		xx				yy	-9.3774	0.1487		xx				yy	-9.975	0.1238		xx			
	xy								xy								xy							
Time (Day)	1	15.406	0.0254*	0.0994	1	16.09	0.0253*	0.079	1	13.284	0.0505	0.1597	1	14.135	0.0501	0.1283	1	14.568	0.0307*	0.121	1	15.41	0.0317*	0.0974
	2	3.1712	0.6249		2	2.5031	0.7084		2	5.1709	0.428		2	4.6081	0.4971		2	3.4928	0.584		2	2.8185	0.6727	
	3	5.7137	0.3704		3	4.9609	0.4491		3	4.634	0.4667		3	3.9634	0.5498		3	5.1322	0.4126		3	4.3903	0.5021	
	4	-6.323	0.5411		4	-8.3741	0.4351		4	-7.1692	0.4754		4	-8.6931	0.4013		4	-5.956	0.552		4	-7.768	0.4566	
	5				5				5				5				5				5			
Age		-0.0337	0.9011	0.9011		0.1125	0.6774	0.6774		-0.16	0.525	0.525		-0.0295	0.9014	0.9014		-0.067	0.7922	0.7922		0.0756	0.7631	0.7631
GCS		-5.7222	0.408	0.408		-3.331	0.637	0.637		-6.953	0.2805	0.2805		-4.6695	0.4596	0.4596		-5.777	0.3769	0.3769		-3.355	0.6097	0.6097
Gender		-11.678	0.1826	0.1826		-13.859	0.1378	0.1378		-8.0944	0.3017	0.3017		-10.254	0.2034	0.2034		-11.03	0.1799	0.1799		-13.26	0.1267	0.1267
Hypothermia		-1.2667	0.8578	0.8578		-2.2374	0.7647	0.7647		1.7055	0.7908	0.7908		0.763	0.9068	0.9068		-0.109	0.9869	0.9869		-1.104	0.8726	0.8726
Genotype					xx & xy	10.687	0.0295*	0.0295*					xx & xy	8.8227	0.0632	0.0632					xx & xy	10.37	0.0306*	0.0306*
					yy								yy								yy			
Time (Day)					1	15.291	0.0204*	0.0838					1	13.404	0.0385*	0.1283					1	14.498	0.0244*	0.101
					2	3.1577	0.6163						2	5.1617	0.4127						2	3.4795	0.5735	
					3	5.7424	0.3543						3	4.5908	0.4555						3	5.1416	0.3973	
					4	-6.3761	0.5246						4	-7.1625	0.4614						4	-5.994	0.5363	
					5								5								5			
Age						-0.0539	0.8213	0.8213						-0.1449	0.5114	0.5114						-0.078	0.7285	0.7285
GCS						-5.9073	0.3728	0.3728						-6.8034	0.2727	0.2727						-5.868	0.3494	0.3494
Gender						-11.395	0.1695	0.1695						-8.2883	0.2688	0.2688						-10.88	0.1631	0.1631
Hypothermia						-0.919	0.8888	0.8888						1.4216	0.8121	0.8121						0.0739	0.9904	0.9904
RS4941185 (n=17)																								
Genotype	xx	0.2584	0.9713	0.0539	xx & xy	-20.845	0.0132*	0.0132*	xx	-3.9618	0.5695	0.0853	xx & xy	-18.369	0.0221*	0.0221*	xx	-0.836	0.9046	0.0608	xx & xy	-19.95	0.0143*	0.0143*
	yy	20.865	0.0193*		yy				yy	16.255	0.0534		yy				yy	19.406	0.0249*		yy			
	xy								xy								xy							
Time (Day)	1	14.143	0.0644	0.0429*	1	14.232	0.0478*	0.0294*	1	14.139	0.0554	0.0709	1	12.639	0.0737	0.0775	1	13.729	0.0691	0.0609	1	13.409	0.0609	0.0483*
	2	4.1107	0.5474		2	4.0988	0.5387		2	7.6182	0.2541		2	7.4735	0.2586		2	5.0801	0.4508		2	4.9947	0.4487	
	3	9.2006	0.2064		3	9.3492	0.1785		3	9.1381	0.1937		3	8.8325	0.1952		3	9.3359	0.193		3	9.4486	0.1696	
	4	-18.85	0.0986		4	-18.68	0.0895		4	-14.599	0.1777		4	-15.266	0.1541		4	-16.56	0.138		4	-16.51	0.1273	
	5				5				5				5				5				5			
Age		-0.0961	0.6375	0.6375		-0.0962	0.6238	0.6238		-0.1428	0.4712	0.4712		-0.1314	0.4786	0.4786		-0.096	0.621	0.621		-0.093	0.6118	0.6118
GCS		1.1649	0.8541	0.8541		1.0188	0.8533	0.8533		-2.9649	0.6289	0.6289		-1.7043	0.7425	0.7425		-0.269	0.9646	0.9646		-0.101	0.9844	0.9844
Gender		-15.258	0.0753	0.0753		-15.293	0.0641	0.0641		-11.271	0.1524	0.1524		-11.886	0.1172	0.1172		-14.5	0.0763	0.0763		-14.71	0.0607	0.0607
Hypothermia		-0.134	0.9816	0.9816		-0.1114	0.9841	0.9841		1.1248	0.841	0.841		0.9322	0.8586	0.8586		0.5712	0.9176	0.9176		0.545	0.9168	0.9168

* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001

Table 4-23 continued

Primary Mixed Models Analyses of BCL-2 SNP and Cerebral Bloof Flow (CBF)																							
		Right Hemiphere								Left Hemiphere								Global					
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS7236090 (n=16)																							
Genotype	xx	-10.196	0.1572	0.0306*	yy & xy	13.142	0.1119	0.1119	xx	-11.802	0.0848	0.0224*	yy & xy	15.048	0.0549	0.0549	xx	-10.94	0.1174	0.0255*	yy & xy	14.095	0.0797	0.0797
	yy	33.154	0.0273*		xx				yy	30.924	0.0272*		xx				yy	32.005	0.0261		xx			
	xy								xy								xy							
Time (Day)	1	7.3425	0.2957	0.0626	1	5.0414	0.4948	0.1196	1	5.263	0.4194	0.0952	1	3.1601	0.6429	0.1834	1	6.3223	0.3503	0.08	1	4.0672	0.5675	0.1508
	2	-0.7715	0.9048		2	-3.7369	0.5965		2	0.6139	0.919		2	-2.327	0.7221		2	-0.006	0.9993		2	-3.088	0.6502	
	3	1.6447	0.8141		3	0.3919	0.9585		3	0.8311	0.899		3	-0.4858	0.9446		3	1.5456	0.8193		3	0.1819	0.9801	
	4	-20.905	0.0378*		4	-21.077	0.0504		4	-18.9	0.0439		4	-18.762	0.0597		4	-19.58	0.0432		4	-19.76	0.0566	
	5				5				5				5				5				5			
Age		-0.1288	0.5721	0.5721		-0.1482	0.615	0.615		-0.0971	0.6473	0.6473		-0.1101	0.6895	0.6895		-0.105	0.6273	0.6273		-0.122	0.6665	0.6665
GCS		-4.5303	0.49	0.49		-0.4731	0.9536	0.9536		-4.6262	0.4518	0.4518		-0.6873	0.9282	0.9282		-4.713	0.4531	0.4531		-0.677	0.9311	0.9311
Gender		-9.5444	0.2824	0.2824		-8.3376	0.4515	0.4515		-10.066	0.2289	0.2289		-9.2841	0.3738	0.3738		-9.928	0.2444	0.2444		-8.944	0.4032	0.4032
Hypothermia		10.271	0.2214	0.2214		7.2829	0.464	0.464		12.293	0.1247	0.1247		9.877	0.2942	0.2942		11.389	0.1611	0.1611		8.7349	0.3648	0.3648
RS8083946 (n=17)																							
Genotype	xx	-7.3836	0.3444	0.0666	yy & xy	11.358	0.1435	0.1435	xx	-6.1527	0.3909	0.1086	yy & xy	9.4463	0.1813	0.1813	xx	-5.987	0.4214	0.0903	yy & xy	9.5053	0.1973	0.1973
	yy	10.08	0.087		xx				yy	9.0932	0.1194		xx				yy	9.9345	0.0942		xx			
	xy								xy								xy							
Time (Day)	1	10.333	0.0794	0.036*	1	10.296	0.1083	0.0634	1	10.078	0.0902	0.0767	1	9.9207	0.1138	0.1198	1	10.455	0.0816	0.0564	1	10.379	0.1094	0.0948
	2	0.045	0.9937		2	-2.1648	0.7216		2	2.8075	0.63		2	0.9886	0.8699		2	1.1148	0.8478		2	-0.951	0.8775	
	3	3.6312	0.5196		3	2.9244	0.633		3	3.5163	0.5428		3	3.0009	0.6229		3	3.7798	0.5138		3	3.2456	0.6036	
	4	-18.786	0.0392*		4	-18.296	0.0603		4	-14.119	0.1069		4	-13.499	0.1398		4	-15.97	0.0745		4	-15.1	0.1116	
	5				5				5				5				5				5			
Age		-0.1602	0.5947	0.5947		-0.176	0.5705	0.5705		-0.1911	0.4705	0.4705		-0.2021	0.4552	0.4552		-0.152	0.5858	0.5858		-0.164	0.5657	0.5657
GCS		5.0599	0.5551	0.5551		4.6393	0.5991	0.5991		2.2562	0.7645	0.7645		1.7274	0.8221	0.8221		3.873	0.6266	0.6266		3.2686	0.6878	0.6878
Gender		-5.9122	0.5856	0.5856		-6.2113	0.5785	0.5785		-3.7882	0.6907	0.6907		-4.1792	0.6674	0.6674		-6.032	0.55	0.55		-6.516	0.5289	0.5289
Hypothermia		0.207	0.981	0.981		-0.8223	0.9263	0.9263		1.3822	0.8553	0.8553		0.41	0.9575	0.9575		0.8153	0.919	0.919		-0.257	0.9748	0.9748

* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001

Table 4-23 continued

Primary Mixed Models Analyses of BCL-2 SNP and Cerebral Bloof Flow (CBF)																							
		Right Hemiphere								Left Hemiphere								Global					
Variables		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test		coefff	P	P for Type-3 Test
RS899968 (n=16)																							
Genotype	xx	6.2847	0.5709	0.0135*	xx & xy	16.296	0.0033**	0.0033**	xx	2.4261	0.8381	0.0906	xx & xy	12.631	0.0264*	0.0264*	xx	4.8442	0.6673	0.0273*	xx & xy	15.194	0.0068**	0.0068**
	yy	-15.036	0.0124*		yy				yy	-12.163	0.0558		yy				yy	-14.19	0.0216*		yy			
	xy								xy								xy							
Time (Day)	1	11.522	0.0531	0.0316*	1	12.491	0.0285*	0.0269*	1	10.116	0.1223	0.1342	1	10.534	0.0841	0.1182	1	10.979	0.0807	0.0722	1	11.793	0.0467*	0.0617
	2	2.9725	0.5882		2	3.0938	0.5694		2	4.0022	0.5242		2	4.0974	0.5051		2	3.1699	0.5912		2	3.2915	0.5706	
	3	6.8882	0.2097		3	7.2882	0.1792		3	4.3525	0.474		3	4.5408	0.4429		3	5.929	0.3074		3	6.2549	0.272	
	4	-10.698	0.2009		4	-9.2049	0.2383		4	-9.2479	0.3192		4	-8.5818	0.318		4	-9.441	0.2827		4	-8.197	0.3147	
	5				5				5				5				5				5			
Age		-0.0671	0.8014	0.8014		-0.0707	0.7846	0.7846		-0.1086	0.7026	0.7026		-0.1102	0.6872	0.6872		-0.052	0.8471	0.8471		-0.054	0.8346	0.8346
GCS		2.6891	0.7292	0.7292		1.1387	0.8715	0.8715		-0.8968	0.9134	0.9134		-1.489	0.8412	0.8412		1.1079	0.8872	0.8872		-0.08	0.991	0.991
Gender		-15.056	0.1242	0.1242		-15.68	0.0988	0.0988		-11.809	0.2473	0.2473		-12.053	0.2176	0.2176		-14.77	0.1345	0.1345		-15.28	0.1088	0.1088
Hypothermia		1.4491	0.8468	0.8468		2.7847	0.6871	0.6871		3.0005	0.7068	0.7068		3.498	0.6311	0.6311		2.1753	0.7731	0.7731		3.187	0.6457	0.6457
RS949037 (n=16)																							
Genotype	xx	-15.396	0.0293*	0.048*	xx & xy	7.2886	0.2432	0.2432	xx	-13.163	0.0535	0.0898	xx & xy	6.2218	0.2937	0.2937	xx	-14.05	0.0432*	0.068	xx & xy	6.9612	0.2528	0.2528
	yy	-10.874	0.0729		yy				yy	-9.7183	0.1015		yy				yy	-10.7	0.078		yy			
	xy								xy								xy							
Time (Day)	1	15.239	0.0294*	0.0707	1	14.891	0.568	0.1518	1	14.177	0.0376*	0.1299	1	13.171	0.0711	0.26	1	14.935	0.0332*	0.1052	1	13.92	0.0642	0.2102
	2	4.0494	0.539		2	2.0267	0.7856		2	7.5935	0.262		2	5.9295	0.4166		2	5.4327	0.4222		2	3.5311	0.6334	
	3	10.323	0.1543		3	9.5542	0.2385		3	9.8666	0.1711		3	9.0326	0.2482		3	10.557	0.1514		3	9.6293	0.2305	
	4	-5.6796	0.5552		4	-4.5352	0.6753		4	-2.7409	0.7724		4	-2.1513	0.8347		4	-3.122	0.7452		4	-2.468	0.8152	
	5				5				5				5				5				5			
Age		0.0614	0.8618	0.8618		-0.1025	0.7797	0.7797		0.05	0.8785	0.8785		-0.0792	0.8135	0.8135		0.0753	0.8215	0.8215		-0.065	0.8503	0.8503
GCS		-1.9496	0.7821	0.7821		-1.7687	0.8135	0.8135		-3.9102	0.5529	0.5529		-3.7288	0.5898	0.5898		-2.688	0.6878	0.6878		-2.476	0.7268	0.7268
Gender		-12.902	0.2101	0.2101		-11.728	0.2768	0.2768		-10.485	0.2659	0.2659		-9.434	0.3363	0.3363		-12.89	0.1868	0.1868		-11.73	0.2506	0.2506
Hypothermia		5.8289	0.5419	0.5419		0.1542	0.9873	0.9873		7.3716	0.4083	0.4083		2.5831	0.7707	0.7707		6.6351	0.4643	0.4643		1.5064	0.9686	0.9686
* p≤ 0.05; ** p≤ 0.01; *** p≤ 0.0001																								

4.3 SUMMARY INDEX

Tables 4-55 to 4-57 is an index of the statistically significant and trending findings presented in the results section. These tables are cataloged by dependent variable, covariates, and independent variables.

Table 4-24: Index of Significant Findings for Dependent Variables

Dependent Variable	Index of Significant Findings in Full/ Primary Model Findings
Neuropsychological Outcomes	
GOS	*rs12968517,* **rs17759659, rs1801018, rs949037, time, age, GCS,** *gender,* **race**
DRS	*rs12968517,* **rs17759659, rs1801018, rs949037, time, age, GCS,** *race, hypothermia*
Mortality	**rs12968517, rs17759659, rs1801018,** *rs7230970, rs949037,* **time, age, GCS,** *race,* **hypothermia**
NRS-R	**rs17756073,** *rs4456611,* **age, seizure**
Trails A	**rs12968517, rs1381548, rs3810027,** *rs7236090, rs8083946,* **GCS, hypothermia**
Trails B	**rs1381548,** *rs17756073, time,* **GCS, hypothermia**
Bcl-2 Protein	
with Outliers	*age*
without Outliers	*rs12454712, rs1481031,* **rs17756073, rs7236090, time, gender, seizure**
Neurometabolites	
Lacate	*rs1381548, rs8083946,* **time, gender**
Pyruvate	**rs17759659, rs1801018, rs4941185, rs949037, time**
LP Ratio	**rs17759659, rs1801018,** *rs8083946,* **time, gender**
CBF	
Right Hemisphere	**rs1026825,** *rs12454712,* **rs1381548, rs1481031, rs17756073, rs1801018,** *rs3810027,* **rs4456611, rs4941185, rs7236090,** *rs8083946,* **rs899968, rs949037, time, gender**
Left Hemisphere	**rs1026825,** *rs12968517,* **rs*1381548*, rs1481031, rs17756073, rs1801018, rs4941185, rs7236090, rs899968,** *rs949037,* **time,** *age,* **GCS,** *gender, hypothermia*
Global	**rs1026825, rs1381548, rs1481031, rs17756073, rs1801018, rs4456611, rs4941185, rs7236090, rs899968,** *rs949037,* **time,** *age, GCS,* **gender**
BOLD= statistically significant (P≤ 0.05) *Italicized=* marginally significant (P= 0.05 -0.09)	

Table 4-25: Index of Significant Findings for Covariates

Covariates	Index of Significant Findings in Full/ Primary Model Findings
Time	**rs1026825, rs12454712, rs12968517**, **rs1381548, rs1481031, rs17756073, rs17759659, rs1801018, rs1944419, rs3810027, rs4456611, rs4941185, rs7236090, rs8083946, rs899968, rs949037, GOS, DRS, Mortality,** *Trails B,* **bcl-2 protein without outliers, lactate, pyruvate, LP ratio, CBF Rt.Hemisphere, CBF, Lt. Hemisphere, CBF Global**
Age	**rs12454712, rs12968517, rs17756073, rs17759659, rs1801018, rs1944419,** *rs4456611,* **rs4941185, rs7236090, rs949037, GOS, DRS, Mortality, NRS-R,** *bcl-2 protein with outliers, CBF, Lt. Hemisphere, CBF Global*
GCS	**rs12454712, rs12968517, rs1381548,** *rs1481031,* **rs17756073, rs17759659, rs1801018, rs1944419,** *rs3810027,* **rs4941185, rs7236090,** *rs8083946,* **rs949037, GOS, DRS, Mortality, Trails A, Trails B**, **CBF Lt. Hemisphere,** *CBF Global*
Gender (Male)	**rs1026825**, *rs12968517, rs1481031,* **rs17756073,** *rs17759659,* **rs1801018,** *rs4456611, rs4941185,* **rs7236090, rs8083946,** *rs899968,* **rs949037,** *GOS,* **bcl-2 protein without outliers, lactate, LP ratio, CBF Rt.Hemisphere,** *CBF Lt. Hemisphere,* **CBF Global**
Race (Caucasian)	**rs17759659, rs1801018,** *rs7230970, rs7236090, rs949037,* **GOS,** *DRS, Mortality*
Hypothermic	**rs12454712, rs12968517, rs1381548, rs1481031, rs17756073, rs17759659, rs1801018, rs3810027, rs4941185, rs7230970, rs7236090, rs8083946,** *rs949037, DRS,* **Mortality, Trails A, Trails B**
Hypoxia	
Hypotensive	
Seizures	*rs1026825,* **rs1481031**, *rs17756073, rs17759659, rs4456611, rs7236090* , **rs899968, NRS-R, bcl-2 protein without outliers**
APOEε4	
BOLD= statistically significant (P≤ 0.05) *Italicized*= marginally significant (P= 0.05 -0.09)	

Table 4-26: Index of Significant Findings for Independent Variables

	Independent Variable	Index of Significant Findings in Full/ Primary Model Findings
	SNP's	
3' end	RS17756073	**NRS-R,** *Trails B,* **bcl-2 protein without outliers, CBF Rt.Hemisphere, CBF, Lt. Hemisphere, CBF Global, time, age, GCS, gender, hypothermia**, *seizure*
I	RS4456611	*NRS-R,* **CBF Rt.Hemisphere, CBF Global, time,** *age, gender,seizure*
I	RS1026825	**CBF Rt.Hemisphere, CBF, Lt. Hemisphere, CBF Global, time, gender,** *seizure*
I	RS7230970	*Mortality,* **time, age, GCS,** *race,* **hypothermia**
I	RS899968	**CBF Rt.Hemisphere, CBF, Lt. Hemisphere, CBF Global, time,** *gender,* **seizure**
I	RS4941185	**pyruvate, CBF Rt.Hemisphere, CBF, Lt. Hemisphere, CBF Global, time, age, GCS,** *gender,* **hypothermia**
I	RS12454712	*bcl-2 protein without outliers, CBF Rt.Hemisphere,* **time, age, GCS, hypothermia**
I	RS1481031	*bcl-2 protein without outliers,* **CBF Rt.Hemisphere, CBF, Lt. Hemisphere, CBF Global, time,** *age, GCS , gender,* **hypothermia**, **seizure**
I	RS3810027	**Trails A,** *lactate, CBF Rt.Hemisphere,* **time,** *GCS,* **hypothermia**
I	RS8083946	*Trails A, lactate, LP ratio, CBF Rt.Hemisphere,* CBF Global, **time,** age, *GCS,* **gender,** race, **hypothermia,** hypoxia, hypotension, seizure
I	RS1944419	**time, age, GCS**
I	RS12968517	*GOS, DRS,* **Mortality, Trails A,** *CBF, Lt. Hemisphere,* **time, age, GCS,** *gender,* **hypothermia**
I	RS7236090	*Trails A,* **bcl-2 protein without outliers, CBF Rt.Hemisphere, CBF, Lt. Hemisphere, CBF Global, time, age, GCS, gender,** *race,* **hypothermia**, *seizure*
I	RS1381548	**Trails A, Trails B, CBF Rt.Hemisphere, CBF, Lt. Hemisphere, CBF Global,** *time,* **GCS**, **hypothermia**
I	RS17759659	**GOS, DRS, Mortality, pyruvate, LP ratio, time, age, GCS,** *gender,* **race, hypothermia,** *seizure*
I	RS949037	**GOS, DRS,** *Mortality,* **pyruvate, CBF Rt.Hemisphere,** *CBF, Lt. Hemisphere, CBF Global,* **time, age, GCS, gender,** *hypothermia*
5' end	RS1801018	**GOS, DRS, Mortality, pyruvate, LP ratio, CBF Rt.Hemisphere, CBF, Lt. Hemisphere, CBF Global, time, age, GCS,** *gender,* **race, hypothermia**
	BOLD= statistically significant (P≤ 0.05) *Italicized*= marginally significant (P= 0.05 -0.09)	

4.4 SUMMARY OF FINDINGS TABLES

Tables 4-58 to 4-62 are summary tables of the statistically significant and trending findings presented in the results section. These tables allow for the comparison of the SNP with the genotype with interptrative outcomes and statistics for each of the analyses.

Table 4-27: Summary of Findings for Global Functional Outcomes

Summary of Findings: Global Functional Outcomes				
	SNP	**GOS**	**DRS**	**Mortality**
3’	rs1801018	Good*	Good*	Survival*
|	variant (AA & AG)	p=0.0002	p=0.0002	Type 3 X^2 =0.0035 OR: 5.01 (0.4024 to 2.8194)
|	rs949037	Good*	Good*	Survival†
|	variant (TT & CT)	p=0.0168	p=0.0255	Type 3 X^2 =0.0551
|				CC Z=0.1145 OR: 0.333 (-2.468 to 0.2661)
|				TT Z=0.2827 OR:1.853 (-0.509 to 1.742)
|	rs17759659	Good*	Good*	Survival*
|	variant (GG & AG)	p<0.0001	p<0.0001	Type 3 X^2 =0.0031 OR: 5.05 (0.3189- 2.9202)
|	rs12968517	Poor*	Poor*	Mortality *
|	variant (CC)	p=0.0242	p=0.0277	Z=0.0115 OR: 6.378 (0.4154 to 3.2897)
|	rs7230970	-	-	Survival†
|				Type 3 X^2 = 0.054
|	homozygous variant (CC)			CC Z=0.0548 OR: 0.191 (-3.3382 to 0.0339)
5’	Wild Type (TT)			TT Z=0.0582 OR: 0.299 (-2.4578 to 0.042)
				* statically significant P<0.05 or Z<0.05 or X^2<0.05
				† statically trending P= 0.05-0.09 or Z=0.05-0.09 or X^2=0.05-0.09

Table 4-28: Summary of Findings: Cognitive-Behavioral Outcomes

Summary of Findings: Cognitive-Behavioral Outcomes					
	SNP	**Allele**	**NRS-R**	**Trails A**	**Trails B**
3’	rs17756073	variant (GG & AG)	Poor* p=0.0331		Poor † p=0.0516
|	rs4456611	variant (CC & CT)	Good† p=0.065		
|	rs1381548	variant (AA & AG)		Poor* p=0.0366	Poor* p=0.0391
|	rs12968517	variant (TT & CT)		Poor* p=0.0289	
|	rs7236090	homozygous wild type (TT) p=0.0396 homozygous variant (CC) p=0.0387		Good † P for F test p= 0.0577	
|	rs3810027	homozygous wild type (CC) p=0.0196 homozygous variant (GG) p=0.0351		Poor* P for F test P=0.032	
|	rs8083946	homozygous variant (AA)		Poor* p=0.0285	
5’					
				* statically significant P<0.05 † statically trending P= 0.05-0.09	

Table 4-29: Summary of Findings: Bcl-2 Protein Concentrations Without Outliers

Summary of Findings: Bcl-2 Protein Concentrations			
	SNP	**Allele**	**Bcl-2 Protein without outliers**
3’	rs12454712	variant (TT & CT)	Increase† p=0.0646 (Good)
|	rs17756073	homozygous variant (TT)	Decreased* p=0.0084 (Poor)
|	rs1481031	homozygous wild type (AA)	Decreased* p=0.018 (Poor)
|	rs7236090	variant (TT & CT)	Increase* p=0.0112 (Good)
5’			
			* statically significant P<0.05 † statically trending P= 0.05-0.09

Table 4-30: Summary of Findings: Neurometabolite Concentrations

Summary of Findings: Neurometabolite Concentrations				
	SNP	**Lactate**	**Pyruvate**	**LP Ratio**
3’	rs8083946 homozygous wild type (GG)	Decrease* p=0.037 (Good)		Decrease* p= 0.0388 (Good)
|	rs3810027 variant (GG & CG)	Increase† P= 0.0791 (Poor)		
|	rs17759659 variant (AA & AG)		Increase* p= 0.0011 (Good)	Decrease† p= 0.0791 (Good)
|	rs1801018 variant (GG & AG)		Increase* p= 0.0175 (Good)	Decrease* p= 0.024(Good)
|	rs949037 variant (CC & CT)		Increase* p= 0.0032 (Good)	
5’				
				* statically significant P<0.05 † statically trending P= 0.05-0.09

Table 4-31: Summary of Findings: Cerebral Blood Flow

Summary of Findings: Global Cerebral Blood Flow					
	SNP	**Allele**	**Global CBF**	**Right Hemisphere CBF**	**Left Hemisphere CBF**
3'	rs17756073	variant (GG & AG)	Decrease* p= 0.0075 (Poor)	Decrease* p= 0.0116 (Poor)	Decrease* p= 0.0086 (Poor)
|	rs4456611	variant (CC & CT)	Increase* p= 0.0306 (Good)	Increase* p= 0.0295 (Good)	Increase† p= 0.0632 (Good)
|	rs1026825	variant (AA & AG)	Increase* p= 0.0287 (Good)	Increase* p= 0.024 (Good)	Increase* p= 0.0296 (Good)
|	rs899968	variant (AA & CC)	Increase* p= 0.0068 (Good)	Increase* p= 0.0033 (Good)	Increase* p= 0.0264 (Good)
|	rs4941185	variant (AA & AG)	Decrease* p= 0.0143 (Poor)	Decrease* p= 0.0132 (Poor)	Decrease* p= 0.0221 (Poor)
|	rs1481031	variant (GG & AG)	Increase* p= 0.0055 (Good)	Increase* p= 0.0046 (Good)	Increase* p= 0.0105 (Good)
|	rs7236090	homozygous variant (TT)	Increase† p= 0.0797 (Good)		Increase† p= 0.0549 (Good)
|	rs1381548	variant (AA & AG)	Increase* p= 0.0177 (Good)	Increase* p= 0.0216 (Good)	Increase* p= 0.0187 (Good)
|	rs949037	homozygous variant (CC)	Decrease* p= 0.0432 (Poor)	Decrease* p= 0.048 (Poor)	Decrease† p= 0.0535 (Poor)
|	rs1801018		Decrease* (Poor)	Decrease* (Poor)	Decrease* (Poor)
|		P for F test	p=0.003	p=0.021	p=0.0099
|		homozygous wild type (AA)	p=0.0003	p=0.0002	p=0.0021
5'		homozygous variant (GG)	p=0.0031	p=0.0007	p=0.0248
					* statically significant P<0.05 † statically trending P= 0.05-0.09

5.0 DISCUSSION

The findings of each specific aim of this study support the claim that there is a relationship between BCL-2 genotypes and outcomes after TBI; both the biological/ clinical variables in the acute phase of treatment and the long term global functional and cogitative-behavioral neuropsychological outcomes.

5.1 HWE

All of the SNP's analyzed in this study were in HWE for the overall genotyped sample (n=230) or was not significantly out of HWE, with the exception of rs8083946. TBI subjects are assumed to be representative of the general population (i.e. TBI is a random event and there are no known genes that place a person at an increased risk). According to HapMap data (July 2008) for the CEU population, based on a chromosome sample center of 120, the allele frequency for rs8083946 is A=0.333 and G=0.667. This study found that based on a chromosome sample of 460, the A(X)=0.4375 and G(Y)=0.6227 with 14 subjects (6.09%) being undetermined. The discrepancy in the findings may be related to issues with the assay or genotyping error. To note, this SNP also had the highest frequency of undetermined genotypes among the 17 SNP's analyzed, which furthers the support that there were issues with the assay. In summary, caution

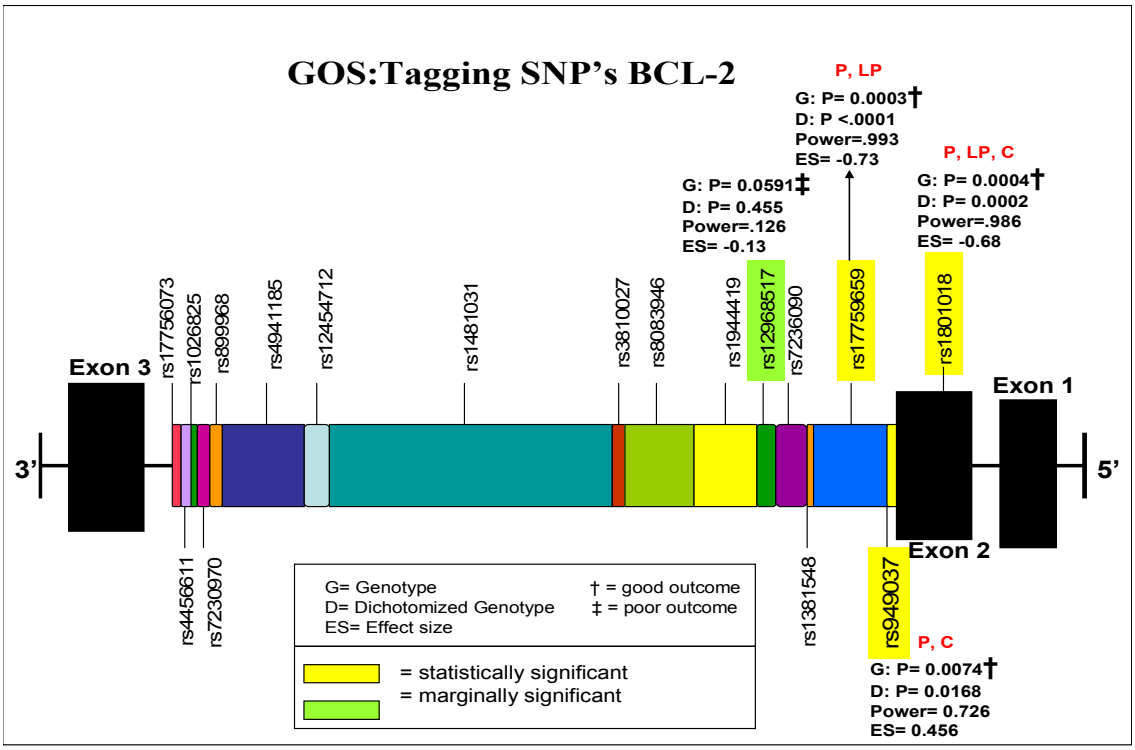
needs to be employed when drawing conclusions that surround SNP rs8083946 due to HWE violations which calls to questions the validity of the data.

5.2 NEUROPSYCHOLOGICAL OUTCOMES

5.2.1 GOS & DRS & Mortality (Refer to figures 5-1 to 5-3)

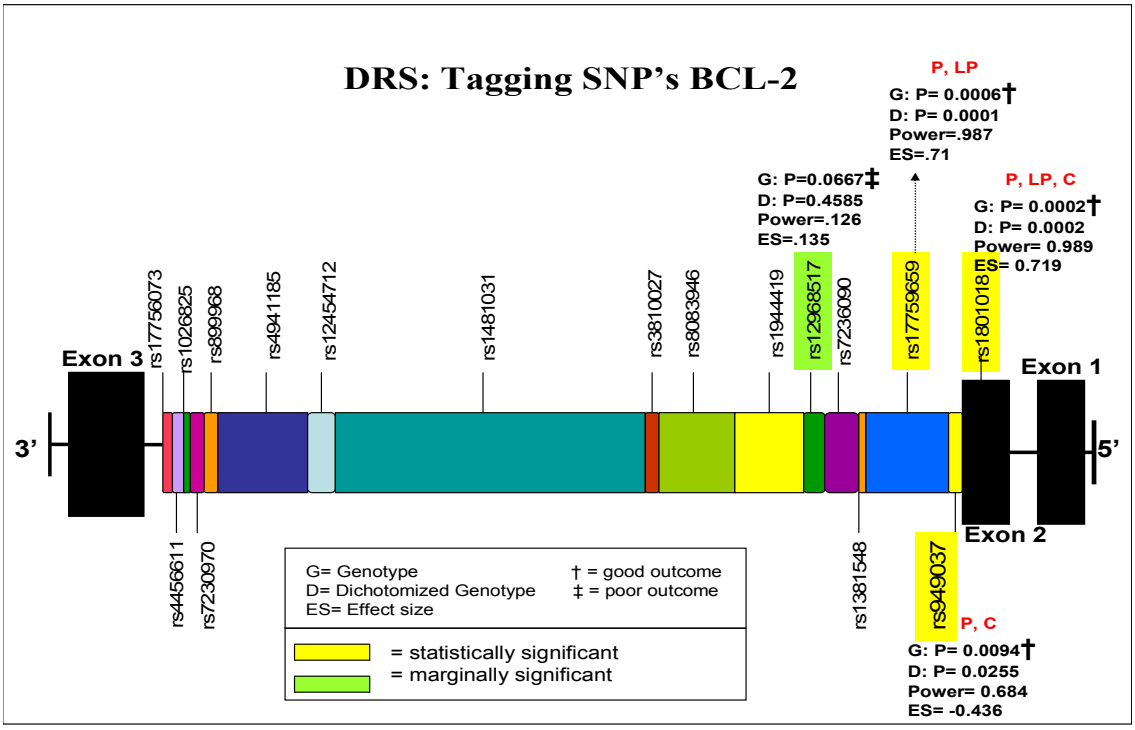
This study shows that there is a relationship between BCL-2 genotypes and global functional outcomes. SNP's rs17759659, homozygous variant (GG); rs949037, homozygous variant (TT); and rs1801018 homozygous wild type (AA) allele were all associated with increased GOS and decreased DRS (good outcomes). All of the SNP's were statistically significant on both genotype and dichotomized genotype with post hoc power estimates from 0.684-0.989. There is increased confidence that the sample size was adequate to support the conclusion that these genotypes for these SNP's are related to better outcomes. SNP's rs17759659 and rs1801018 in the mortality analyses were both statistically significant on the genotype analyses and have a power of 1 for each. SNP rs17759659, homozygous variant (GG) genotype in a TBI patient is 5.05 times (95% CI: 0.3189- 2.9202) more likely to survive. TBI patients with rs1801018 homozygous wild type (AA) allele are 5.01 times (95% CI: 0.4024-2.8194) more likely to survive their injuries. However, SNP rs949037 genotypes was marginally significant in the mortality analysis with homozygous variant (TT) being 0.412 times (95% CI: -1.9451 to 0.1728) more likely to survive the injury. The findings are consistent for these SNP's among the global functional outcome data. Biologically, these 3 SNP's may be related to exon 2. There is substantial linkage disequilibrium (LD), spanning 27, 235 base pairs, for rs1801018

with rs17759659 (r^2=.634) and rs949037 (r^2=.687). Rs1801018, rs17759659, and rs949037 were also implicated in the neurometabolite and cerebral blood flow analyses, which may provide a physiological explaination to the GOS, DRS, and mortality findings.



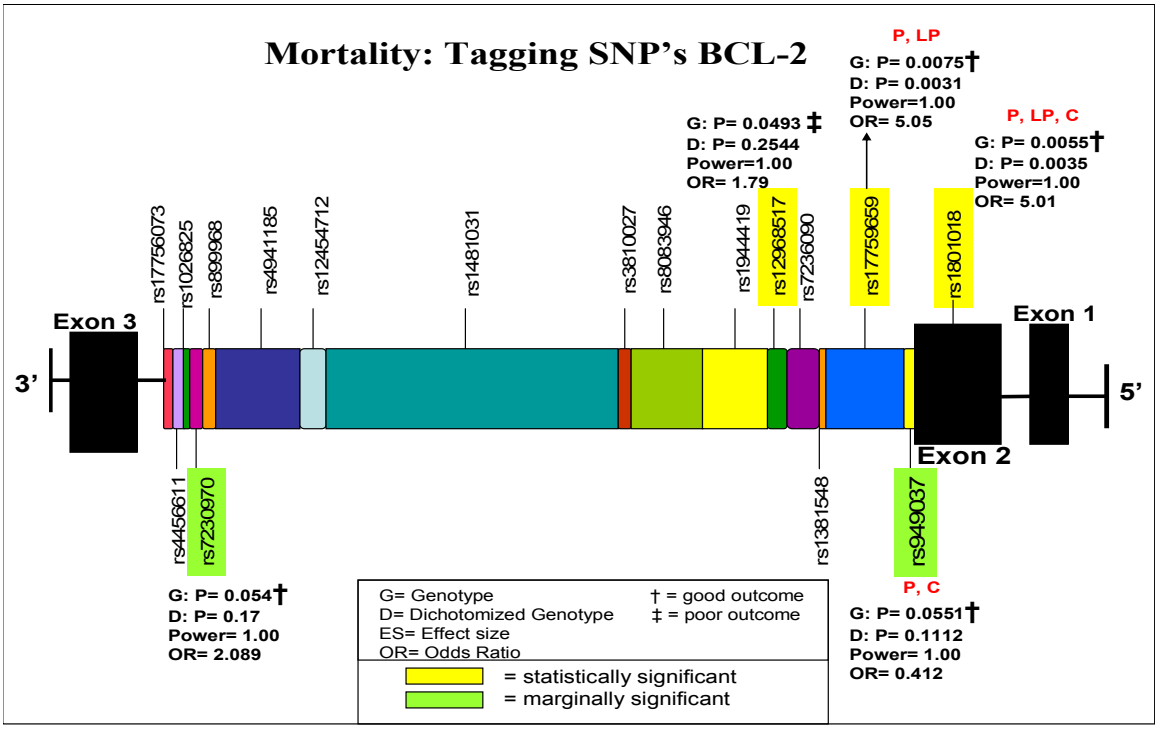
B= BCL-2 Protein; C= Cerebral Blood Flow; L= Lactate; LP= Lactate Pyruvate Ratio; P=Pyruvate

Figure 5-1: GOS and BCL-2 Tagging SNP's



B= BCL-2 Protein; C= Cerebral Blood Flow; L= Lactate; LP= Lactate Pyruvate Ratio; P=Pyruvate

Figure 5-2: DRS and BCL-2 Tagging SNP's



B= BCL-2 Protein; C= Cerebral Blood Flow; L= Lactate; LP= Lactate Pyruvate Ratio; P=Pyruvate

Figure 5-3: Mortality and BCL-2 Tagging SNP's

Rs12968517 genotypes was marginally significant in the GOS and DRS analyses with lower post hoc power estimate (Power= 0.126). The trend supports that the homozygous wild type (CC) allele was associated with decreased GOS and increased DRS (poor outcomes). Due to the lower power associated with this finding the conclusions are limited. However, in the mortality analyses there was statistical significance, SNP rs12968517, homozygous wild type (CC) genotype is associated with a 1.79 times (95% CI: -0.4325 to 1.5934) increased likelihood to die from the TBI. SNP's rs12968517 compared to the findings in the GOS and DRS analysis, this SNP is more sensitive to survival/death than global functioning on a continuum. The LD correlations of rs12968517 with the SNP's rs1801018, rs17759659, and rs949037 are not available in the HapMap database. This indicates that there is most likely a different "surrogate function" for this SNP than the others implicated in these global functional outcomes analyses. This lack of correlation or LD may explain why is rs12968517 associated more strongly with survival/mortality than with other global functioning measures that assess activities of daily living. SNP's rs7230970 was a SNP of interest only in the global functional outcome of mortality being marginally significant with post hoc power of 1.0. Both rs7230970 homozygous wild type (CC) and homozygous variant (TT) genotypes were associated with 2.089 times (95% CI: -0.3661 to 1.8404) decreased mortality in a TBI patient when compared to those who were heterozygous (CT). SNP's rs7230970 position is distant from the cluster of significant SNP's for global functioning. The LD with the highest correlation with this SNP is rs956572 r^2=0.598 in the same intron region as rs7230970.

The covariates of interest in this study among the global functional outcomes (time, age, GCS, race, and hypothermia) are supported by the literature (Cifu et al., 1996a; Cifu, Kaelin & Wall, 1996b; Pillai, Kolluri & Praharaj, 2003; Jiang, Gao, Li, Yu, & Zhu, 2002; Girard et al.,

1996; King, Carlier & Marion, 2005; Labi et al. 2003). Caucasians in this study have higher GOS scores and there for better outcomes which may be related to genetic differences, however, the under-representation of non-caucasians may have biased these data.

5.2.2 Cognitive Behavioral Outcome Measures

5.2.2.1 NRS-R

SNP's rs17756073 and rs4456611 are the two SNP's of interest in the NRS-R analyses. (Refer to figure 5-4). Despite the proximity of these two SNP's the LD is not available in the HapMap database. The surrogate "function" of the two tagging SNP's is likely to be different.

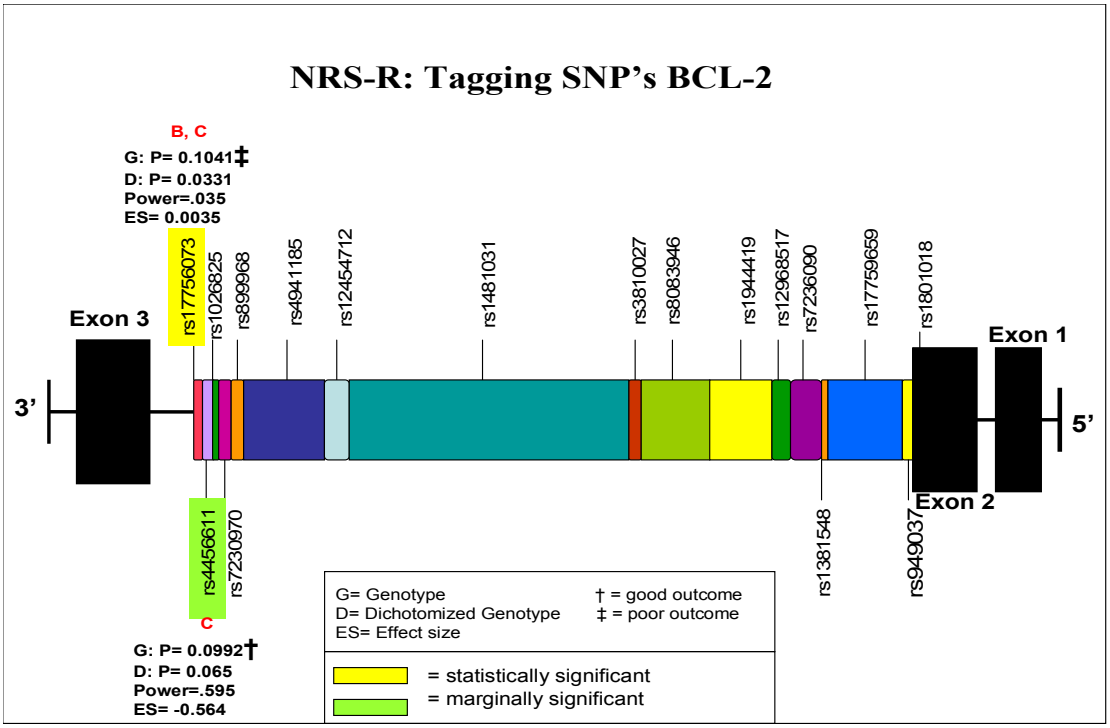
SNP rs17756073 is a SNP of interest in the NRS-R outcome measures. The homozygous wild type (AA) genotype was associated with a decrease in NRS-R scores and therefore is associated with better outcomes. Subjects with the presence of the variant allele (GG or AG) had significantly higher NRS-R scores; worse outcomes. The post hoc power for this analysis was very low (power= 0.035) therefore what we can say is limited. The LD for this SNP covers 3,530 base pairs in the intron region. In the subsequent sub-studies, while having smaller sample sizes but high post hoc power and large ES, found that rs17756073 the homozygous wild type genotype (AA) or homozygous variant (GG) were both related to decrease in bcl-2 protein concentrations, or based on the literature worse outcomes, which is in contrast to the NRS-R findings. However, the homozygous wild type genotype (AA) in the global CBF analysis, which was related to increased CBF corroborates the findings that for NRS-R, rs17756073 AA alleles are associated with good outcomes.

SNP rs4456611 in the primary (full) model resulted in trends towards significance the presence of the variant allele (CC or CT) was associated with lower NRS-R scores, indicating

better outcomes. The post hoc power for this analysis was power=0.595. This SNP covers a LD region of 14,775 base pairs in the same intron region. As subsequent sub-studies would suggest, NRS-R scores as a representation of cognitive behavioral outcomes maybe related at CBF. Our findings suggest that global CBF for SNP's rs4456611 the presence of the variant allele (CC or CT) was related to an increase in CBF which may be associated with good outcomes.

SNP's rs17756073 and rs4456611 were also implicated in the bcl-2 protein and cerebral blood flow analyses, which may provide a physiological explanation to the NRS-R findings.

Increasing age and preadmission seizures were consistently associated with poor outcomes in this study; the sub-sample findings were consistent with the literature.



B= BCL-2 Protein; C= Cerebral Blood Flow; L= Lactate; LP= Lactate Pyruvate Ratio; P=Pyruvate

Figure 5-4: NRS-R and BCL-2 Tagging SNP's

5.2.2.2 Trails Making Tests

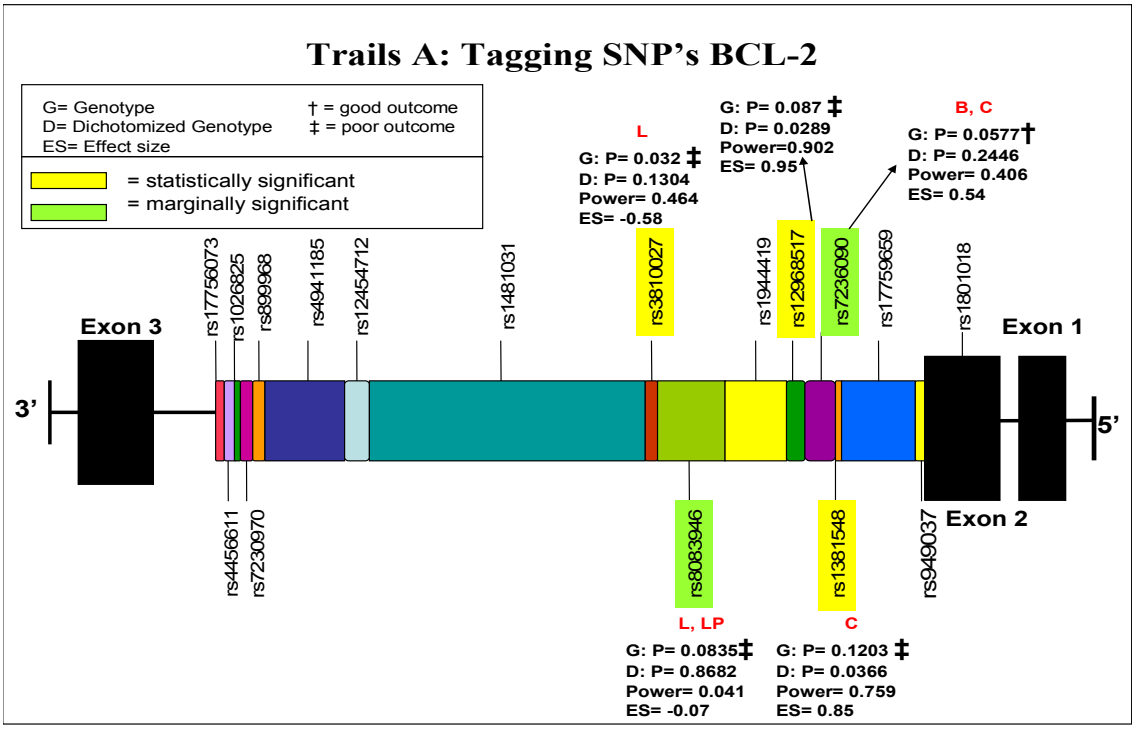
Trails A

SNP's rs12968517 homozygous wild type (CC), rs13815487 homozygous wild type (GG), and rs7236090 homozygous for wild type (TT) or the variant (CC), all were associated with decrease Trails A scores and was therefore associated with better outcomes (attention). (Refer to figure 5-5). Despite being located in the same region they cover a span of 15, 425 base pairs. The LD for rs12968517 compared to rs13815487 or rs7236090 are both unavailable in the HapMap data base. The LD between rs13815487 and rs7236090 is very low correlation (r^2=0.091). The highest published LD correlation by HapMap for SNP rs13815487 is r^2=0.173. None of the surrogate SNP's for rs12968517 are shared by rs13815487 and rs7236090 at greater than r^2= 0.186 correlation. These SNP's appear to be independent of each other.

Based on the biology of LD, the action/ surrogate action of rs12968517 is likely encompass the surrogate SNP for Trails A. The SNP is statistically significant for the dichotomized genotype analysis (P=0.0289) with an adequate post hoc power of 0.902. This study supports that the region of BCL-2 tagged by rs12968517 is associated with decreased Trails A score; better outcomes. The action/ surrogate action of rs13815487 is likely to be independent for Trails A because of the low correlation with other SNP's. The SNP was found to be statistically significant for lower Trails A score, for the dichotomized genotype analysis (P=0.0366) with a post hoc power of 0.759. SNP rs7236090's action/ surrogate action is likely to encompass a surrogate SNP's for Trails A. This analysis was trending towards significance for the genotypes analysis (P=0.0577) with a low post hoc power of 0.406, while not conclusive, it is of interest for future studies.

SNP's rs3810027 and rs8083946 are 1,943 base pairs apart. For both rs3810027 and rs8083946 analyses were underpowered (power= 0.464; power= 0.041, respectively). SNP's rs3810027 homozygous for wild type (CC) and the variant (GG) and rs8083946 homozygous

variant (AA) had higher Trails A scores and were therefore associated with poorer outcomes (attention). LD of rs8083946 with rs3810027 r^2=0.505. They act for surrogates for 6 out of 7 SNP's with an LD > 0.6 [rs7243091 (for rs8083946 only); rs1165975; rs1296167; rs9630855; rs7231914; rs4987768; rs7240326]. While the analyses for these SNP's were underpowered, future studies with larger sample sized may implicate these SNP's and/or their surrogates as SNP's of interest in poor attention after TBI. SNP's rs3810027 and rs8083946 were also implicated in the lactate concentration sub-study. The presence of rs3810027 variant allele (GG or CG) was associated to an increase in lactate, which is consistent with the increase Trails A scores being associated with poor outcomes. SNP rs8083946 homozygous allele GG was related to lower lactate levels, which would be associated with good outcomes however, rs8083946 may have been a problematic assay. Based on these findings in the Trails A analyses and in concordance with the lactate findings the underling mechanism for lactate concentration regulation and bcl-2 may also affect attention after TBI. CBF may also provide a physiological underpinnings.



B= BCL-2 Protein; C= Cerebral Blood Flow; L= Lactate; LP= Lactate Pyruvate Ratio; P=Pyruvate

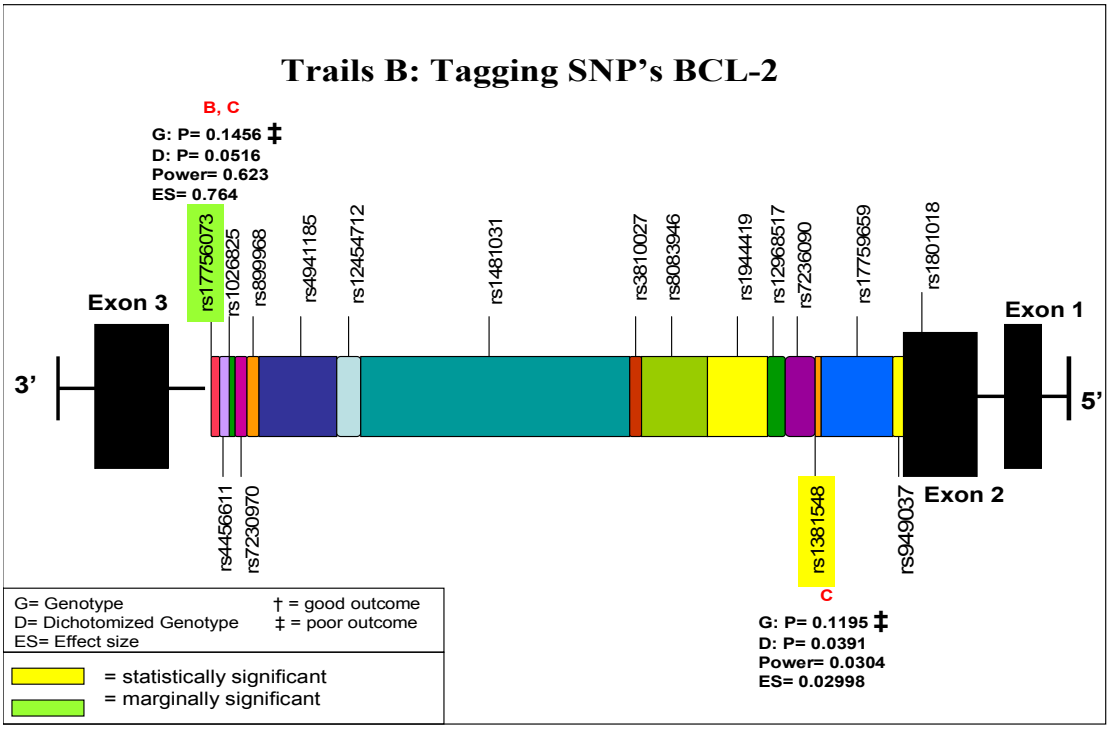
Figure 5-5: Trails A and BCL-2 Tagging SNP's

Trails B

SNP's rs1381548 homozygous wild type (GG) and rs17756073 homozygous wild type (AA) were associated with significantly lower Trails B completion times therefore better outcomes (mental flexibility). (Refer to figure 5-6). As discussed in Trails A, SNP's rs1381548 highest know LD with another SNP's is r^2=0.173. Therefore, rs1381548 is likely to have an independent action associated with better Trails B scores. Despite statistical significance for the genotype analysis (P=0.0391) the post hoc power was very low (Power= 0.0304). SNP rs17756073 is a surrogate for 3 SNP's with LD r^2 >0.6 (range 0.778-0.958) (rs4987834, rs4987828, rs4987827). These SNP's are likely to act in concert with each other. The statistical significance was trending (P=0.0516) and post hoc power (0.623) for the analyses of this SNP with the Trails B data were indicative of a trend in these data. Based on the biological and clinical data sub-studies, Trails B shares in common the significant SNP's indicated in bcl-2

protein concentration and CBF which may serve as the underlying biological explanation for the mental flexibility results.

Commonalities in the Trails Making neuropsychological outcomes were that rs1381548 was significant in both Trails A and Trails B analyses and covariates of interest. The presence of the homozygous variant allele (AA) for SNP rs1381548 was associated with higher test scores. There were three main themes in the covariates for the Trails A and Trails B data; time, GCS, and hypothermia. Time was sensitive for Trails B tests alone. When the test was administered closer to time of injury (3 month evaluation compared to 12 months post injury) subjects had longer Trails B time scores indicating that less mental flexibility and this is reflected in the literature (Spikeman et al., 2000). One finding that was counterintuitive was that higher GCS scores on admission were associated with higher Trails A and Trails B scores (longer times); poor outcomes. This finding may be related to the higher number of subjects with high GCS (n=19; 70.4%) vs. low number with low GCS (n=8; 29.6%) which can skew the results. For both Trails A and Trails B the subjects who did not receive a hypothermia intervention were found to have higher scores, this supports the literature that hypothermia is neuroprotective after TBI (Mcilvoy, 2005).



B= BCL-2 Protein; C= Cerebral Blood Flow; L= Lactate; LP= Lactate Pyruvate Ratio; P=Pyruvate

Figure 5-6: Trails B and BCL-2 Tagging SNP's

While the explanation for the neuropsychological outcomes may be rooted in the function of exon 2, intronic mRNA stability, the mechanism the regulates bcl-2 protein concentrations or CBF, as implicated in subsequent sub-studies, our findings could be explained by different SNP's having different functions in different parts of the brain. This hypothesized difference in the SNP's function stems from the biology of bcl-2 and the apoptosis pathways. For example, caspases are active in different regions of the brain and that bcl-2 protein is expressed differently in different regions of the brain (Clark et al, 1997). Cognitive-behavioral function is believed to be rooted in different parts of the brain than motor functioning or survival. Therefore, the implication of different SNP's maybe related to these factors.

5.3 BIOLOGICAL/ CLINICAL DEPENDENT VARIABLES

5.3.1 Bcl-2 Protein

As discussed in the methods, the bcl-2 protein concentration data were analyzed with and without outliers. The demographics of the two subjects with the bcl-2 protein concentration outliers were representative of the entire sample. As stated in the methods and results, the two samples with higher concentrations were within normal limits of the ELISA. However, the two samples were outliers to the dataset. The data was analyzed with and without bcl-2 protein concentration outliers. The findings suggested that there were no statistically significant or trending SNP's when the data was analyzed with the outliers. In contrast, the analyses without the outliers revealed SNP's that were statistically significant and/or trending SNP's (rs17756073, rs12454712, rs1481031, and rs7236090). (Refer to figure5-7).

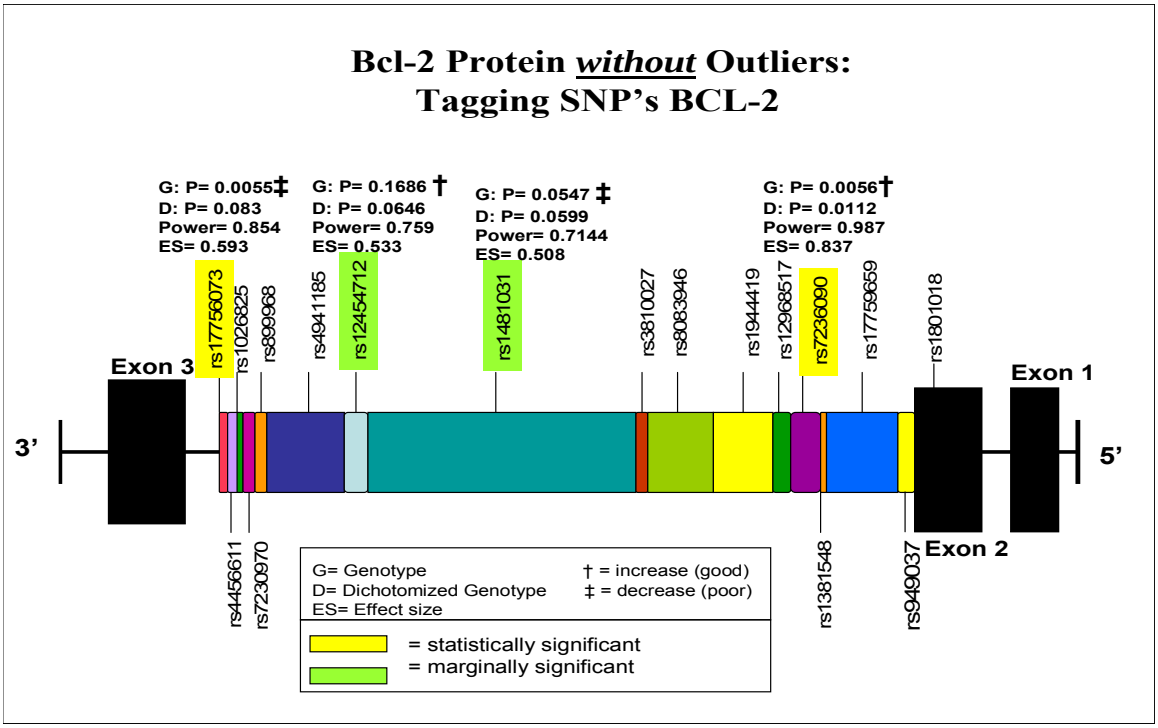


Figure 5-7: Bcl-2 Protein Concentrations and BCL-2 Tagging SNP's

The findings of this study may be related to small sample size (n=41) or the small cell sizes of covariates (gender and preadmission seizure). Methodologically, the piece of protein that is being interrogated for in the ELISA test may not encompass all of the neuroprotective nature of bcl-2, the piece may not be specific to the bcl-2α isoform and also be analyzing bcl-2β, or that the SNP's chosen may not be the optimal SNP's to analyze the piece of protein that is begin analyzed.

SNP's rs12454712 homozygous wild type (TT), rs17756073 homozygous wild type (AA) and homozygous variant (GG), rs1481031 homozygous wild type (AA), and rs7236090 homozygous wild type (CC) and homozygous variant (TT) were all associated with statistically significant or a trending decrease in bcl-2 protein concentrations.

SNP rs17756073 is a surrogate for 3 additional SNP's with LD r^2 >0.6 (range 0.778-0.958) (3,530 base pair range) in the same intron region. The analyses for this SNP had an adequate post hoc power (Power=0.854).

SNP rs1481031 is a surrogate for 19 SNP's with r^2>0.6 (range of r^2=0.611-0.811) and spans 21,042 base pairs. All of the surrogate SNP's are located to the same intron region, and are likely to be involved with mRNA stability. The analyses for this SNP had a post hoc power of 0.759.

SNP rs7236090 is a surrogate for 1 additional SNP rs8089538 (r^2=0.663) in the intron region. Among the SNP's analyzed, this SNP was most significant for both the genotype and dichotomized genotype analyses, had the largest post hoc power (0.987) and ES (0.837). This SNP was 1,031,902 base pairs from the exon 2 region. There was significant LD decay that

implies that it is not associated with the exon 2 function, however, more research needs to be done to further explore this relationship.

Surrogate SNP's for rs12454712 have no known SNP's with $r^2 > 0.154$ and is located in the intron region. The analyses for this SNP had a power of 0.7144.

The three covariates of interest in these analyses were time, gender, history of pre-admission seizures. In the analyses of the SNP's of interest, bcl-2 protein concentrations spiked typically on days 2, 4, and 5 compared to day 6. This finding additional empirical evidence to the literature that bcl-2 protein peaks between day 2 and 5 following TBI in humans (Uzan et al, 2006; Yang & Xue, 2004).

This study found that the nine females compared to the 33 males had statistically significant (or trending) lower bcl-2 protein concentrations in all of the full models analyzed. The age range for the females was 20-60 years with mean of 35 years. The finding that females have lower bcl-2 protein concentrations compared to males is counterintuitive compared to the literature that suggests that there is a connection between higher bcl-2 protein concentrations and estrogen (Soustiel, Palzur, Nevo, Thaler, & Vlodavsky, 2005; Wise, Dubal, Wilson, & Rau, 2000; Zhang, L., et al., 2004) and that bcl-2 is theoretically more neuroprotective in females (Soustiel et al., 2005; Wagner, et al, 2005; Wise et al.,2000). However, estrogen can also be found in males as testosterone is known to be converted to estradiol in the brain (Behl & Manthey, 2000). It is plausible that the males could have higher estrogen levels than the females and that preinjury and postinjury concentrations may differ. Estrogen is reported to have neuroprotective effects by maintaining cerebral blood flow, antioxidant properties, impeded excitotoxic injury and promoting the production of growth factor (Soustiel et al., 2005; Wagner, et al, 2005; Wise et al., 2000). In murine studies, bcl-2 is found to increase in the presence of

estrogen/ estrogen treatment thus believed to inhibit apoptosis (Soustiel et al., 2005; Wise et al., 2000; Zhang, L., et al., 2004). Estradiol increased bcl-2 expression and decrease cerebral infarct (hypoxic-ischemic damage) in non diabetic female mice and was overall significantly neuroprotective compared to males (Zhang, L., et al., 2004). In estrogen-treated animals, there was a marked and significant reduction of apoptosis in comparison with non-treated animals. Soustiel and colleagues (2005) furthers found that estrogen treatment mice had a reduction caspase 3, a significant increase in bcl-2 expression, and no effect on bax expression after experimental TBI. Estrogen is therefore likely to have its action in the caspase dependent pathway of apoptosis.

Pre-admission seizures are common after severe TBI. About 25 percent of patients with brain contusions or hematomas and about 50 percent of patients with penetrating head injuries will develop immediate seizures (seizures that occur within the first 24 hours of the injury) (NINDS, 2008). In our study, 9 of the 42 subjects used in the bcl-2 protein analyses had a documented history of a pre-admission seizure. This event was significantly related to having lower bcl-2 protein concentrations. The relationship of positive seizures with low bcl-2 protein concentrations (and presumably poor outcomes based on the literature) is suspected (Henshall, 2007; Henshall & Simon, 2005; Shinoda et al., 2003). The mechanism by which seizure and low bcl-2 protein concentrations are connected may be that the intrinsic pathway of apoptosis is triggered after seizures by the rise in intracellular calcium (Henshall, 2007; Henshall & Simon, 2005; Shinoda et al., 2003). In the rat model epilepsy model in the hippocampus there is evidence of the release of cytochrome c, apaf-1, and caspase 9 and-3, and with the rise in intracellular calcium activation of pro-apoptotic bcl-2 family members (bad, bim, and bid) (Henshall, 2007; Henshall & Simon, 2005). The extrinsic apoptosis caspase dependent pathway

and the role of TNF as the trigger has also been implicated in cell death post- seizure (Shinoda et al., 2003)

Future studies would 1) increase the enrollment, gender diversity, and subjects with a positive pre-admission history of seizure 2) examine bcl-2 genotypes and bcl-2 protein with neuropsychological outcomes after TBI. Based on the literature and the SNP's of interest in this study, the hypothesis would be that the SNP's with low bcl-2 concentrations would be associated with poor outcomes 3) re-examine the relationship with protein concentrations and gender and pre-admission seizures, and related those findings to the neuropsychological outcomes.

5.3.2 Neurometabolites

The neurometabolite findings are clustered into two groups: lactate and LP ratio results vs pyruvate and LP ratio results.

SNP's rs8083946 homozygous wild type (GG) was associated with a decrease in lactate concentrations and a decrease in LP ratios. SNP's rs3810027 homozygous wild type (CC) was associated with a decrease in lactate concentrations alone. (See figures 5-8 and 5-10). The lactate acid analyses were under powered (rs3810027, power= 0.397; rs8083946, power=0.441). The LP ratio analyses for rs8083946, were underpowered as well (power=0.397).The SNP's of interest in the lactate acid analyses, SNP's rs3810027 and rs8083946 are neighboring SNP's with 1943 base pairs separating the two. The correlation of the LD between the two SNP's is r^2=0.505. They share 6 of 7 surrogate SNP's with r^2>0.6 [rs7243091 (for rs8083946 only); rs1165975; rs1296167; rs9630855; rs7231914; rs4987768; rs7240326].

SNP's rs17759659 homozygous wild type (GG) and rs1801018 homozygous wild type (AA) were significant for a decrease in pyruvate concentrations and increase in LP ratios. SNP

rs949037 homozygous wild type (TT) was significant for a decrease in pyruvate concentrations alone. (See figures 5-9 and 5-10). There is substantial LD for rs1801018 with rs17759659 (r^2=.634) and rs949037 (r^2=.687). SNP rs1801018 and rs949037 share 3 surrogates SNP's (rs1462129, rs1893806, and rs2051423). rs1801018 correlated with each of the 3 surrogates at r^2= 0.737 and rs949037 correlates with the surrogates at r^2=0.932. The LD correlation with rs949037 with rs17759659 is unknown. The analyses for the pyruvate data were adequately powered [rs17759659, power= 0.96659; rs1801018, power=0.756; rs949037, power=0.963] as well as the LP ratio data [rs17759659, power= 0.856; rs1801018, power=0.727] given the exploratory nature of the analyses. SNP rs4941185 homozygous wild type (AA) was marginally significant for an increase in pyruvate concentrations. HapMap does not contain any known SNP's in LD with rs4941185. The highest correlation for this SNP is at r^2>0.09.

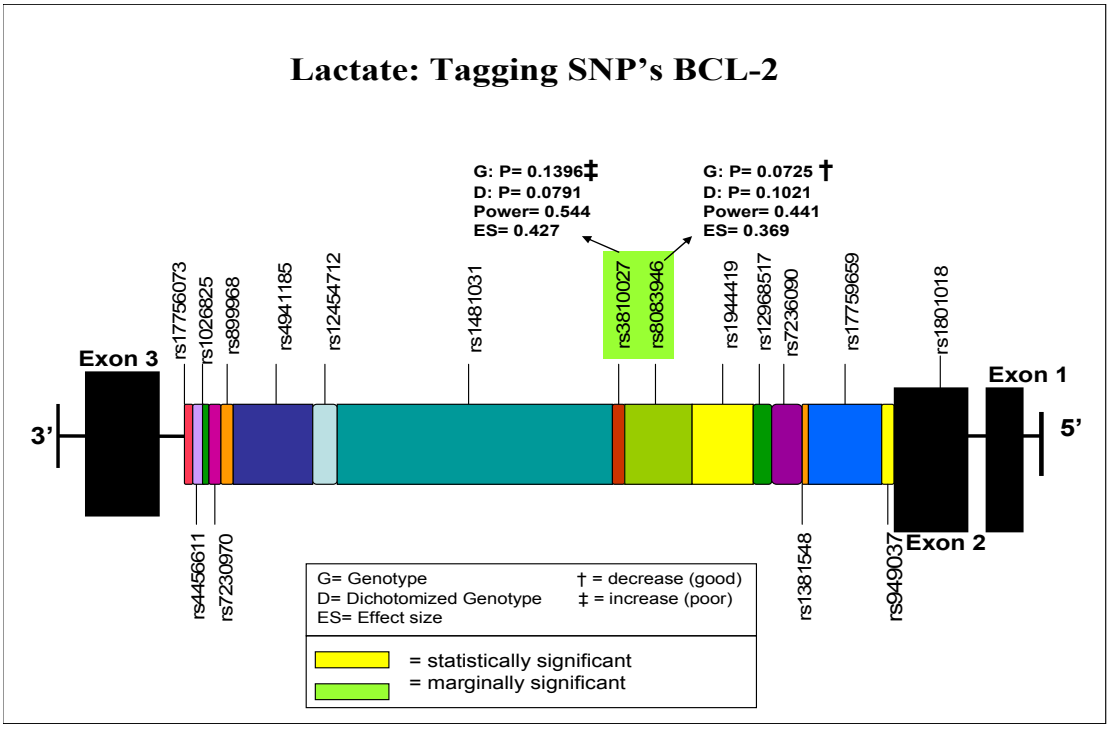


Figure 5-8: Lactate and BCL-2 Tagging SNP's

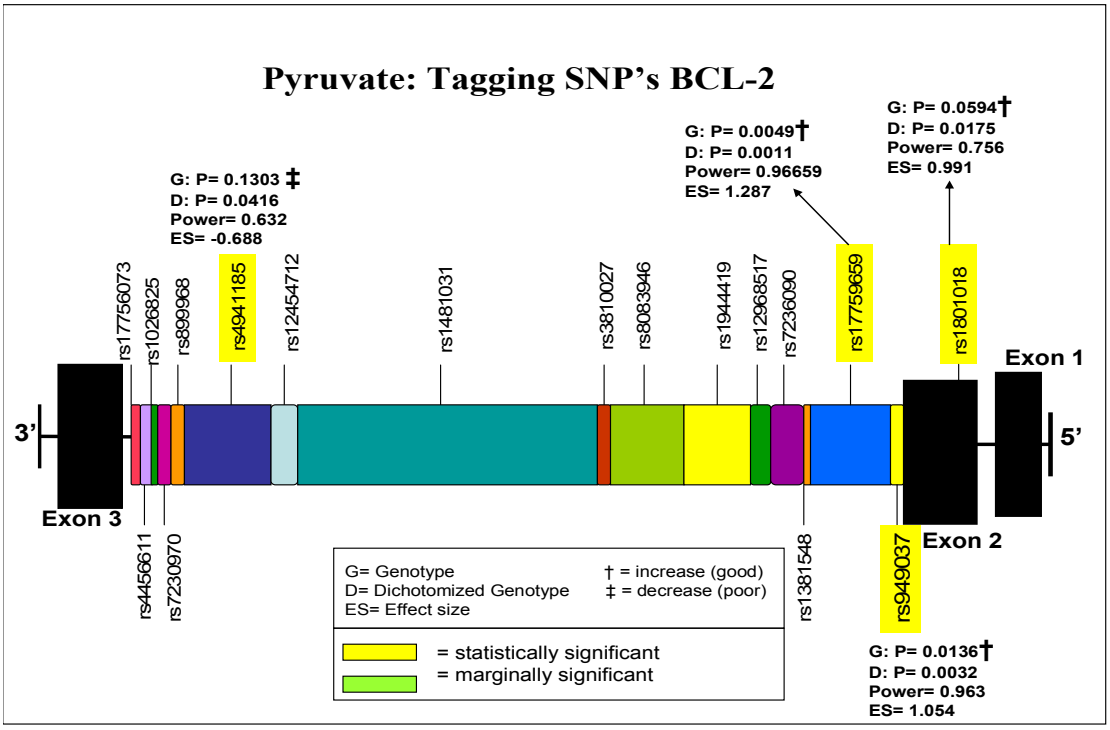


Figure 5-9: Pyruvate and BCL-2 Tagging SNP's

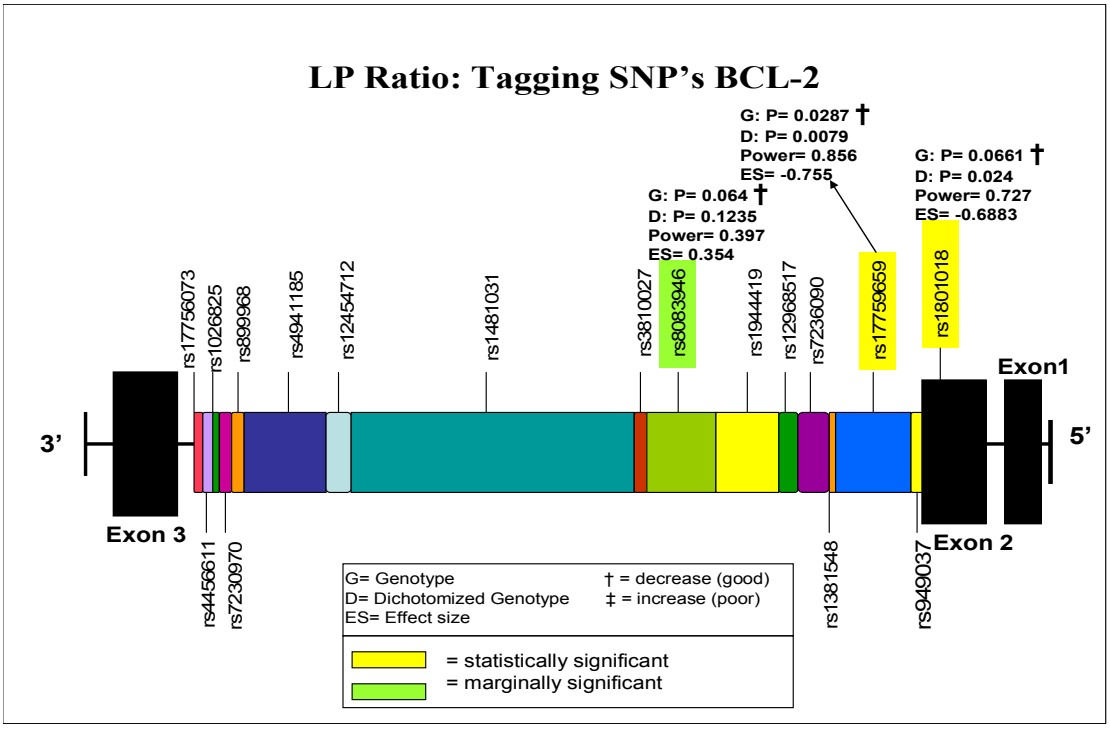


Figure 5-10: LP Ratio and BCL-2 Tagging SNP's

The covariates of interest in the neurometabolite analyses were time (for lactate, pyruvate, and LP ratio) and gender (for lactate and LP ratio). Time is consistent with the literature that lactate concentrations and LP ratio were significantly higher days 1 and 2 post injury in relation to day 5 (Pyruvate was only significantly higher on day 1 than day 5). Females in this study were found to have significant higher lactate concentrations and LP ratios. This is in contrast to the literature and theoretical underpinnings that suggests that females have lower lactate and LP ratio due to the neuroprotective effects of estrogens (Wagner et al., 2004; Wagner, 2005; Mclean & Nunez, 2008). The one mechanism by which the female hormones, estrogen, is neuroprotective is receptor –dependent and receptor independent modulation of increased bcl-2 expression (Dubal, Shughrue, Wilson, Merchenthaler, & Wise, 1999; Mclean & Nunez, 2008; Singer, Rogers, & Dorsa, 1998; Stoltzner, Berchtold, Cotman, & Pike, 2001; Zhao, Wu, Brinton, 2005).

Until this study there have been no studies that examine the relationship between BCL-2 genotypes and lactate, pyruvate or L/P ratio after TBI. Lactate, pyruvate, and lactate/pyruvate ratio (L/P) are important because they are energy- related metabolites and biochemical indicators of brain injury and cerebral anoxia (Kerr et al., 2003; Wagner et al., 2004; Wagner et al., 2005; Yu et al., 2005). As a consequence of TBI, brain metabolism increases as well as energy demands and glycolysis (Chen, T. et al, 2000). This increase need for energy occurs simultaneously with the ischemia like changes that increase lactic acid due to the anaerobic glycolysis which deplete energy (Kerr et al., 2003; Wagner et al., 2004; Wagner et al., 2005; Werner & Engelhard, 2007; Yu et al., 2005). The anaerobic metabolism depletes the mitochondria ATP-stores as well as impairs membrane permeability that is dependent on energy. These events are coupled with additional secondary injury events (terminal membrane

depolarization and the excessive release of excitatory neurotransmitters, depolarization of Ca2+ and Na+ channels) that lead to the activation of capsize cascades and ultimately necrosis or apoptosis (Werner & Engelhard, 2007). In this study the SNP's (rs8083946 and rs3810027) that were indicated as having significantly decreased lactate and/ or LP ratios, would hypothetically be indicative of better outcomes because of the increase in energy within the mitochondria of the cells. The literature asserts that elevated lactate is related to poor outcomes, diminished cerebral blood flow, elevated intracranial pressure, and ischemia (Goodman, Valaka, Gopinath, Uzura, & Robertson, 1999; Kerr et al., 2003; Wagner et al., 2004; Wagner et al., 2005; Yu et al., 2005). Future studies would need to further explore this hypothesis.

Lactate and pyruvate are often considered in ratio because they are antagonistic to each other (Kerr et al., 2003; Wagner et al., 2004; Wagner et al., 2005). Pyruvate is an intermediate in the metabolism of glucose. It is a potent ROS scavenger. Pyruvate is protective against ROS in neuronal tissue (Kerr et al., 2003; Wagner et al., 2004; Wagner et al., 2005; Yu et al., 2005). Increased pyruvate is associated with decreased cerebral ischemia (Yu et al., 2005). The relationship with pyruvate and bcl-2 is more clear in that it is known that pyruvate can act on the caspase independent cascade and inhibit the transport and activation on p53, thus increasing the expression of bcl-2 (Lee Y., 2003; Lee, Y. 2004). Based on the findings of this study the hypothesis could be made that SNP's rs17759659, rs1801018, and rs949037, because they were associated with a decrease in pyruvate concentrations and/ or increase in LP ratios, would be associated with poor outcomes. Future studies would need to further explore this hypothesis.

Limitations to this sub-study include the small sample size and small sample representation of females. In addition, this sub-study was conducted on 36 Caucasian subjects due to the lack of variability in the race covariate.

5.3.3 Cerebral Blood Flow

In the global CBF analyses the findings reveal that of the 14 SNP's in the primary (full model) analyses there were 10 SNP's with statistical or trending significance. Of the 10 SNP's 8 were associated with a decrease in global CBF [rs1026825 homozygous variant (AA) and homozygous wild type (GG); rs1381548 homozygous variant (GG); rs1481031 homozygous wild type (AA); rs17756073 dichotomized analysis homozygous variant (GG); rs1801018 homozygous wild type (AA) and homozygous variant (GG); rs4456611 in the dichotomized analysis homozygous wild type (TT); rs899968 homozygous wild type (CC); rs949037 homozygous variant (CC) and homozygous wild type (TT)]. The remaining two SNP's rs4941185 homozygous wild type (GG) and rs7236090: homozygous variant (TT) were associated with an increase in global CBF. (See figure 5-11).

Covariates of interest in these analyses were time and gender. Overall, time day one had the highest CBF compared to day 5, which is supported in the literature. Gender, the findings suggest that males have lower CBF than females which is consistent with the literature. The female sex hormones of progesterone and estrogen are believed to have neuroprotective and anti-apoptotic effects which contribute to the maintenance of CBF after TBI and stroke (Pettus, Wright Stein & Hoffman, 2005; Roof & Hall, 2000a; Roof & Hall, 2000b; Roof, Hoffman, & Stein, 1997; Stein & Hoffman, 2003). However, of the 17 subjects in the study 4 were female, which may have some degree of biased the data due to low cell count.

The analyses of the CBF data were exploratory in nature. Sample size is a significant limitation of what can be elucidated from the findings. The ES for these analyses was quite large, however, power was not able to be calculated due to the inability at calculate the rho (estimate R correlation [autocorrelation] secondary to the small sample size). The clustering of the

significant SNP's suggest that global CBF is sensitive to both bcl-2 coding in exon 2 (rs949037, rs1801018), as well as, mRNA stability (all of these SNP's are positioned in the intro region between exon 2 and exon 3 and have no know surrogates that are in LD with any of the exons: rs17756073, rs4456611, rs1026825, rs899968, rs4941185, rs1481031, rs7236090, rs1381548).

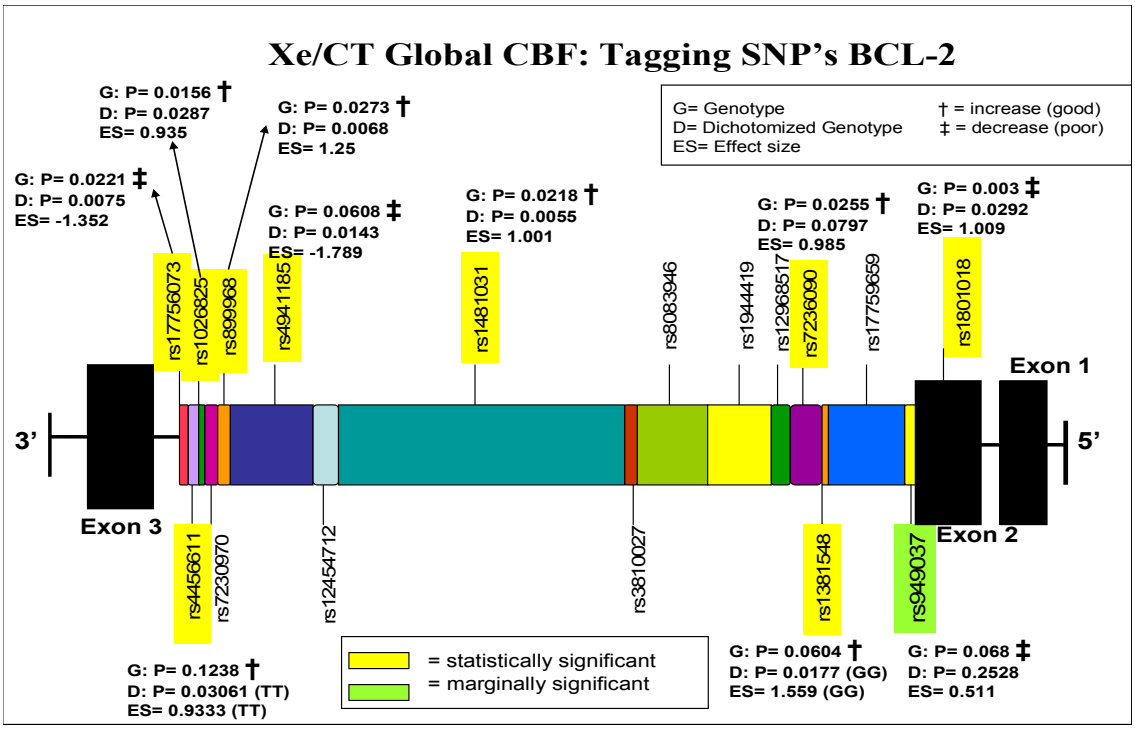


Figure 5-11: Global CBF and BCL-2 Tagging SNP's

Cerebral ischemia, a consequence of secondary brain injury, is related to the reduction or complete loss of CBF (McLaughlin & Marion, 1996). Ischemic changes result in the release of cytochrome c leading to apoptosis occurs (Zhao, Steinberg, & Sapolsky, 2007). In bcl-2 knockout mice, homozygous knockout mice have significantly larger infarct area more severe reduction in CBF (Hata, Gillardon, Michaelidis, & Hossmann, 1999). Seven of 17 subjects in this study were enrolled in a hypothermia protocol. Our study found that 1 SNP, rs1381548 homozygous variant (GG), had a trending relationship with decreased CBF. The literature asserts that the conflicting of evidence in the literature that hypothermia can increase bcl-2

protein expression after global ischemia (Zhang, Z. et al., 2001) versus hypothermia only inhibiting bax expression 4 hours after ischemia but having no effect on other proteins (Eberspacher et al., 2003). The inconsistencies in the literature maybe related to differences in the effect of hypothermia on apoptosis in focal versus global ischemia (Yenari, et al., 2002; Zhao et al., 2005).

5.4 COVARIATES

5.4.1 Hypothermia

This dissertation study found that hypothermia was related to DRS, mortality, Trails A and Trails B. The subjects who did not receive the hypothermia intervention had lower DRS scores; and lower mortality; good outcomes. For both Trails A and Trails B, the subjects who did not receive the hypothermia intervention had higher scores, which indicated poor outcome. The inconsistent findings maybe reflective of multiple analyses, small sample sizes, or the literature. The literature debates the efficacy of the hypothermia intervention in the treatment of secondary injury after severe TBI. The use of hypothermia as an intervention for the treatment and management of severe TBI is controversial. The inconsistent results are shrouded in the operational definition of hypothermia; deep hypothermia vs. mild hypothermia (Jermitsky, Omert, Dunham, Protetch, & Rodriguez, 2003; Jiang et al, 2002).

Hypothermia as in intervention in TBI is believed to decreases neurotransmitter release, reduces metabolic requirements for oxygen and glucose, normalizes pH, decreases inflammatory precursors, and preserves brain tissue and blood brain barrier (Mcilvoy, 2005). From the CBF

literature there is conflicting evidence that hypothermia can increase bcl-2 protein expression after global ischemia (Zhang, Z. et al., 2001) versus hypothermia only inhibiting bax expression 4 hours after ischemia but having no effect on other proteins (Eberspacher et al., 2003). The inconsistencies in the literature maybe related to differences in the effect of hypothermia on apoptosis in focal versus global ischemia (Yenari, et al., 2002; Zhao et al., 2005).

The action of mild hypothermia on the mitochondria was found to be related to the reduction in the number of morphologically apoptotic neurons by nearly half in 48 hours compared to normothermic models (Xu, Yenari , Steinberg, & Giffard, 2002). Along with this caspase-3, -8, and -9 activity was significantly decreased and cytochrome c translocation was reduced (Xu et al, 2002). However, in this study there was a significant reduction in cJun N-terminal kinase activation but not change in bcl-2 protein concentrations; however it is clear that hypothermia is an intervention for apoptosis (Xu et al, 2002). Other studies have found that BCL-2 is upregulated in the presence of hypothermia (Yenari et al., 2003). One murine stroke model study used a gene therapy used a neurotropic herpes simplex viral vector system containing bipromoter vectors to transfer BCL-2 genes to neurons found that BCL-2 improved neuron survival was protective against stroke in a normothermic environment. In the hypothermic intervention significant neuroprotection was associated with BCL-2 upregulation suppressing cytochrome c release, caspase activation, and DNA fragmentation (Yenari et al., 2003). The results of this dissertation support that hypothermia is implicated in both neuropsychological outcomes after TBI. The cell size for hypothermia throughout all of the analyses fluctuated, and particularly for specific aims 2, the low cell count may have influenced the findings.

5.4.2 APOEϵ4

While APOEε4 was indicated as a covariate because the literature suggests that there may be a synergistic relationship between APOEε4 and BCL-2 as a representative of apoptosis (Belton et al., 2003; Moulder et al., 1999; Ong et al., 2003; Shimohamma et al., 2001; Wei et al., 1999) APOEε4 did not meet the a priori cut-off significance of $p<0.2$ for any of the analyses. APOEε4 was therefore omitted from all of the primary (full model) analyses. In future studies the potential synergistic relationship between BCL-2 and APOE/APOEε4 can be further explored by including APOEε4 in the model regardless of preliminary statistics as well as assess for an interaction of the variables.

5.5 LIMITATIONS

There are several limitations to these series of studies conducted for this dissertation. Sample size was a challenge given the subjects available with sub analyses data. The sample sizes fluctuate greatly in the primary models from 17 to 141 subjects. However, the fixed effects estimates were robust for some of the exploratory studies and adequate post hoc power was noted. Missing data, especially for the covariates of hypotension, hypoxia, and seizure were problematic.

While the demographics of the over all sample were representative of TBI in the greater Pittsburgh area, the generalizability of the data is limited due to the lack of racial and gender diversity in the sample. Multicenter study or a match case control design may help address these issues.

The global functional outcomes data over the 24 months outcome periods were biased towards death as an outcome (GOS, DRS, and mortality). Death carries over and crosses time, so that if a patient dies prior to the 3 month outcome, the death outcome was recorded for the remainder of the outcome points regardless if the 24 months post injury time point arrived. Time was biased/ weighted towards the representation of death. A prime example of this bias was the mortality data 3 months [n=51 (25.4% of 230 subjects with 1.5% missing data)] versus 24 months [n=58 (28.9% of 230 subjects with 41.3% missing data)].

5.6 CONCLUSIONS

The results of this study show that BCL-2 genotypes are associated with global functional outcomes and neurobehavioral outcomes after severe TBI. The global functional outcomes may be related to the coding region of exon 2. The cognitive behavioral outcomes, which were not analyzed directly with neurometabolite and CBF data, share significant SNP's of interest and potential mechanisms of action by which cognitive behavioral outcomes are mediated by the underlying neurometabolite and CBF biological functioning. This study also asserts that there is a significant relationship between BCL-2 genotypes and bcl-2 protein concentrations, neurometabolite concentrations, and CBF after severe TBI. The relationship between BCL-2 proteins and bcl-2 protein concentrations was significant, this was to be expected because genes ultimately code for protein, however, what was not expected is that the SNP's of interest were not associated with mRNA transcription thus protein synthesis. While our SNP's had a significant relationship with bcl-2 protein concentrations and mechanism by which our SNP's are functioning, even in the surrogate role as a tagging SNP, has yet to be fully elucidated; mRNA

stability maybe one function. The neurometabolites of lactate, pyruvate and LP ratio were empirically associated with BCL-2 genotypes. On the biological level we would expect this because bcl-2 and these neurometabolites reside in the mitochondria, and that the neurometabolites which are associated with the metabolism of the organelle and is effected by Ca2+ , that the energy balance of ATP/ ADP and Ca2+ influx also effects the functioning of bcl-2. Cerebral ischemia, a consequence of secondary brain injury, is related to the reduction or complete loss of CBF (McLaughlin & Marion, 1996). The literature asserts that for bcl-2 knockout mice, homozygous knockout mice have significantly larger infarct area more severe reduction in CBF (Hata et al., 1999). After brain injury, ischemic changes result in the release of cytochrome c leading to apoptosis (Zhao, Steinberg, & Sapolsky, 2007). The CBF analyses findings support that BCL-2 genotypes are significantly associated with CBF.

A pattern of significant SNP's was revealed in the globals function outcomes, neurometabolite and CBD studies. The pieces of the BCL-2 gene coded for by the blocks of DNA tagged by the significant SNP's at the 5' end (rs17759659, rs949037, and rs1801018) may act as surrogates for exon 2. The initiation codon sires are in the exon 2 region at amino acids -31 and -58 which are associated with the structural flexible loop domain which is associated with phosporylation (Bruckheimer, Cho, Sarkiss, Herrmann & McDonnell, 1998; Reed, 1997). Phosporylation in the loop is required for bcl-2's to function in an anti-apoptotic manner at its full capacity (Ruvolo, Deng, & May, 2001).

5.7 FUTURE DIRECTIONS

This study was a first step in exploring BCL-2 genotypes and how they affect both acute care biological/ clinical outcomes and long term neuropsychological outcomes after severe TBI in adults. Future directions in original research studies or secondary analyses should assess:

1. interaction of SNP's and covariates

2. analyzing the SNP with biological/ clinical marker and an outcome measure (i.e. SNP/ Bcl-2 protein concentration/GOS)

3. exploring gene/gene interaction of anti- versus pro-apoptosis genes (i.e., BCL-2 versus BAX).

4. amino acid sequence versus the genomic sequence to further elucidate the surrogate function of the tagging SNP

5. additional tagging SNP's should be considered in the analyses, as the 17 SNP's used in this study only covered the surrogate function of 1 of the 3 known exons: exon 2

6. the hypothesis that the SNP's, which are positioned in the intron region and cover surrogate function of other SNP's in the intron region, are related to mRNA instability needs to be explored empirically

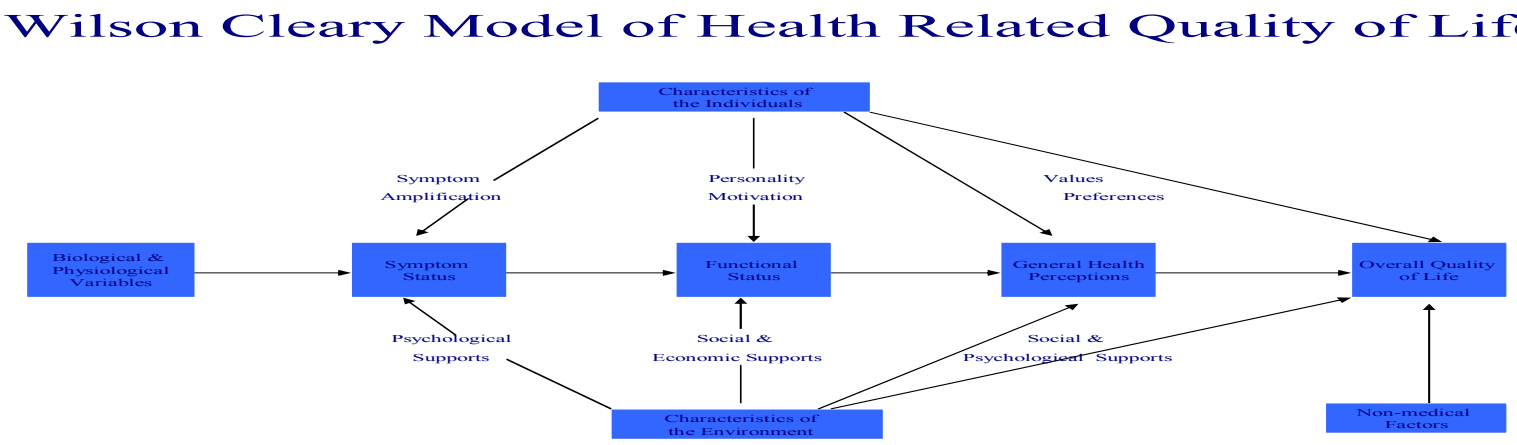
7. additional clinical dependent variables should be examined (i.e. intracranial pressure, cerebral perfusion pressure, admission pupillary reaction)

8. this study should be expanded to other TBI populations, (i.e. pediatrics) and to other neurotrauma conditions (i.e. traumatic spinal cord injury)

9. *in vitro* studies of BCL-2 variants

APPENDIX A

WILSON CLEARY MODEL



Relationship among measures of patient outcomes in health-related quality of life conceptual model; Wilson Cleary Model.

Wilson & Cleary (1995)

APPENDIX B

IRB APPROVAL MEMO

University of Pittsburgh
Institutional Review Board

Exempt and Expedited Reviews

University of Pittsburgh FWA: 00006790
University of Pittsburgh Medical Center: FWA 00006735
Children's Hospital of Pittsburgh: FWA 00000600

3500 Fifth Avenue
Suite 100
Pittsburgh, PA 15213
Phone: 412.383.1480
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TO: Ms. Nicole Zangrilli Hoh

FROM: Christopher M. Ryan, Ph.D., Vice Chair

DATE: October 4, 2006

PROTOCOL: BCL-2 Genotypes and Outcomes after Traumatic Brain Injury

IRB Number: 0607083

The above-referenced protocol has been reviewed by the University of Pittsburgh Institutional Review Board. Based on the information provided in the IRB protocol, this project meets all the necessary criteria for an exemption, and is hereby designated as "exempt" under section 45 CFR 46.101(b)(4).

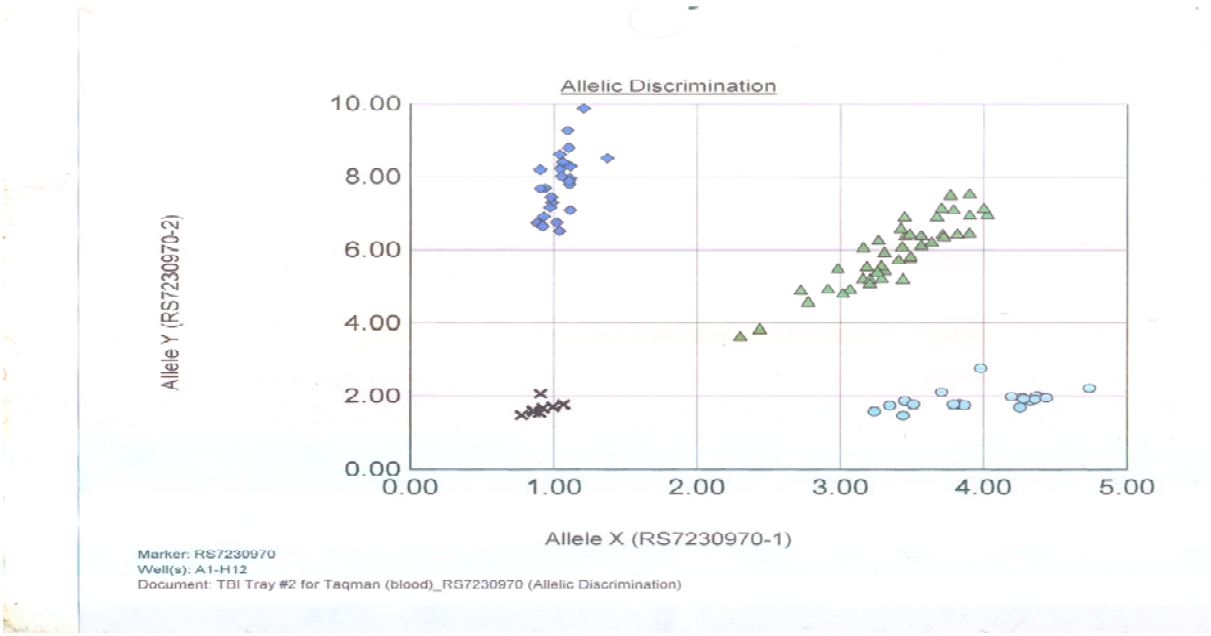
- If any modifications are made to this project, please submit an 'exempt modification' form to the IRB.
- Please advise the IRB when your project has been completed so that it may be officially terminated in the IRB database.
- This research study may be audited by the University of Pittsburgh Research Conduct and Compliance Office.

Approval Date: October 4, 2006

CR:kh

APPENDIX C

EXAMPLE OF TAQMAN® PLOT



APPENDIX D

GLASGOW OUTCOME SCALE (GOS)

Glasgow Outcome Scale	
1	DEAD
2	VEGETATIVE STATE Unable to interact with environment; unresponsive
3	SEVERE DISABILITY Able to follow commands/ unable to live independently
4	MODERATE DISABILITY Able to live independently; unable to return to work or school
5	GOOD RECOVERY Able to return to work or school

APPENDIX E

DISABILITY RATING SCALE (DRS)

Patient Name_____
Rater_____
Date Completed_____

Disability Rating Scale (DRS)

Arousability, Awareness, & Responsivity

Eye Opening
- ❑ 0 Spontaneous
- ❑ 1 To Speech
- ❑ 2 To Pain
- ❑ 3 None

Communication Ability
- ❑ 0 Oriented
- ❑ 1 Confused
- ❑ 2 Inappropriate
- ❑ 3 Incomprehensible
- ❑ 4 None

Motor Response
- ❑ 0 Obeying
- ❑ 1 Localizing
- ❑ 2 Withdrawing
- ❑ 3 Flexing
- ❑ 4 Extending
- ❑ 5 None

Cognitive Ability for Self Care Activities
Knows how and when to feed, toilet or groom self

Feeding
- ❑ 0.0 Complete
- ❑ 0.5
- ❑ 1.0 Partial
- ❑ 1.5
- ❑ 2.0 Minimal
- ❑ 2.5
- ❑ 3.0 None

Toileting
- ❑ 0.0 Complete
- ❑ 0.5
- ❑ 1.0 Partial
- ❑ 1.5
- ❑ 2.0 Minimal
- ❑ 2.5
- ❑ 3.0 None

Grooming
- ❑ 0.0 Complete
- ❑ 0.5
- ❑ 1.0 Partial
- ❑ 1.5
- ❑ 2.0 Minimal
- ❑ 2.5
- ❑ 3.0 None

Dependence on Others

Level of Functioning
Physical & cognitive disability
- ❑ 0.0 Completely Independent
- ❑ 0.5
- ❑ 1.0 Independent in special environment
- ❑ 1.5
- ❑ 2.0 Mildly Dependent-Limited assistance *Non-resident helper*
- ❑ 2.5
- ❑ 3.0 Moderately Dependent-moderate assist *Person in home*
- ❑ 3.5
- ❑ 4.0 Markedly Dependent *Assistance with all major activities, all times*
- ❑ 4.5
- ❑ 5.0 Totally Dependent *24 hour nursing care*

Psychosocial Adaptability

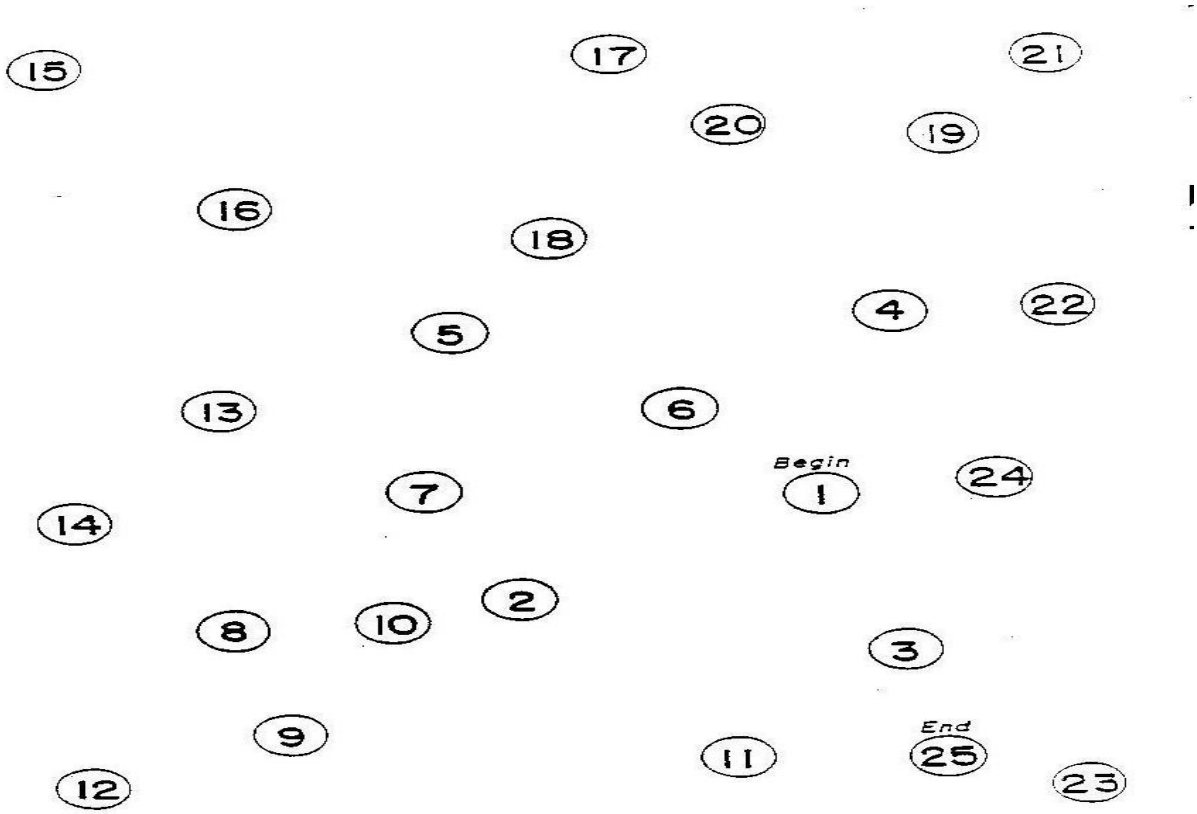
Employability
As full time worker, homemaker, student
- ❑ 0.0 Not Restricted
- ❑ 0.5
- ❑ 1.0 Selected jobs, competitive
- ❑ 1.5
- ❑ 2.0 Sheltered workshop, Noncompet.
- ❑ 2.5
- ❑ 3.0 Not Employable

Total Score (sum all scores) _____

Revised 2/99 Santa Clara Valley Medical Center

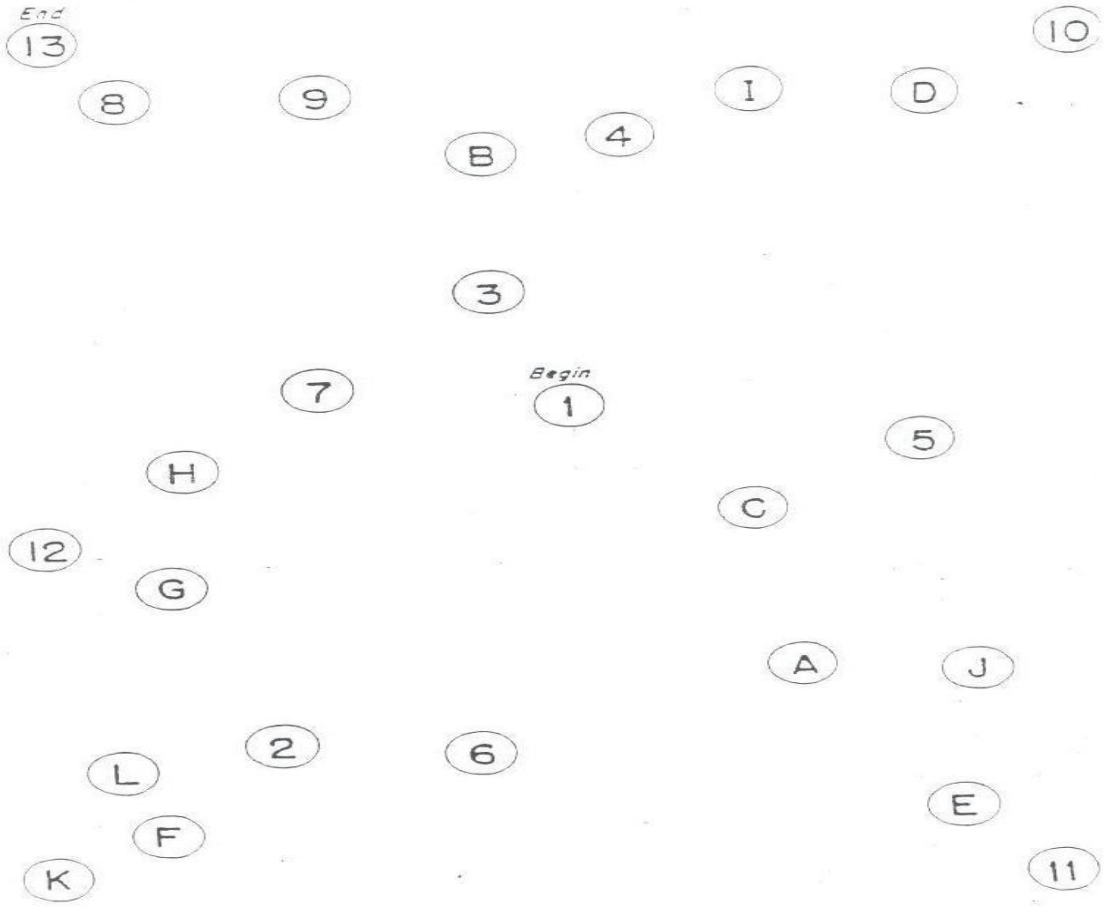
APPENDIX F

TRAILS A



APPENDIX G

TRAILS B



APPENDIX H

GLASGOW COMA SCALE (GCS)

GLASGOW COMA SCALE

Patient Name: _____
Rater Name: _____
Date: _____

Activity		Score
EYE OPENING		
None	1 = Even to supra-orbital pressure	
To pain	2 = Pain from sternum/limb/supra-orbital pressure	
To speech	3 = Non-specific response, not necessarily to command	
Spontaneous	4 = Eyes open, not necessarily aware	_____
MOTOR RESPONSE		
None	1 = To any pain; limbs remain flaccid	
Extension	2 = Shoulder adducted and shoulder and forearm internally rotated	
Flexor response	3 = Withdrawal response or assumption of hemiplegic posture	
Withdrawal	4 = Arm withdraws to pain, shoulder abducts	
Localizes pain	5 = Arm attempts to remove supra-orbital/chest pressure	
Obeys commands	6 = Follows simple commands	_____
VERBAL RESPONSE		
None	1 = No verbalization of any type	
Incomprehensible	2 = Moans/groans, no speech	
Inappropriate	3 = Intelligible, no sustained sentences	
Confused	4 = Converses but confused, disoriented	
Oriented	5 = Converses and oriented	_____
	TOTAL (3–15):	_____

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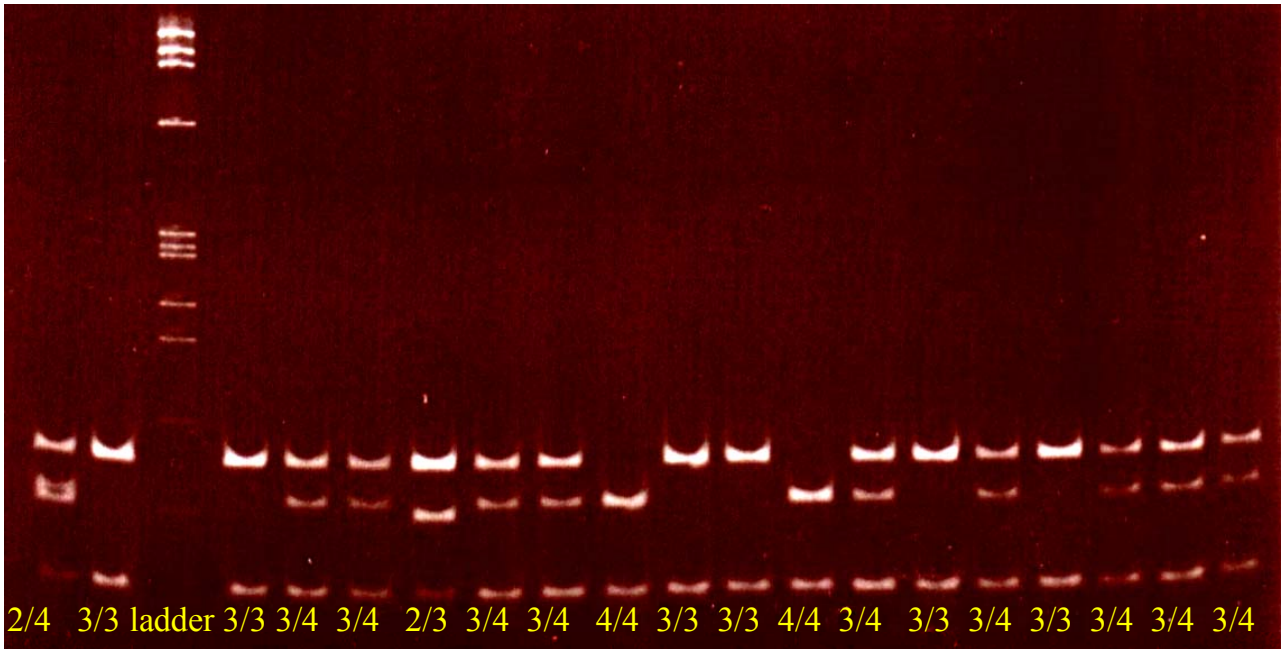
APPENDIX I

EXAMPLE OF APOE GENOTYPE COLLECTION

DV: APOE GENOTYPES



Restriction Fragment Length Polymorphism (RFLP)

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