Monoaminergic and Neurotrophic Gene Variation Associated with Fronto-Limbic Circuitry affect Mood and Cognitive Recovery Post-TBI

by

Michelle D. Failla

BS, Truman State University, 2007

Submitted to the Graduate Faculty of

University of Pittsburgh in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2014

UNIVERSITY OF PITTSBURGH

SCHOOL OF MEDICINE

This dissertation was presented

by

Michelle D. Failla

It was defended on

November 10, 2014

and approved by

Dr. Kirk Erickson, PhD

Dr. Etienne Sibille, PhD

Dr. Yvette P. Conley, PhD

Dr. C. Edward Dixon, PhD

Dr. Jordan Grafman, PhD

Dissertation Advisor: Dr. Amy K. Wagner, MD,

Associate Professor of Phvsical Medicine and Rehabilitation

MONOAMINERGIC AND NEUROTROPHIC GENE VARIATION ASSOCIATED WITH FRONTO-LIMBIC CIRCUITRY AFFECT MOOD AND COGNITIVE RECOVERY POST-TBI

Michelle D. Failla, PhD

University of Pittsburgh, 2014

Following traumatic brain injury (TBI), ~80% of individuals will experience cognitive deficits, and ~50% will experience post-TBI depression (PTD). Identifying individual risk patterns for these complications is important for preventive treatment and early intervention. In uninjured populations, individuals with depression have distinct accompanying cognitive deficits. Importantly, dysregulation of fronto-limbic regions may, in part, explain the co-occurrence of both depressive and cognitive symptoms. The work presented investigates biological factors that influence survival after severe TBI, and among survivors, the presence and severity of PTD and/or cognitive deficits. The scientific framework under which this work was completed proposes targeted interactions between monoaminergic and neurotrophic gene variation that may lead to cognitive deficits and depressive symptoms. In addition to cognition, serotonergic (5-HT) and dopaminergic (DA) signaling both contribute to depressed mood and anhedonia, suggesting genetic variation in their signaling pathways may modulate PTD risk. Brain-derived neurotrophic factor (BDNF), a ubiquitous neurotrophin involved in neuronal survival and synaptic plasticity, is implicated in depression and cognitive dysfunction, and BDNF interacts with 5-HT/DA signaling in mood and cognitive processes. The work presented examines monoaminergic-neurotrophic biomarkers for predicting PTD risk and cognitive deficits. Serum and cerebrospinal fluid (CSF) BDNF levels, and fronto-limbic atrophy, were examined as possible biomarkers of PTD and cognitive deficits. A battery of targeted genes were examined for their proposed roles in survival, depression, cognition, and/or modulation of fronto-limbic connectivity. The data show variation within monoaminergic genes was associated with PTD incidence (serotonin transporter, 5-HTTLPR) and cognitive deficits post-TBI (dopamine D2 receptor, *DRD2* and *COMT*). When investigating BDNF associations with PTD, we discovered that variation in *BDNF* interacts with

age to influence TBI survival, and acute BDNF levels were consistent biomarkers for TBI survival. Among TBI survivors, acute BDNF levels were associated with chronic cognitive performance and depressive symptoms severity, suggesting early neurotrophic support may facilitate chronic recovery. Investigating fronto-limbic regional brain volumes identified significant relationships to PTD and suggested non-uniform fronto-limbic atrophy patterns that may explain PTD susceptibility. Overall, this work supports that monoaminergic-neurotrophin genetic variability affects individual risk for PTD and related cognitive deficits, possibly through relationships with fronto-limbic circuitry.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

There are a number of people for which I must thank for their support during the writing of this dissertation. First, I must thank the CNUP, the CNBC, and all the support staff that keep us all moving along. I was lucky to have a wonderful, supportive dissertation committee. I was also lucky to conduct this research in a fantastic environment, especially because of the people I got to work with everyday: Lauren Kessler, Megan Miller, Anne Ritter, Raj Kumar, Matt Diamond, Jen Boles, Kristin Graham, and Shannon Juengst. I was privileged to mentor a number of amazing students, like Akash Goyal and John Myrga. Finally, I had the wonderful honor of being Dr. Amy Wagner's first graduate student. I have to thank her for helping me get to this point and providing so many learning experiences!

More importantly, I must thank my friends and family. Nicole Scheff has been a fantastic, supportive, and most importantly, sometimes the only person who knows what to say to keep me sane. Jamey Maniscalco and Caitlin Kirkwood, for all the late nights studying in first year and for the continued support and friendship. My family has been incredibly understanding and guided me in ways I cannot express. Most importantly, my amazing husband, Tony, for cooking me dinner when I couldn't stop writing, for listening at all hours of the night, and for being my constant partner in life.

1.0 INTRODUCTION

Traumatic brain injury (TBI) results in either a transient or permanent neurological deficit resulting from mechanical insult to the brain due to an external force. Approximately 1.7 million people per year in the US sustain a TBI ("CDC - Injury - TBI - TBI in the US Report," n.d.). An estimated 5.3 million Americans are currently living with disabilities resulting from TBI, with an estimated national cost to society of $48.3 billion per year. As there is a spike in incidence during late adolescence/young adulthood ("CDC - Injury - TBI - TBI in the US Report," n.d.), the incurred difficulties resulting from a TBI can lead to decades of lost opportunities for employment and productivity, significantly burdening survivors and their families. Several factors influence patient reintegration in society and quality of life following injury, but cognitive deficits and depressive symptoms can have the greatest impact on recovery. Following a TBI, 80% of individuals will experience cognitive deficits (McCullagh and Feinstein, 2004). Similarly, individuals with TBI are at a significantly greater risk for depression compared to the general population. In the U.S., 6.7% of the general population will experience an episode of major depression each year (Kessler et al., 2005), while the rate of depression in TBI ranges from 10-77% within the first year post-injury(Rosenthal et al., 1998); a recent, well characterized study reported a rate of 53% (Bombardier et al., 2010a). Further, those with PTD are at increased risk for suicide (León-Carrión et al., 2001). With the addition of an increasing rise in TBI among the U.S. military population,

where TBI has become the ‘signature wound’, understanding cognitive deficits and depression following injury will be increasingly important.

Identifying risk for complications in an individual’s recovery is integral in development of effective treatment and early intervention. Risk assessment tools are currently lacking and clinical and injury-related variables provide little insight. In the work described in this narrative, survival, mood, and cognitive recovery post-TBI is studied across multiple disciplines and modalities to discern relevant biological mechanisms behind post-TBI depression (PTD) and associative cognitive deficits, in order to aid in identifying and treating an at-risk, highly complex, population. The results of this work will advance both clinical investigation into monoaminergic and neurotrophin pathology of TBI as well as basic research into fronto-limbic structural vulnerabilities and their involvement in depressive symptomology and cognitive impairments post-TBI.

1.1 CHRONIC RECOVERY FOLLOWING TBI

The effects of TBI are heterogeneous, and in addition to damage from the direct injury, the mechanical disruptions associated with TBI facilitates the development of secondary pathologic processes in the brain, including inflammation, excitotoxicity, ischemia, edema, and many chronic secondary signaling changes (Park et al., 2008). Traditionally, treatments and translational research in TBI, has focused primarily on acute neurological injury and neuroprotection. Yet, therapies that are neuroprotective in experimental TBI, have consistently failed to translate into successful treatments in the clinical setting (Ikonomidou and Turski, 2002; Narayan et al., 2002). This apparent disconnect may be due to heterogeneity in injury as well as a lack of consideration

in preclinical studies for important covariates like age or sex. However, one of the most critical limitations of successfully translating experimental research to clinical trials is the lack of understanding of TBI as a chronic, yet rehabilitation sensitive, disease.

To date, there has been a lack of emphasis in the literature on mechanisms of chronic dysfunction after TBI. Contemporary research now suggests that TBI, even mild, concussive injuries, is not simply a transient and static syndrome from which people recover, but rather a chronic and evolving disease state. Our own work suggests that TBI pathology has a dynamic time course in which secondary complications and symptoms can arise and require ongoing management(Failla et al., 2014). Within this chronic disease framework, therapeutic plasticity and recovery mechanisms interplay with ongoing neurodegenerative and other chronic state pathology to affect symptoms, complications, and function (Dixon et al., 1999; S.N. Niogi et al., 2008; Sidaros et al., 2008). Common functional impairment and disability following TBI can vary and lead to faster recovery patterns (months for motor function (Katz et al., 1998)) compared to longer recovery patterns (years for cognitive/mood recovery (Hammond et al., 2004)). This evolving understanding of the dynamic nature of TBI requires a paradigm shift in both experimental and clinical TBI studies and understanding of chronic states of TBI pathology and associated sequelae.

The work described here focuses on closed head traumatic brain injuries, (as opposed to those that penetrate the skull), and most of the resultant injuries are wide-spread with complex, evolving diffuse axonal injury (DAI). Thus, these injuries tend to follow a multi-stage injury/recovery time-line with three important stages: primary injury (disruption and physical injuries to brain tissue), secondary injury (induction of biochemical cascades resulting in dysfunction and apoptosis), and a chronic repair state characterized by both degeneration and reorganization of the brain. The timeline for these phases is likely tied to severity of injury. The

primary injury complex TBI itself is the result of biomechanical forces, complicated by contact between the brain tissue and the skull (resulting in compression of tissue at the impact and stretching of tissue at the opposite pole), and angular rotation. Angular rotation occurs even with linear blows to the head as the brain is tethered to the base of the skull at medial, inferior frontal regions at the base of the brain (near and including the pituitary stalk) (Bayly et al., 2005). This rotational force often results in DAI. DAI results from microstructural damage of axons resulting in axonal disruptions and disconnections (Carrera and Tononi, 2014; Hernández, 2006). Importantly, longer axonal tracts in the brain are more susceptible to these rotational forces (Sabet et al., 2008). Due to the inherent vulnerability in the brain, it is likely that injury severity plays a role in the development and degree of the resulting DAI. Severity of injury is classically measured by the Glasgow Coma Scale (GCS (Teasdale and Jennett, 1974a)) score, taken acutely at varying time-points following the injury. While GCS has been shown to be related to a number of acute and chronic dysfunctions (McNett, 2007; Udekwu et al., 2004a), there is no significant evidence of injury severity relating to PTD (Maller et al., 2010).

Even with the association between injury severity and chronic complications, there is difficulty with chronic complication prognostication that precludes clinician abilities to define early interventions. The population with TBI can be quite heterogeneous; individuals can have markedly different outcomes despite similar injury types and/or clinical management complicating standard treatment development. This is especially true when examining chronic complications like mood and cognitive recovery post-TBI, where dynamic and temporal variation in the pathophysiology associated with TBI can alter the mechanisms associated with recovery.

1.1.1 Post-TBI Depression

PTD sufferers comprise a large subset of those with depression. Importantly, there is evidence to suggest a unique pathology in PTD compared to major depressive disorder (MDD), yet there is a limited understanding of the unique underlying biological mechanisms that lead to PTD. Many features of major depression, including apathy, insomnia, psychomotor agitation or retardation, slowed thinking, poor concentration, and fatigue are reported frequently after TBI. As these symptoms can also be features of TBI, MDD, pre-injury functioning, or medication side effects, it can be difficult to discern causation. Several hypotheses have been produced regarding this alarming rate of depressive symptoms following TBI including the presence of premorbid depression/mood disorders, location of the injury (damage to fronto-limbic or serotonergic neurons), and/or a psychogenic reaction to the injury. While individuals with premorbid mood disorders are at greater risk for depression following TBI (**Chapter 2, 3**), this does not explain the substantial risk seen in individuals with no history of mood disorders and may suggest different risk factors stratified by premorbid history. Several other factors have been suggested to indicate risk for PTD such as lower education, unemployment, and minority status (Jorge and Arciniegas, 2014). Yet, again, this does not explain the still increased risk in individuals with none of these identified risk factors.

Interestingly, there has not been conclusive evidence to suggest that location of injury predicates PTD development. While some studies support the notion that injury foci in left frontal/medial regions relate to PTD development (Jorge et al., 2004), this is not conclusive and, again, does not explain susceptibility in a large number of PTD sufferers (Maller et al., 2010). In the experimental literature, there is a lack of anatomical relationships to mood complications post-TBI, but this may be due to the paucity of research specifically assessing mood complications post-

TBI. One study has shown depressive-like behavior following frontal lobe damage in an experimental TBI model (Moritz et al., 2014).

Other theories posit that a subject's awareness of their injury induced deficits predicts development of PTD (Malec et al., 2010). Yet, this theory of a proposed reactive PTD has been called into question. Interestingly, PTD can develop at early chronic time-points (1 month post-injury) before a patient may fully realize cognitive/functional deficits (Bombardier et al., 2010a). Consistent with the clinical studies, there is evidence in experimental models that depressive-like symptoms are present early after injury (Cope et al., 2011; Fenn et al., 2013) and persist (Washington et al., 2012). While awareness of deficits may be a factor in PTD, it does not fully explain the etiology of PTD. Similarly, current leaders in the field have hypothesized that, due to awareness of deficits theory, subjects with more mild injuries would have greater incidence of PTD, but this has not had consistent support in the literature (Maller et al., 2010). In experimental TBI, there is also no supported relationship between injury severity and development or severity of depression or anxiety (Washington et al., 2012).

As there is not conclusive evidence supporting PTD mechanisms or risk, it is not surprising that there is a severe lack of efficacious treatment paradigms for PTD. Treatments used for Major Depressive Disorders (MDD) are not necessarily effective in the context of PTD (Warden et al., 2006). The most common treatments include antidepressants, yet, recent studies show reduced efficacy and increased adverse events, especially with selective serotonin reuptake inhibitors (SSRI) (Fann et al., 2009a; Lanctôt et al., 2010; Lee et al., 2005). Recent work examining physical activity as a possible therapeutic in PTD has shown promising results (Hoffman et al., 2010; Wise et al., 2012). This lack of effective treatments may indicate unique pathologies involved in PTD

development compared to MDD, emphasizing the need for a greater understanding of the underlying mechanisms of PTD in order to aid treatment.

1.1.2 Post-TBI Cognition

Cognitive impairment post-TBI can impact return to work and community reintegration (Jennifer Fleming, Leigh Tooth, Mary, 1999). Cognitive dysfunction following TBI is the most common complaint cited, even years after TBI (Hoofien et al., 2001). Multiple neuropsychological domains of cognition are impaired following TBI, including attention, arousal, memory, and executive control. Cognitive deficits mirror severity of injury as indicated by the extent of DAI, generalized atrophy, and the location and extent of focal injuries (Katz and Alexander, 1994; Wilson et al., 1995).

Studies have demonstrated that following TBI, individuals with depressive symptomology perform worse on a number of cognitive measures (Bornstein et al., 1989; Gfeller et al., 1994; Rapoport et al., 2002; Satz et al., 1998), across domains (attention, memory, and executive function). We report important findings regarding unique patterns of PTD-associated cognitive deficits in **Chapter 2**. Many symptoms overlap between TBI-related cognitive deficits and PTD like slowed thinking and poor concentration. Similarly, symptoms like apathy, insomnia, psychomotor agitation or retardation, and fatigue are reported frequently after TBI and can influence mood and cognition. For individuals with TBI, remittance of depressive symptoms may lead to improved cognitive performance (Fann et al., 2001), suggesting overlapping biological pathways or at least interacting symptomology.

Memory problems are common following TBI. Explicit memory involves the conscious acquisition and retention of new information, while implicit learning involves skill or knowledge

acquisition without explicit awareness of how the material is learned. Explicit learning-memory is hippocampal dependent (Brandeis et al., 1989), and hippocampal damage is associated with reproducible deficits in spatial and temporal memory processing. Explicit learning-memory is vulnerable to the effects of TBI, while implicit learning-memory relatively resistant to impairment because it is hippocampal independent and involves both cortical and subcortical regions (Buckley, 2005; Vakil, 2005). Implicitly learned information is often considered relatively "inflexible", where carry-over of implicitly learned knowledge can be limited (Schmitter-Edgecombe, 2006). Cognitive rehabilitation interventions appear to improve attention, memory, social communication skills, and executive function (Cicerone et al., 2011). In the clinical population, cognitive rehabilitation paradigms have shown significant promise but have not been subjected to the rigor of experimental studies to characterize their mechanisms of action, effectiveness, or capacity to enhance efficacy of other interventions (Cicerone et al., 2011). Importantly, implicit tasks are more resistant to external factors like anxiety or fatigue, yet interference with explicit feedback during implicit learning can adversely affect task performance after TBI (Vakil, 2005). Thus, understanding how mood can impact cognitive rehabilitation will be important for successful cognitive rehabilitation.

In uninjured populations, individuals with depression also have distinct accompanying cognitive deficits, likely due to common pathology. Individuals with depression consistently show working memory deficits and reduced cognitive flexibility and complain of memory problems and difficulty concentrating (Gotlib and Joormann, 2010). Several studies suggest an attentional bias to negative stimuli, consistent with an emotional congruence theory (Peckham et al., 2010). Importantly, individuals with depression show greater attention or distractibility by negative stimuli, possibly explaining propensity for rumination (Joormann et al., 2006). Similarly, this lack

of task-switching ability may indicate a loss of cognitive control in individuals with depression (Fales et al., 2008). Individuals in TBI often exhibit difficulties in cognitive control (cognitive flexibility, inhibition, and task switching) that could further aggravate these predispositions to negative stimuli or explain relationships to PTD.

While the resultant cognitive impairments following TBI have been well-studied (Levin et al., 1982), identifying individual patterns in recovery and response to rehabilitation is still difficult. In fact, the recent international guidelines for cognitive rehabilitation post-TBI (Bayley et al., 2014), emphasize the need for individualized cognitive rehabilitation paradigms. With regard to cognitive recovery post-TBI, numerous acute care clinical trials have been negative, yet all of these trials failed to examine how genetic factors interplay in TBI recovery. Understanding the unique relationships between cognition and mood recovery may aid in treatment identification.

1.2 INTERACTING MONOAMINERGIC AND NEUROTROPHIC SIGNALING: IMPLICATIONS FOR PTD AND COGNITIVE DEFICITS POST-TBI

The overall goal of the work presented here to identify individual factors that influence the presence and severity of cognitive deficits and/or PTD concurrently. In this vein, we examined genetic variation that, through modulation of monoaminergic or neurotrophic signaling, may impact fronto-limbic circuitry, and thus, influence mood or cognition post-TBI. Factors underlying individual variation in recovery, both in depression and cognition, following TBI are poorly understood. We specifically targeted monoaminergic signaling due to the role of serotonin (5-HT) and dopamine (DA) in the hallmark symptoms of PTD, depressed mood and anhedonia (Dunlop and Nemeroff, 2007; Martinowich and Lu, 2008). Similarly, 5-HT and DA signaling modulates

cognitive function(Olvera-Cortés et al., 2008). As TBI induces a hypo-monoaminergic state, genetic variation in 5-HT/DA pathways may then be relevant to recovery. Brain-derived neurotrophic factor (BDNF), a neurotrophin involved in neuronal survival and synaptic plasticity, is implicated in depression and cognition and interacts with 5-HT/DA signaling (Nibuya et al., 1996)·(Guillin et al., 2001). There are major gaps in our understanding of how these pathways converge to influence PTD and associated cognitive deficits post-TBI. We suggest that without studying these interacting pathways, treatments and prevention measures for PTD and cognitive recovery will remain fragmentary and inadequate.

1.2.1 Dopaminergic functioning in TBI recovery

Dopaminergic signaling is critically important for attention and cognitive control. A number of studies implicate DAergic signaling in attention, response inhibition, and working memory (Braver and Cohen, 2000). DA neurons project from the ventral tegmental area (VTA) to subcortical areas like the nucleus accumbens (NAc, also called the ventral striatum) and amygdala, as well as fronto-limbic cortical areas like the dorsolateral prefrontal cortex (DLPFC). DA has long been implicated in reward processing, with strong associations with attentional properties, prediction, stimulus-reward pairing (learning (Graybiel, 2005)), and motivational states for reward acquisition (Berridge and Kringelbach, 2008) that may underlie an organisms ability to experience pleasure. As such, there is mounting evidence supporting a role for DA in depressive symptomology. Some current antidepressants may partially act through improving DA function (bupropion (Cooper et al., 1980)) and some evidence that serotoninergic targeting antidepressants can have partial affinity for DA targets (Damsa et al., 2004). Similarly, there is comorbidity of depression and Parkinson's

disease (with loss of DAergic neurons) (Santamaría et al., 1986). Studies show conflicting results in positron emission tomography (PET) studies of striatal DAT binding (Laasonen-Balk et al., 1999; Meyer et al., 2001) but do tend to suggest reduced DA neurotransmission in depression (Nutt, 2006). Given this evidence, DA is an attractive link between depressive symptomology and cognitive function post-TBI.

Dysregulated DAergic signaling may explain many of the persistent cognitive deficits in TBI (James W Bales et al., 2009). The facets of cognition that seem most influenced by TBI are often associated with frontal lobe areas that have dense projections to the striatum. PET studies characterizing DA function showed D2 binding was correlated to several aspects of cognition, such as working memory in healthy populations (Aalto et al., 2005). Overall, TBI induces a chronic hypo-DAergic state that relates to cognitive deficits even outside of overt damage to DA neurons (James W Bales et al., 2009).

TBI studied have demonstrated a number of lines of DA dysfunction. Single photon emission tomography studies in adult TBI show reduced binding of the dopamine transporter (DAT) at 4-5 months post-TBI (Donnemiller et al., 2000; Scanlon et al., 2009). DAT regulates action of DA neurotransmission, taking up extracellular DA into presynaptic terminals following DA release (Torres et al., 2003); studies in mice lacking DAT suggest DAT is determinant of extracellular DA levels (Perona et al., 2008). Decreased levels of DAT in the prefrontal cortex have also been confirmed in a rat model of TBI (H.Q. Yan et al., 2002), suggesting a compensatory action by DA neurons in order to increase DA signaling post-TBI. In TBI, tyrosine hydroxylase (TH), a rate-limiting enzyme in the synthesis of DA is upregulated in presynaptic terminals in the frontal cortex (Yan et al., 2001) and in the striatum after very severe injury (James W Bales et al., 2009). This finding is also suggestive of a compensatory mechanism in presynaptic DA neurons

trying to increase DA neurotransmission post-TBI. Interestingly, real time neurotransmission studies evaluating stimulated DA release in the striatum still demonstrate decreased evoked DA overflow and altered DA clearance kinetics. The dopamine receptor D2 serves an auto-receptor function in the striatum (Tang et al., 1994). Human single photon emission tomography studies show reduced binding of D2 post-TBI (Donnemiller et al., 2000). Catechol-O-methyl transferase (COMT), an enzyme involved in degradation of DA in the synapse, can also impact DA signaling by modulating active DA. In addition, there is evidence for COMT in executive function following TBI (Lipsky et al., 2005a). Multiple DA agonists, including Methylphenidate, Amantadine, and Bromocriptine have beneficial effects on cognition and place learning post-TBI (James W Bales et al., 2009; Frenette et al., 2012). While methylphenidate (MPD) has been used primarily to aid in attentional processes and overall cognitive recovery post-TBI, there is new evidence of a positive effect on mood in TBI (Kim et al., 2011). Similarly, methylphenidate has shown promise in treating post-stroke depression (Grade et al., 1998; Lazarus et al., 1992).

1.2.2 Serotonergic functioning in TBI recovery

Serotonin (5-hydroxytryptamine, 5-HT) has long been implicated in cognition and mood. Serotonergic projections from the raphe nucleus of the brainstem have wide-spread connections throughout the brain, especially in regions important for mood and cognition. Serotonergic function has long been implicated in depressive symptomology; individuals with depression have reduced 5-HT metabolites in CSF, decreases in serotonin uptake and transporter binding in post-mortem brain tissue and platelets, changes in expression of serotonergic receptors(Owens and Nemeroff, 1994). Similarly, SSRIs target the serotonin transporter (5-HTT), blocking 5-HTT, and effectively increasing 5-HT levels. Acute tryptophan (precursor for serotonin) depletion in humans

results in depressive symptoms (Toker et al., 2010) and cognitive deficits (response inhibition) (Drueke et al., 2013; Rubia et al., 2005). Targeted serotonergic depletion in the marmoset results in deficits in reversal learning (Clarke et al., 2007) and other studies show serotonergic modulation of task-switching and cognitive control (Lamar et al., 2012). Transcription of the 5-HTT gene, coded by *SLC6A4*, is modulated by a variation in the serotonin transporter linked polymorphic region (5-HTTLPR). The *SLC6A4* S-allele (44 bp deletion in the promoter region, 5-HTTLPR) is a risk allele for depression, though results from meta-analyses are mixed (Karg et al., 2011; Risch et al., 2009). The 5-HTTLPR is thought to modulate amygdala reactivity and amygdala connectivity with the anterior cingulate and orbitomedial prefrontal cortex such that S-carriers have increased reactivity of the amygdala and decreased orbitomedial prefrontal cortex control over amygdala activity (Pezawas et al., 2005). However, S-carriers also so show greater cognitive resilience in healthy subjects, performing better in social cognitive measures and attention tasks (Canli and Lesch, 2007).

A recent study implicated 5-HTTLPR in resiliency following TBI (Graham et al., 2013). Trait resiliency refers to individual variation with stress management and mood regulation and reaction within challenging and stressful situations. Serotoninergic signaling impacts resiliency in conjunction with the modulation of the stress response (and thus, modulation of cortisol) (Lesch, 2011; Pariante and Lightman, 2008). Much of the literature on resiliency has focused on stress and reactivity of the HPA Axis. Activation of the HPA axis during stress results in increased levels of cortisol and glucocorticoids that act on the hippocampus (Jacobson and Sapolsky, 1991). Increased exposure of the brain to stress hormones can have lasting depressive effects, immunosuppression, and cognitive impairments (Lupien et al., 2009; Pariante and Lightman, 2008). Importantly, resiliency in individuals is associated with rapid induction of the HPA axis with subsequent

adaptation and termination of this response (de Kloet et al., 2005). This literature suggests that adaptability of this biological signaling could have lasting effects on depressive symptomology and cognitive resiliency.

There is a paucity of literature on how TBI effects the serotonergic system, and relationship of serotonergic function to both mood and cognitive performance post-TBI has not been well studied. Several studies suggest a hypo-neurotransmission state following TBI, including monoaminergic neurotransmitter systems, that likely impacts chronic TBI pathology and cognitive dysfunction (James W Bales et al., 2009). In the acute stages of TBI, rapid increase in extracellular serotonin are followed by a chronic hypo-neurotransmission state(Busto et al., 1997). One study showed serum serotonin profiles in TBI suffers fall below control subject levels during recovery, with even lower levels observed in PTD (Wozniak et al., 2010). While agonists targeting the serotonin 5-HT1a receptor seem to confer some benefit in cognitive recovery in experimental TBI (Kline et al., 2002), SSRIs do not seem to enhance cognitive recovery in animal models, nor do they tend to improve depressive behaviors (Wang et al., 2011). In PTD, some studies have suggested that anatomic location of injury, specifically left orbitomedial frontal lobe lesions that directly affect serotonergic innervation (Jorge et al., 1993), is a major contributor to PTD development, but this pathology is not apparent in a number of PTD cases (Maller et al., 2010).

1.2.3 Neurotrophic support: impact on monoaminergic function and TBI recovery

BDNF, a neurotrophin involved in neuronal survival and synaptic plasticity, affects depression, cognition, and TBI pathology (Martinowich and Lu, 2008). BDNF has a significant role in cognitive and behavioral processes. BDNF is ubiquitous in the hippocampus where it plays a critical role in synaptogenesis and maintenance, particularly in long-term potentiation with

activity-dependent secretion of BDNF (Kovalchuk et al., 2002). Similarly, BDNF may be an underlying substrate for persistent long-term memory storage (Bekinschtein et al., 2008a, 2008b). As such, animal studies with conditional BDNF knock-out mice show impairment in hippocampal-dependent cognition and behavior, but there is a much less established role for BDNF in hippocampal-independent cognition (Bath and Lee, 2006).

In depression, serum BDNF levels are decreased in untreated depression but increase with antidepressant treatment, indicating the viability for BDNF serum levels as a biomarker of depression (Hashimoto, 2010; Karege et al., 2002; Sen et al., 2008). Yet, serum BDNF may be more reflective of BDNF function in the hippocampus and BDNF signaling has differing roles in the hippocampus compared to mesolimbic reward circuits. In the hippocampus, BDNF expression levels are decreased in correlation with stress and depression (Pittenger and Duman, 2007). BDNF signaling in the hippocampus is also implicated in mechanisms of antidepressant treatment (D'Sa and Duman, 2002). In rat models, intracerebral BDNF infusions have antidepressant effects, while decreased BDNF signaling results in decreased hippocampal neurogenesis (Siuciak et al., 1997).

In TBI, serum BDNF is acutely decreased, correlating with severity of injury (Kalish and Phillips, 2010). Hippocampal BDNF is chronically decreased in TBI (Chen et al., 2005). Importantly, therapies thought to increase BDNF expression in the brain, like environmental enrichment (Chen et al., 2005) and exercise (Griesbach et al., 2009) are promising therapies for cognitive recovery post-TBI. Hippocampal BDNF expression is linked to spatial memory in experimental TBI studies (Griesbach et al., 2009).

BDNF interacts with monoaminergic signaling. BDNF can mediate DAergic synaptic plasticity in the striatum, and nucleus accumbens (Guillin et al., 2001). BDNF injections during reward cueing can have a 'stamping-in' effect and can induce reward-seeking behaviors(Lu et al.,

2004). Similarly, BDNF signaling also interacts with serotonergic function. TrkB receptor activation by BDNF increases 5-HT neurotransmission and dendritic branching (Mamounas et al., 2000), (Horch and Katz, 2002). As increased synaptic 5-HT increases 5-HT receptor activation, these receptors signal phosphorylation of cAMP response element binding protein (CREB) (Martinowich and Lu, 2008), (Conti et al., 2002) and induce CREB-mediated increases in BDNF transcription and neuronal survival (Tao et al., 1998), (Walton and Dragunow, 2000).

1.3 NEUROCIRCUITRY UNDERLYING POST-TBI COGNITION AND DEPRESSION

Alterations in fronto-limbic networks are implicated in depression and TBI (Kraus et al., 2007; Maller et al., 2010). In uninjured populations, significant dysregulation in an anatomically interconnected network of fronto-limbic regions are hypothesized to contribute to depression pathology and successful remittance of depression implicates a coordinated restructuring of this circuit (Mayberg, 2003). Consistent with this hypothesis, individuals with major depressive disorder have reduced fronto-limbic volumes in structures like the hippocampus (Sheline et al., 2003), anterior cingulate, and orbitomedial frontal cortex (Koolschijn et al., 2009).

Atrophy is a common issue in TBI (MacKenzie et al., 2002). In PTD, a few studies have examined regionally specific atrophy in fronto-limbic regions like the hippocampus (David F. Tate and Bigler, 2000) and the anterior cingulate (Chen et al., 2008a). Recent work has shown in moderate-severe TBI, the degree of fronto-limbic structure atrophy for the hippocampus and other regions, was associated with PTD (Hudak et al., 2011). Interestingly, their work also shows regional grey matter atrophy is closely linked to damaged WM tracts post-TBI (Warner et al.,

2010) suggesting overall reductions in fronto-limbic connectivity. Recent reviews suggest overlapping brain regions of vulnerability between depression and TBI, including decreased white matter integrity in tracts within fronto-limbic circuits (uncinate fasciculus (UF), cingulum gyrus (CG), and fimbria-fornix (Maller et al., 2010)). The CG, UF, and superior longitudinal fasciculus (SLF) have all been studied in cognitive deficits post-TBI (Turken et al., 2008) and are implicated in circuits relating to depression (Hamani et al., 2011; Mayberg, 2003; Mettenburg et al., 2012). With evidence of reduced structural integrity and connectivity in fronto-limbic circuits post-TBI, we propose possible alterations in functional connectivity of these circuits.

Functional MRI (fMRI) studies also support altered fronto-limbic connectivity in depression and TBI. In depression, studies have identified altered connectivity in circuits containing the anterior cingulate, hippocampus and amygdala (Zeng et al., 2012; Zhu et al., 2012). In TBI, disruption of long-range axons may result is a loss of structural connectivity and subsequently affect functional connectivity (Sharp et al., 2011). Resting-state fMRI studies have identified correlated patterns of activity in a number of brain regions at rest that comprise a default mode network (Greicius et al., 2009). While there are no resting-state fMRI studies in PTD, in a pair of studies by Palacidos et al., resting-state fMRI post-TBI showed altered patterns of fronto-limbic connectivity post-TBI. This study suggested that reduced white matter integrity of the cingulum disrupted the default mode network and correlated with increased connectivity of the frontal portion of the DMN (Eva M Palacios et al., 2013). In this work, cognitive performance correlated to the amplitude of low frequency fluctuations during resting state fMRI (Eva M Palacios et al., 2013). Interestingly, in this study, hippocampal volume was significantly decreased but did not predict cognitive function post-TBI (Eva M. Palacios et al., 2013).

In task-dependent fMRI studies, individuals with TBI show consistent hypoactivation in the frontal lobe during cognitive tasks, and increased bilateral recruitment, suggesting compensatory activation is needed to perform at similar levels to controls on cognitive tasks (Ricker et al., 2001; Sánchez-Carrión et al., 2008). One study showed reduced activation in the medial orbital frontal cortex of control subjects during a working memory task that was attenuated in concussed, depressed individuals (Chen et al., 2008a). These findings may suggest that during cognitive tasks there is interference of altered fronto-limbic networks associated with depressive symptomology following injury.

1.4 WORKING TOWARDS A MONOAMINERGIC AND NEUROTROPHIC HYPOTHESIS FOR RECOVERY POST-TBI

Dopamine, serotonin, and BDNF show alterations in signaling post-TBI that may map to TBI recovery patterns through alterations in function of fronto-limbic circuits. Studies examining targeted monoaminergic-BDNF genes as they relate to regional atrophy and fronto-limbic system disruption in TBI recovery may aid in understanding PTD and cognitive impairment risk post-TBI. The work presented here sought to understand the role of genetic risk within monoaminergic-neurotrophic pathways in PTD and how it relates to neurotrophic support and fronto-limbic atrophy, providing a possible novel mechanism for PTD.

Yet, factors underlying individual variation in recovery following TBI, whether in survival, depression or cognition, are poorly understood. We suggest genetic variation may explain some of this variance. We hypothesize that risk for specific TBI complications (depression and/or cognitive impairment) is modulated by alterations in regionally specific gray matter atrophy post-TBI. As

such, we have selected a battery of targeted genes for their proposed roles in depression, cognition, and/or modulation of fronto-limbic connectivity.

Importantly, we also aim to determine relationships with BDNF serum levels as these levels may be a possible genetically modulated, physiologically relevant biomarker for, survival, PTD, and cognition allowing for high translational potential. In addition to survival, we identify several genetically-modulated intermediate markers for TBI recovery, including PTD, cognitive composite scores, BDNF levels, and structural integrity of fronto-limbic regions.

1.4.1 Monoaminergic and Neurotrophic Genetic Factors in Mood and Cognition Post-TBI

Genetic studies of TBI recovery among survivors are important in order to assess how individual risk profiles affect multidimensional recovery. Similarly, genetic studies can act as a marker of pre-injury risk, though TBI induces a number of epigenetic alterations(Z.-Y. Zhang et al., 2007) that make this interpretation of gene-candidate studies complex. There is evidence of important gene-injury interactions, which make TBI-specific analyses critical. The work presented here proposed exploration of monoaminergic and BDNF genes important for risk in PTD and cognitive recovery post-TBI (see **Table 1** for complete list). We focused on genetic variants for a number of candidate genes thought to be important in monoaminergic function, cognitive function, mood regulation, or fronto-limbic connectivity. Importantly, the majority of these genetic effects have not been examined in PTD.

Table 1. Summary of Monoaminergic and Neurotrophic Candidate Gene Predictions

Gene	Polymorphism	Function; Implications	Prediction to PTD	Prediction to Cognition
SLC6A4	5-HTTLPR	5-HTT expression, fronto-limbic circuitry; depression risk, TBI	S-carriers will show increased PTD risk	S-carriers will show better overall cognitive performance
DAT1	10/9 VNTR	Reduced DAT expression(VanNess et al., 2005); TBI	9/10 heterozygotes will be associated with increased PTD	9- carriers will be associated with decreased cognitive performance
COMT	Val158Met	Reduced COMT activity(Lotta et al., 1995); depression(Baune et al., 2007), TBI(Lipsky et al., 2005a)	Met-homozygotes will be at a greater risk for PTD	Met-homozygotes will have better cognitive performance
DRD2	TaqIA1	Related to D2 binding; depression(Opmeer et al., 2010), TBI(McAllister et al., 2008)	A1-carriers will show reduced risk for PTD	A1-carriers will show better overall cognitive performance
BDNF	Val66Met	Reduced BDNFsecretion(Egan et al., 2003); depression(Martinowich et al., 2007), TBI(Krueger et al., 2011)	BDNF may interact with 5-HTTLPR to modulated PTD	Met-carriers show worse overall cognitive performance

We observe a relationship with *DAT1* on PET binding and cognition (Amy K Wagner et al., 2014), but this has not been investigated in PTD. The 9 allele is associated with decreased DAT expression (Mill et al., 2002) (suggesting a possible reflection of a hypo-dopaminergic state of TBI), yet heterozygotes carry the highest risk in depression (Lopez-Leon et al., 2007). We have also examined genetic associations of the DAT gene (*SLC6A3*, but identified as *DAT1*), specifically of the 40bp variable number of tandem repeats (VNTR) in the 3' UTR in exon 15. The most common alleles, 9 and 10 (although it ranges from 3-11 with additional SNPs within alleles (Miller et al., 2002)), are associated with DAT expression. Specifically, the 10-allele is associated with increased DAT expression(Mill et al., 2002) in control populations. While homozygotes have

been associated with a higher depression risk, 10-homozygotes have higher DA metabolite levels as measured in CSF collected in the acute phase after TBI. Imaging studies in our lab using PET imaging demonstrate reduced DAT binding in 9-allele subjects as measured by ^{11}C-β-CFT in the caudate and putamen. Similarly, 9-allele subjects showed significantly worse performance on measures of executive function post-TBI (Scanlon et al., 2007).

RS4680 is a known functional single nucleotide polymorphism (SNP) in exon 4 of COMT that causes a val/met switch, where val (G)=↑activity=↓DA and more metabolite, and Met (A)=↓activity=↑DA and less metabolite (J. Chen et al., 2004). The Met allele has been associated with depression in a male Swedish population (Åberg et al., 2011), and consistent with the DA hypothesis, met/met individuals make fewer cognitive (perseverative) errors after TBI (Lipsky et al., 2005a). In the *DRD2* Taq1A polymorphism, A1-carriers, with increased basal DA levels (Laakso et al., 2005, p. 2), receptor levels is related to cognitive show worse performance on some cognitive measures following mild TBI (McAllister et al., 2008). We report *DRD2* associations with cognitive composites at 6 and 12 months following moderate to severe TBI [**see Chapter 4**]. While genetic associations with depression and *DRD2* have not been consistent, A2-homozygotes seem to confer the most risk in association with stressful life events (Opmeer et al., 2010), suggesting A1-carriers may also be at less risk for PTD.

We report 5-HTTLPR S-carriers have reduced PTD risk at 6 months post-TBI [**see Chapter 3**]. S-carriers also so show greater cognitive resilience in healthy subjects, performing better in social cognitive measures and attention tasks (Canli and Lesch, 2007), which has been confirmed in a TBI population (Graham et al., 2013). In this vein, we predict that S-carriers would likely show better cognitive performance post-TBI. The *BDNF* gene polymorphism val66met is implicated in several of psychiatric disorders, including major depressive disorder(Martinowich et

al., 2007). *BDNF* has been shown to modulate cognitive recovery post-TBI (Krueger et al., 2011) and response to SSRIs in PTD (Lanctôt et al., 2010). Humans with the BDNF Met allele have relative deficits in episodic memory and abnormal hippocampus activation compared to Val homozygotes (Egan et al., 2003).

In addition to individual gene associations to cognition/depression, there is evidence of epistasis within pairs of targeted genes, suggesting genetic risk profiles that include variation within a number of these genes will be increasingly informative in TBI recovery profile prediction. There is evidence of epistasis between *BDNF* and *DRD2* in personality traits of harm avoidance and novelty seeking (Montag et al., 2010). Similarly, variation within BDNF shows modulation or 'rescue' of 5-HTTLPR effects (Pezawas et al., 2008).

All of our targeted genes (5-HTTLPR (Pezawas et al., 2005), *BDNF* (Kim et al., 2013), *DAT* (Bertolino et al., 2009), *DRD2* (Bartrés-Faz et al., 2002), and *COMT* (Honea et al., 2009)) have shown associations to volume of fronto-limbic regions in healthy populations, often showing epistasis or interacting effects with each other (Montag et al., 2010; Pezawas et al., 2008; Radua et al., 2013). Associations with 5-HTTLPR and frontal-limbic tracts are seen in healthy controls, modulating white matter integrity in the UF (Pacheco et al., 2009) and functional connectivity in fronto-limbic regions (Pezawas et al., 2005). *BDNF* may also influence WM tracts and functional fronto-limbic circuits, but this relationship is less clear (Egan et al., 2003; Kennedy et al., 2009). Additionally, there is evidence of epistasis between the *BDNF* and *SLC6A4* genes in moderating amygdala activation (Pezawas et al., 2005). Interestingly, hippocampal atrophy in major depression tends to be more severe in 5-HTTLPR L-homozygotes (Frodl et al., 2008, 2004) , suggesting a potential 'advantage' for S-carriers.

1.4.2 Biomarker development for PTD and Cognitive Outcomes

The utilization of serum BDNF as a biomarker for PTD could be extremely helpful for clinical evaluation and identification of at-risk subpopulations in TBI. Serum BDNF levels likely provide a genetically modulated biomarker for depression, and possibly PTD. Serum BDNF levels are consistently decreased in depression (Karege et al., 2005). BDNF is able to cross the blood-brain barrier (BBB) in normal conditions (Pan et al., 1998). Consistent with reduced serum BDNF levels being indicative of depressive symptoms, effective antidepressant treatment increases serum BDNF (Sen et al., 2008). Also, the *SLC6A4* and *BDNF* genes influence BDNF levels in both CSF and serum (Bhang et al., 2010). The BDNF Met allele directly affects BDNF secretion, which may affect CSF and/or serum BDNF levels (Egan et al., 2003)·(Lang et al., 2009). Serum BDNF may be an important genetically-modulated tool in understanding neurotrophic support post-injury.

Few studies have examined human BDNF levels after TBI. In adult clinical TBI, acute serum BDNF levels decrease, with a positive correlation to injury severity, within the first 24 hours post-injury (Kalish and Phillips, 2010). Less is known about acute CSF BDNF levels following TBI, but one pediatric TBI study found acute increase in CSF (Chiaretti et al., 2003). In experimental TBI, there is an acute increase in BDNF transcription, yet our lab shows a chronic decrease in hippocampal BDNF protein expression at later time points after experimental TBI (Hicks et al., 1997)·(Chen et al., 2005). Notably, in a study about depression development following stroke, serum BDNF levels were increased during periods of no depressive symptoms, but decreased when research participants presented with clinical symptoms associated with depression (L. Yang et al., 2010), suggesting BDNF levels could be indicative of emerging depressive symptomology in a post-injury state.

Studies have begun to show relationships between serum BDNF levels and cognitive function (Griffin et al., 2011). Specifically in both patient populations and healthy controls (Gunstad et al., 2008), there are relationships to memory and verbal fluency (Dias et al., 2009). Interestingly, individuals with schizophrenia have reduced BDNF levels that recover after weeks of cognitive plasticity training (Vinogradov et al., 2009). Also, animal TBI models show that hippocampal BDNF expression in is correlated to cognitive recovery (Griesbach et al., 2009).

There is some evidence to suggest BDNF levels may also be associated with neural correlates of PTD or cognition. Serum BDNF levels correlates with hippocampal volume in depressed subjects (Eker et al., 2010). In healthy controls, serum BDNF levels correlate with *N*-acetylaspartate, a marker of neuronal integrity (Lang et al., 2007b).

1.5 REHABILOMICS IN SURVIVAL, PTD, AND CONGITIVE DEFICITS POST-TBI

Given the level of complexity in understanding depression and cognition following brain injury, we proposed a Rehabilomics (A K Wagner, 2010) approach to understanding how monoaminergic and neurotrophic signaling may impact depression and cognition following TBI. While we did not intended to examine factors related to survival in this body of work, understanding chronic outcomes like depression and cognitive functioning depend on the course of acute care and survival. In applying the Rehabilomics approach (A K Wagner, 2010; Wagner and Zitelli, 2012), we sought to understand how a wide-range of individual factors (like genetics, age, and sex) that interact with injury parameters that could influence response to rehabilitation and alter outcomes following TBI. It is imperative for clinical studies, in addition to experimental paradigms, to rigorously evaluate important factors in rehabilitation like timing, chronicity of treatment,

maintenance of treatment effects and influences of acute management on chronic care. Much of the work presented here, supports these important caveats. With an integrative approach, the Rehabilomics-framework combined with rigorous experimental models of neurorehabilitation will likely yield rapid improvements in the current state of neurorehabilitation research and clinical care.

Thus, in this work we sought to utilize basic research to identify biomarkers within relevant mechanistic framework that can inform personalized treatments. This is incredibly relevant to understanding complications like depression and cognitive deficits, that are highly interrelated and complex. There is a paucity of research into the unique mechanisms of PTD. With the interactions of acute and chronic factors, impacting mood and cognition post-injury, it will be imperative to examine mood and cognition across recovery utilizing a personalized medicine approach. Novel genetic markers as risk factors in PTD may direct future research in mechanisms behind increased risk for depressive symptoms in the TBI population, specifically directing future animal studies. The work described here we examined genetic risk factors in DA-5-HT-BDNF signaling, allowing us to begin to understand how underlying genetic make-up within interacting pathways influences TBI recovery.

There has been a call for genetic studies in TBI. The TBI population can be quite heterogeneous: patients can have markedly different outcomes despite similar injury types and/or clinical management complicating standard treatment development. This indicates that there is likely variation in the neurobiology associated with both TBI pathophysiology, neurotransmission, and the mechanisms associated with recovery. However, when identifying candidate genes for recovery post-TBI, one important caveat in disease populations, and especially TBI, is to be cognizant of mortality effects. Thus, again, applying a holistic Rehabilomics approach can aid in

identifying individual variation that can affect acute and chronic outcomes. Genetic studies may help to explain some of this heterogeneity and/or aid in identifying particular subsets of the population that are more likely to benefit from a particular treatment.

Results from this work will evaluate how pathways in TBI/PTD interact and allow for new therapeutics for PTD that positively influence interacting, complex systems involved. This work may also be highly important as many rehabilitation paradigms following TBI utilize behavioral and/or cognitive training in PTD therapies. Within this Rehabilomics framework, it is imperative to design studies that not only identify subsets of the injured population who may or may not benefit from a given treatment, but also aim to understand the biological underpinnings of these treatments and disorders as current therapeutics are severely lacking.

2.0 EFFECTS OF DEPRESSION AND ANTIDEPRESSANT USE ON COGNITIVE DEFICITS AND FUNCTIONAL COGNITION FOLLOWING SEVERE TRAUMATIC BRAIN INJURY

Michelle D. Failla, BS; Shannon B. Juengst, PhD;

Patricia M. Arenth, PhD; Amy K. Wagner, MD

2.1 ABSTRACT

Traumatic brain injury (TBI) results in mood and cognitive complications that impact functional recovery. A number of studies have suggested increased presence of cognitive deficits in individuals with post-TBI depression (PTD), but have not examined this relationship in the context of other demographic, medication, or injury effects. We utilize a Rehabilomics framework, to evaluate relationships between PTD and associated demographics (like antidepressant use) on cognitive performance and functional cognition in a prospective cohort with severe TBI. Participants with TBI (n=154) were evaluated for PTD (Patient Health Questionnaire-9), cognitive deficits (cognitive composite score, a battery of 9 neuropsychological assessments) and functional cognition (Functional Independence Measure–Cognition, FIM-Cog) at 6 and 12 months post-injury. Individuals with PTD did not have any measurable cognitive deficits with any of the neuropsychological assessments. However, there were consistent negative effects of antidepressant use across the cognitive battery. Participants with PTD exhibited lower FIM-Cog scores at 12 months post-injury, even after correction for other demographic variables and

cognitive performance. PTD is associated with functional cognitive deficits, but not cognitive performance, with apathy/motivation as likely etiological factors.

2.2 INTRODUCTION

Traumatic brain injury (TBI) is increasingly recognized as a chronic medical condition with accompanying mood and cognitive changes. Both cognitive and mood complications can greatly impact quality of life for patients and influence return to work or school following TBI (Cifu et al., 1997a; Fleming et al., 1999; Juengst et al., 2013; Yasuda et al., 2001). Post-TBI depression (PTD) is the most common neurobehavioral complication following TBI. Individuals with TBI are 10 times more likely than the general population to experience a depressive episode during their first year of recovery (53% (Bombardier et al., 2010a) compared to 6% (Kessler et al., 2005) per 12 months in the general population) and are at greater risk decades later for recurring depressive symptoms (Koponen et al., 2002). Depression can be more debilitating than injury-induced cognitive deficits as one study found that, compared to cognitive deficits, depression was a better predictor of continued disability post-TBI (Whitnall et al., 2006). Importantly, the diagnosis of PTD is of significant concern clinically due to overlap of symptomology with TBI symptoms (slowed thinking, trouble concentrating, fatigue). However studies have shown that measures like the Patient Health Questionairre-9 can be useful in distinguishing between PTD and cognitive deficits (Cook et al., 2011a). Even so, a thorough understanding of the relationship between depression and cognitive deficits post-TBI, especially following more severe injuries, is important for understanding individual functional recovery.

In non-brain injured populations, individuals with depression often have comorbid cognitive deficits, likely due to common pathology (Levin et al., 2007). Meta-analyses indicate individuals with depression consistently have working memory deficits, reduced cognitive flexibility, and complain of memory problems and difficulty concentrating (Gotlib and Joormann, 2010). While individuals with depression report a number of cognitive difficulties, memory deficits are consistently problematic (Burt et al., 1995). Cognitive control, the process defined as directing cognitive processing in the face of ever-changing goals and distractions, is particularly susceptible to mood and motivation (Kouneiher et al., 2009). Interestingly, individuals with depression show the most memory deficits on tasks where cognitive control is necessary (additional attentional components, or timed tests) to focus memory (Hertel, 1998; Hertel and Rude, 1991), suggesting these specific deficits may manifest in functional cognitive impairment. Similar to individuals with depression, individuals with TBI commonly exhibit significant memory, cognitive control, attentional difficulties after their injury (Brooks et al., 1999; Sumit N. Niogi et al., 2008; Perlstein et al., 2006; Vakil, 2005). Similar to hypotheses in uninjured populations, deficits in cognitive control (Fales et al., 2008) (cognitive flexibility, inhibition, and task switching) could further aggravate an attentional bias to negative stimuli (Peckham et al., 2010), increasing risk or duration of depressive symptoms.

Studies consistently suggest that following TBI, individuals with depressive symptomology show worse functional cognition (Bornstein et al., 1989; Gfeller et al., 1994; Rapoport et al., 2002). Apathy, insomnia, psychomotor agitation or retardation, and fatigue are reported frequently after TBI, even in individuals without depression, and can influence cognition(Andersson and Bergedalen, 2002; Bushnik et al., 2008; Larson and Zollman, 2010). For individuals with TBI, one study suggests that remittance of depressive symptoms with

antidepressant treatment can lead to improved cognitive performance (Fann et al., 2001), suggesting overlapping biological pathways or interacting symptomology.

In this study, we sought to develop an inclusive measure of cognitive performance deficits among individuals with PTD, and demonstrate the effect of this relationship on functional cognitive measures. Many of the previous studies that demonstrate worse cognitive performance among individuals with PTD use raw scores obtained from neuropsychological tests and do not correct for demographic or clinical differences in their populations (like age, injury severity, or medication use) (Jorge et al., 2004; Rapoport et al., 2005). To begin to address this gap in the literature, we utilized normative data from healthy populations to adjust raw neuropsychological testing scores for age, education, sex, and race, where appropriate normative were available. Within a Rehabilomics (A K Wagner, 2010) framework aimed at understanding how individual factors integrate to form unique risk profiles for rehabilitation and recovery following TBI, we also examined covariates like age, presence of pre-morbid mood disorders, injury severity, and antidepressant use in associations between PTD and neuropsychological tests. In this study, we evaluated interrelationships between cognitive deficits as examined with neuropsychological tests, functional cognitive impairments, and PTD in the first year following TBI.

2.3 METHODS

2.3.1 Participants

Participants in this study, approved by the University of Pittsburgh's Institutional Review Board, were recruited while receiving care at inpatient and/or outpatient clinics within the University of

Pittsburgh Medical Center (UPMC). All participants sustained a non-penetrating traumatic brain injury (TBI), with evidence of intracranial injury on Computed Tomography (CT). Exclusion criteria included: cardiac arrest prior to admission, documented prolonged hypoxia or hypotension prior to admission, or penetrating TBI. All participants survived for at least one year post-injury and were a subset of a larger study investigating possible biomarkers and genetic factors related to individual recovery following TBI.

Injury severity was described using the best GCS obtained within the first 24 hours post-injury, as the best GCS in 24hrs shows better sensitivity in discriminating cognitive outcomes (Cifu et al., 1997a; Udekwu et al., 2004a). Demographic information, including age, sex, and education, was collected by chart review as well as through participant or caregiver interviews. Similarly, anti-depressant use at 6 and 12 months was extracted from both participant interview and chart review (see **Table 2** for a list of anti-depressants medications considered in this study). Additionally, participants on atypical anti-psychotics were excluded from associations with cognition due to negative effects of these medications on cognitive functioning post-TBI (Elovic et al., 2003; Phelps et al., 2014). A pre-injury history of mood disorders, including depression, bipolar disorder, and anxiety, was established by self-report and chart review.

Table 2. Antidepressant Categories and distribution within population.

Type	Generic	Trade	6 months	12 months
Selective Serotonin Reuptake Inhibitors (SSRI)	Fluoxetine	Prozac	1	3
	Citalopram	Celexa	7	5
	Sertraline	Zoloft	3	4
	Escitalopram	Lexapro	15	9
	Paroxetine	Paxil	3	2
Serotonin Antagonist and Reuptake Inhibitor (SARI)	Trazodone		4	3
Serotonin-Norepinephrine Reuptake Inhibitors (SNRI)	Duloxetine	Cymbalta	1*	4*
	Venlafaxine	Effexor	6	2
	Bupropion	Wellbutrin	1**	1
Noradrenergic and Specific Serotonergic Antidepressant (NaSSA)	Mirtazapine	Remeron	1	1

***Individual was also on Citalopram, **Escitalopram.**

2.3.2 Cognitive Assessment

Participants' functional cognitive impairment was assessed with the FIM-Cog (Dodds et al., 1993) at both 6 and 12 months. FIM-Cog has five component scales: expression, comprehension, social interaction, problem solving, and memory. Each scale is rated from one to seven, with a 5 or lower indicative of need for caregiver assistance. The sum of these five components was considered the FIM-Cog Score.

Cognitive performance was assessed at 6 and 12 months post-injury using a battery of nine neuropsychological tests, targeting 4 domains of cognition (attention, language fluency, memory, and executive function). Trail Making Tests A and B, where participants draw lines between consecutive numbers (Part A) and then between alternating letters and numbers (Part B), was used to measure psychomotor processing speed and cognitive flexibility/task-switching, respectively (RM Reitan and Wolfson, 1985). Digit span, a sub-test from the Wechsler Adult Intelligence Scale-R, measures attention and memory by asking participants to repeat a sequence of numbers forward

and backwards (Glenn J. Larrabee and Curtiss, 1995). Rey-Osterreith Complex Figure Test assesses visuo-spatial episodic memory by asking participants to copy an abstract line drawing from memory (PA Osterrieth, 1944). The California Verbal Learning Test-II (CVLT-II (Delis and et al., 2000)) is a list learning paradigm, with subtests measuring learning, immediate recall, interference, and recognition. Different forms of the CVLT were used at 6 and 12 months to minimize practice effects from repeated administration. The Controlled Oral Word Association (JG Borkowski et al., 1967) and Delis-Kaplan Executive Function Systems (DKEFS) Verbal Fluency assess verbal fluency. In both, participants are asked to name words beginning with a letter (phonemic) or within a subject category (semantic); a third condition in the DKEFS assesses ability to switch between two semantic categories (Category Switching). The Stroop Task (J. R. Stroop, 1935) examines selective attention and cognitive flexibility by asking an individual to name the color of ink a word is printed in, suppressing a habitual response (reading the word) to produce a more effortful response (naming the color of ink). Wisconsin Card Sorting Task (WCST) (Heaton, 1981) requires individuals to sort a series of cards along one of three dimensions, based on verbal accuracy feedback from the administrator, without any information regarding the dimension. The dimension requirements were switched every 10 cards. For this study, perseverative errors were assessed as a measure of executive function.

Raw scores from each test were converted into T-scores using appropriate metrics (i.e education, age, sex, race) based on norms indicated by the test manufacturer. An overall cognitive composite was also utilized, similar to previous studies (see **Chapter 4**). For the cognitive composite, two tests from each domain were used (Attention, Trails A and digit span; Memory, Rey delayed copy, CVLT Long Delay Free Recall; Language Fluency, COWA Animals DKEFS Letter fluency; Executive function, Trails B, Stroop Interference). These tests were selected as

representative measures for their associated domains. T-scores were averaged within each domain to create domain specific sub-scores. To calculate a cognitive composite score, participants had to complete at least one test in each domain. Mean values across domain sub-scores were calculated for the overall cognitive composite score.

2.3.3 Depressive Symptom Assessment

At 6 and 12 months, depressive symptoms were evaluated using the Patient Health Questionnaire-9 (PHQ-9), a brief self-report symptom inventory based on the 9 DSM-IV diagnostic criteria for Major Depressive Disorder (MDD). The PHQ-9 asks participants to rate how often they have experienced symptoms of depression, on a scale between 0 (None) and 3 (Nearly Every Day), over a two-week period. Higher total scores (PHQ-9 Total) reflect a greater number of and/or greater severity of depressive symptoms, with the maximum score being 27. Participants were grouped as "depressed" vs. "non-depressed" using the PHQ-9 questions as they map to DSM diagnostic criteria (previously described) (Fann et al., 2005). For a categorization of depression (PTD), individuals responded positively to at least five symptom questions on the PHQ-9, with at least one pertaining to a cardinal symptom (anhedonia or depression). Compared to the Structured Clinical Interview for DSM Diagnosis (SCID) (Fann et al., 2005), this method has been validated in populations with TBI showing a sensitivity of 93% and a specificity of 89%. Importantly, the PHQ-9 is reliably able to discriminate between chronic TBI and depression symptoms (Cook et al., 2011a).

2.3.4 Statistical Analysis

Data analysis was conducted using Statistical Analysis Software (version 9.4; SAS Institute). Descriptive analysis included mean and standard deviation and/or median for continuous and ordinal variables such as age, GCS, and education. Frequencies were calculated for categorical variables such as sex and antidepressant use. Demographic and relevant clinical information was assessed for relationships with cognitive performance using Student's *t*-tests or ANOVA to compare means. Non-parametric tests (Mann-Whitney and Kruskal-Wallis) were used when appropriate. Pearson's or Spearman's rho (r) correlations were used to assess relationships between two continuous variables. Multivariate linear regression models were used to assess factors influencing cognitive performance or functional cognition. Target variables and covariates were entered into the model and removed in a backwards step-wise fashion when $p>0.2$ to generate final models.

2.4 RESULTS

Specific cohort demographics are shown in **Table 3**. Overall, participants had a GCS (best in 24hrs) of 3-15 (mean GCS, 7.7 ± 2.8, median=7). Participants were aged 16-72 (mean age 34.1±13.8 years) and 18.9% of participants were women. At 6 months post-injury, 38.3% of participants with TBI had PTD, while 30.3% had PTD at 12 months. Participants with PTD tended to have a higher mean age compared to those without PTD ($p=0.061$). Participants with PTD at 12 months tended to have a higher initial best in 24hrs GCS compared to those with no PTD ($p=0.057$), and participants with premorbid mood disorders had higher PTD rates at both 6 (27.9

versus 6.0%, p=0.002) and 12 months (31.4 versus 10.0%, p=0.006). At 6 months, 51% of participants with PTD were on antidepressants while only 26.1% of participants with no PTD were on antidepressants (p=0.007). At 12 months, 40.0% of participants with PTD were taking an antidepressant, compared to 25.0% of those with no PTD (p=0.110).

Table 3. Demographic description of study population.

	Total Population	6 Months			12 Months		
		None (n=71)	PTD (n=44)	p value	None (n=83)	PTD (n=36)	p value
Age, mean±STD	34.1±13.8	32.9±13.8	36.1±13.5	0.061	34.4±13.8	36.5±13.3	0.153
GCS, median	7	7	8	0.493	7	8	0.057
Sex, # (%) Males	177 (83.9)	61 (85.9)	33 (75.0)	0.146	67 (80.7)	27 (75.0)	0.482
Race, # (%) Caucasian	192 (91.9)	67 (94.4)	40 (90.9)	0.485	77 (92.8)	32 (88.9)	0.493
Education, mean±STD	13.0±1.9	13.1±1.9	12.7±1.9	0.178	13.1±1.8	12.7±2.0	0.246
Premorbid Mood Disorders, # (%)		4 (6.0)	12 (27.9)	*0.002*	8 (10.0)	11 (31.4)	*0.006*
Antidepressant Use, # (%)		18 (26.1)	22 (51.0)	*0.007*	20 (25.0)	14 (40.0)	0.110

STD, Standard Deviation; PTD, Post-TBI Depression; GCS, Glasgow Coma Scale

Table 4 summarizes bivariate associations between PTD, neuropsychological tests and FIM-COG at both time-points. Participants with PTD had no significant cognitive deficits at 6 months post-TBI in any individual neuropsychological test administered. At 12 months, participants with PTD had better cognitive performance on the Rey immediate copy test (p=0.027) compared to participants with no PTD. There were no other significant associations between neuropsychological tests and PTD at 12 months.

Table 4. Bivariate Depression Associations with Cognitive Performance and Cognitive Functioning Post-TBI

			6 MONTHS			12 MONTHS	
Depression		**None**	**PTD**	**p value**	**None**	**PTD**	**p value**
Neuropsychological Tests (mean±SEM)							
Overall Composite		40.8±1.0*	40.4±1.1	0.813	41.7±1.1	40.1±1.8	0.459
CVLT	Total 1-5	33.6±1.7	36.5±2	0.280	34.3±1.5	34.7±2.4	0.871
	SDFR	34±2	37.8±2.5	0.578	36.6±2	36.5±3.2	0.971
	LDFR	35.1±2.1	37±2.6	0.725	37.9±2.2	33±2.9	0.195
Stroop	Interference	53.7±1.4	53±1.3	0.208	54.2±1.1	51.5±1.9	0.214
	Color-word	42.9±1.5	39.8±1.7	0.538	42±1.5	39.2±2.1	0.284
	Color	36.2±1.3	35±1.4	0.725	36.7±1.3	35±1.7	0.455
	Word	34.4±1.3	31.9±1.5	0.212	34.7±1.2	33±1.7	0.431
DKEFS	Cat Total	39.7±1.5	40.7±1.5	0.676	37.2±1.4	34.8±1.8	0.330
	Cat Switch	39.9±1.7	38±2.1	0.490	40±1.7	36.1±2.2	0.187
Rey	Immediate	29.4±1.6	30.3±1.7	0.704	29.5±1.3	35.1±2.4	***0.027***
	Delay	41.8±1.5	42.1±1.7	0.911	43.8±1.4	44.3±2.1	0.833
WCST	Perseverative Errors	49.3±2.3	44.2±2.8	0.163	50±2	52.3±2.9	0.523
Trails A		36±1.7	36.6±2	0.809	36±1.9	36.8±3.4	0.818
Trails B		41.6±1.9	39.4±1.9	0.423	40±2.1	38.6±2.7	0.708
Digit Span	Forwards-Backwards	41.7±1	41.8±1.4	0.946	42.4±1.2	41.8±1.9	0.770
COWA	Animal Category	33±2	34.8±2.4	0.583	34.3±1.7	33.7±3.1	0.860
Functional Independence Measure – Cognition (mean±SEM, median)							
Overall		31.4±0.5, 33	30.6±0.4, 31	***0.018***	31.2±0.5, 33	30.4±0.5, 30.5	***0.015***
Memory		5.9±0.2, 6	5.6±0.2, 6	***0.029***	5.9±0.2, 6	5.6±0.2, 6	***0.020***
Problem Solving		5.9±0.2, 7	5.7±0.1, 5.5	**0.052**	6.1±0.1, 7	5.7±0.2, 6	***0.013***
Social Interaction		6.5±0.1, 7	6.3±0.1, 6	**0.066**	6.4±0.1, 7	6.1±0.2, 6	***0.016***
Expression		6.5±0.1, 7	6.5±0.1, 7	0.366	6.5±0.1, 7	6.6±0.1, 7	0.433
Comprehension		6.3±0.1, 7	6.4±0.1, 7	0.389	6.4±0.1, 7	6.5±0.1, 7	0.343

GCS, Glasgow Coma Scale; PTD, Post-TBI Depression; SEM, Standard Error of the Mean; CVLT, California Verbal Learning Test; SDFR, Short Delay Free Recall; LDFR, Long Delay Free Recall; DKEFS, Delis-Kaplan Executive Function Systems; Cat, Category; WCST, Wisconsin Card Sorting Task; COWA, Controlled Oral Word Association.

Similarly, there was no association between PTD and overall cognitive composite score at either time-point. Participants with PTD had lower FIM-COG scores at 6 ($p=0.018$, median of 31 compared to 33) and 12 ($p=0.015$, median of 30.5 compared to 33) months post-TBI. FIM-COG components were examined for associations with PTD. At 6 months, FIM-Memory was lower in participants with PTD ($p=0.029$). At 12 months, PTD was associated with FIM-Memory, FIM-Problem-Solving, and FIM-Social Interaction ($p<0.02$ all comparisons).

As age, GCS, premorbid mood disorders, and antidepressant use were associated with PTD, we examined PTD associations after adjusting for these variables. Linear regression models were examined for each individual neuropsychological test (**Table 5**). Age and presence of premorbid mood disorders did not prove to be consistent contributors to the models and were omitted. Similar to bivariate analysis described in **Table 4**, PTD was not significantly associated with any of the individual neuropsychological tests except for the Stroop Interference score at 12 months. Interestingly, antidepressant use was a consistent predictor of performance on neuropsychological tests, with antidepressant use being associated with worse cognitive performance on the CVLT, Stroop Color, DKEFS Category Total, Rey delayed copy, and COWA at 6 months ($p<0.05$ for all comparisons) and the Stroop Word, Trails A, and Trails B at 12 months ($p<0.05$ for all comparisons). GCS was also a consistent predictor of performance on multiple individual neuropsychological tests (**Table 5**). We then examined neuropsychological test performance by both PTD and antidepressant use in order to understand possible interactions in predictions of cognitive performance. PTD*Antidepressant Use interactions were examined in the linear regression models present in **Table 5**, but this interaction was not significant in any of the examined models (data not shown).

Table 5. Linear regression models of neuropsychological tests.

		GCS		Antidepressant Use*		PTD**	
		Beta	**p**	**Beta**	**p**	**Beta**	**p**
6 Months							
CVLT	Total 1-5	1.039	***0.016***	-8.339	***0.002***	0.093	0.669
	SDFR	1.207	***0.015***	-11.417	***0.000***	0.130	0.607
	LDFR	0.994	*0.059*	-12.193	***0.000***	0.047	0.856
Stroop	Interference	-0.184	0.622	-1.128	0.601	-0.059	0.724
	Color-word	0.346	0.416	-4.375	*0.077*	-0.062	0.743
	Color	0.280	0.438	-4.216	***0.045***	0.054	0.735
	Word	0.556	0.137	-2.777	0.197	0.023	0.889
DKEFS	Cat Total	0.349	0.345	-6.797	***0.005***	0.243	0.203
	Cat Switching	0.569	0.224	-4.916	*0.098*	-0.066	0.778
Rey	Immediate Copy	0.428	0.332	0.518	0.846	0.227	0.284
	Delay Copy	0.740	*0.066*	-4.938	***0.042***	0.029	0.879
WCST	Perseverative Errors	0.339	0.576	-4.217	0.293	-0.366	0.267
Trails A		1.026	***0.025***	-2.759	0.326	0.085	0.697
Trails B		1.350	***0.005***	-3.958	0.171	-0.080	0.720
Digit Span	Forwards-Backwards	1.057	***0.001***	-2.545	0.142	-0.013	0.923
COWA	Animals Category	0.576	0.317	-8.590	***0.010***	0.288	0.242
12 Months							
		Beta	p	Beta	p	Beta	p
CVLT	Total 1-5	1.16	***0.008***	-3.274	0.235	-0.042	0.855
	SDFR	1.454	***0.012***	-5.814	0.116	-0.416	0.179
	LDFR	1.593	***0.015***	-0.397	0.92	-0.514	0.119
Stroop	Interference	-0.058	0.886	2.447	0.315	-0.387	***0.037***
	Color-word	0.286	0.584	-1.919	0.496	-0.056	0.808
	Color	-0.398	0.375	-2.513	0.299	0.125	0.527
	Word	0.012	0.976	-4.612	***0.036***	0.058	0.742
DKEFS	Cat Total	0.031	0.938	-2.713	0.292	0.063	0.772
	Cat Switching	0.222	0.65	-3.241	0.301	0.089	0.735
Rey	Immediate Copy	0.767	*0.084*	-2.769	0.306	0.149	0.516
	Delay Copy	0.85	*0.059*	-1.647	0.546	0.011	0.962
WCST	Perseverative Errors	0.023	0.971	-1.704	0.668	0.15	0.647
Trails A		1.327	*0.05*	-10.459	***0.01***	0.551	*0.067*
Trails B		0.677	0.32	-10.543	***0.013***	0.497	0.111
Digit Span	Forwards-Backwards	0.197	0.644	-3.548	0.155	0.17	0.383
COWA	Animals Category	0.045	0.947	-7.097	*0.064*	0.381	0.198

*Reference category is no antidepressant;**Reference category is no PTD;
GCS, Glasgow Coma Scale; PTD, Post-TBI Depression; CVLT, California Verbal Learning Test; SDFR, Short Delay Free Recall; LDFR, Long Delay Free Recall; DKEFS, Delis-Kaplan Executive Function Systems; Cat, Category; WCST, Wisconsin Card Sorting Task; COWA, Controlled Oral Word Association.

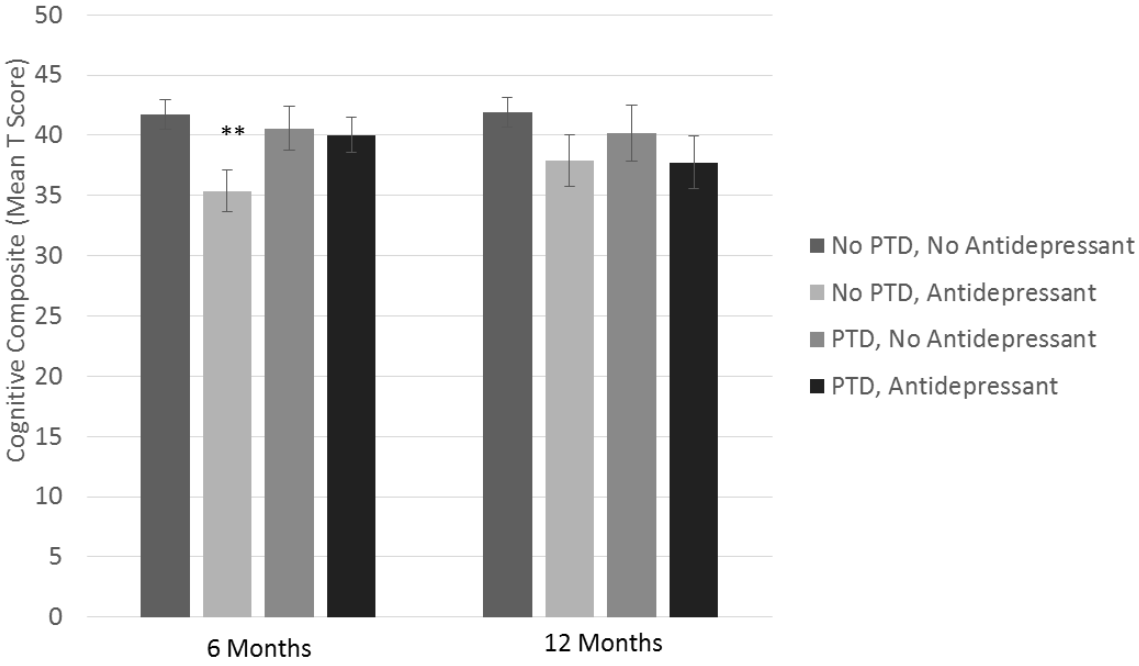


Figure 1. Cognitive composite scores (T score on y-axis) are shown in groups of participants based on PTD and antidepressant use at 6 and 12 months post-injury.

At 6 months, participants with no PTD, but who are on antidepressants perform worse on cognitive composite measures, compared to all other groups (**compared to no PTD, no antidepressant use, p=0.002, compared to PTD, no antidepressant use, p=0.048, compared to PTD, antidepressant use, p=0.050). At 12 months, there were no significant differences.

We examined overall cognitive composite total scores by both PTD and antidepressant use in order to understand possible interactions in predictions of cognitive performance. **Figure 1** shows cognitive performance on cognitive composite scores by PTD and antidepressant use at 6 and 12 months post-TBI. Within subjects with no PTD, those on antidepressants had significantly worse cognitive performance at 6 months (p=0.002). There were no significant effects at 12

months. This interaction, PTD*Antidepressant use, was a trend in our model of cognitive composite scores at 6 months post-injury (**Table 6**), meeting criteria of $p<0.2$ to stay in the model. Our multivariate linear regression model also demonstrated that age, GCS, and education were also associated with cognitive composite scores. At 12 months, only age, GCS, and education were associated with cognitive composites.

Table 6. Linear regression models for overall cognitive composites at 6 and 12 months post-injury.

Variable	Beta	Standard Error	t value	p value
6 Months				
Age	-0.08478	0.05354	-1.58	0.1168
GCS	0.7464	0.25265	2.95	***0.004***
Education	0.89902	0.36506	2.46	***0.0157***
PTD	-1.32184	1.87498	-0.7	0.4826
Antidepressant Use	-5.95378	2.06092	-2.89	***0.0048***
PTD*Antidepressant Use	5.5098	3.03042	1.82	*0.0724*
12 months				
Age	-0.12801	0.06335	-2.02	***0.0475***
GCS	0.94555	0.31182	3.03	***0.0035***
Education	1.23474	0.50676	2.44	***0.0176***

GCS, Glasgow Coma Scale; PTD, Post-traumatic brain injury depression

Effects of antidepressant use, PTD, cognitive deficits on functional cognitive impairment were then investigated. In **Figure 2**, we show that participants with no PTD, who are also taking antidepressants, have worse FIM-Cog scores compared to participants with no PTD who are not on antidepressants (6 months, $p<0.0001$; 12 months, $p=0.008$).

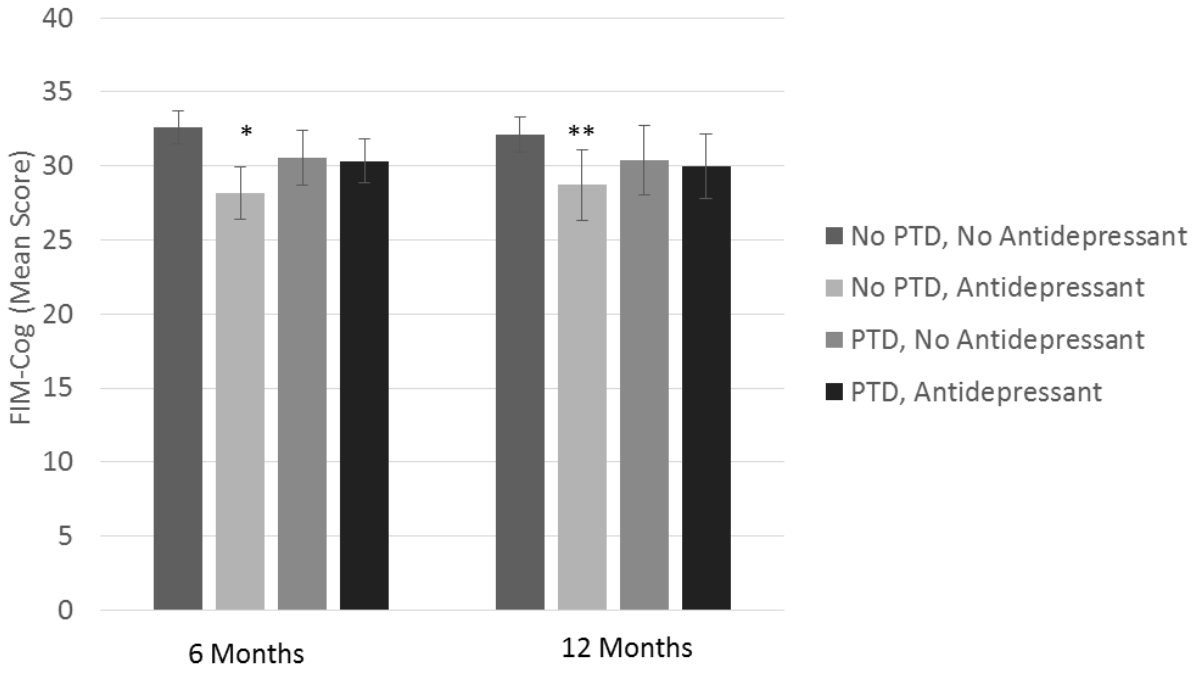


Figure 2. Functional cognition scores (mean score on y-axis) are shown in groups of participants based on PTD and antidepressant use at 6 and 12 months post-injury.

At 6 and 12 months, participants with no PTD, but who are on antidepressant, have lower functional cognition compared to no PTD, no antidepressant use (*$p<0.0001$, **$p=0.008$).

In **Table 7**, models predicting functional cognition were examined. At 6 months, after adjusting for race, cognitive composites and the interaction of PTD*Antidepressant Use remained in the model, with cognitive composites being significant component. At 12 months, age, GCS, education, cognitive composites and PTD predicted functional cognition. There were no significant interactions at 12 months.

Table 7. Linear regression models for functional cognition (FIM-Cog Total) at 6 and 12 months post-injury.

Variable	Beta	Standard Error	t value	p value
6 Months				
Race	1.26773	0.49389	2.57	***0.0119***
Cognitive Composite	0.24908	0.03725	6.69	***<0.0001***
PTD	-1.27808	0.72994	-1.75	*0.0833*
Antidepressant Use	-1.95009	0.84102	-2.32	***0.0226***
PTD*Antidepressant Use	1.69613	1.19088	1.42	0.1577
12 months				
Age	-0.05802	0.02753	-2.11	***0.0393***
GCS	0.23944	0.1382	1.73	*0.0883*
Education	-0.34395	0.21436	-1.6	0.1138
Cognitive Composite	0.31099	0.048	6.48	***<0.0001***
PTD	-1.31959	0.76733	-1.72	*0.0906*

GCS, Glasgow Coma Scale; PTD, Post-traumatic brain injury depression

2.5 DISCUSSION

This study sought to identify cognitive performance susceptible to effects of PTD and the relationship of these deficits and PTD in functional cognition. In this study, there was no significant evidence that cognitive performance deficits associated with PTD, yet individuals with PTD had significantly more functional cognitive impairment. Importantly, cognitive performance was highly associated with antidepressant use, where individuals on antidepressants performed significantly worse on neuropsychological tests, even when correcting for injury severity and PTD.

While some studies demonstrate that individuals with depressive symptomology following TBI perform worse on a number of cognitive measures (Bornstein et al., 1989; Gfeller et al., 1994; Rapoport et al., 2002), other studies report no significant relationship (Satz et al., 1998). Similar

to previous literature, we show no PTD-associated cognitive performance deficits (as measured by neuropsychological cognitive composite scores), but confirm a significant impact on functional cognitive impairment (as measured by the FIM-Cog). In the context of Rehabilomics, we aimed to understand how cognitive deficits and PTD as "complications" from the injury that might impact functional cognition. We had hypothesized that depression might induce or worsen cognitive deficits, but instead, PTD is more important in functional cognition.

It is important to note, participants with PTD were still cognitively impaired (greater than one standard deviation below average), but exhibited no greater cognitive impairment compared to participants with no PTD. Given this finding, it may implicate the lack of motivation present in individuals with PTD, who, despite having comparatively similar cognitive performance, still demonstrated more functional impairment compared to those without PTD. Individuals with PTD may not feel able or motivated to compensate for cognitive deficits as much as their non-PTD counterparts, requiring greater assistance in daily tasks. Of note, the FIM is a measure of functional performance, capturing what an individual does in his or her daily life, not what he or she is capable of doing. Neuropsychological tests of memory more specifically measure an individual's actual cognitive capacity, but do not necessarily translate to functional daily tasks in real life. Our findings suggest that those who develop PTD may have comparable cognitive capacity, but as a result of depressive symptomology, this capacity does not translate into similar functional performance. While individuals with depression also tend to complain of cognitive difficulties more, after controlling for depressive symptoms, complaints of cognitive difficulties was significantly associated with lower performance on neuropsychological assessments, suggesting the perception of cognitive abilities may play a role in functional cognitive impairment (Chamelian

and Feinstein, 2006). These findings highlight the need to address PTD, as it may exacerbate the functional consequences of cognitive deficits.

The relationship between PTD and functional cognition, even without significant cognitive impairment, may be due to other symptoms associated with PTD. Symptoms like apathy, insomnia, psychomotor agitation or retardation, and fatigue are reported frequently after TBI and can influence cognitive function. This overlapping symptomology could greatly influence functional cognition without manifesting in cognitive deficits. Importantly, these overlapping symptoms can also make identification of PTD difficult, though the PHQ-9 has been shown to differentiate between cognitive symptoms and PTD (Fann et al., 2005). Identifying relationships between mood and cognitive difficulties may also help to delineate risk for PTD with or without cognitive deficits.

Multiple studies have suggested that individuals with PTD have worse cognitive deficits compared to individuals without PTD (Bornstein et al., 1989; Gfeller et al., 1994; Jorge et al., 2004; Rapoport et al., 2002, 2005). However, many of these studies examined raw neuropsychological data (Jorge et al., 2004; Rapoport et al., 2005). As age, sex, education, and race can all affect cognitive performance on these neuropsychological evaluations, the previous literature may overestimate cognitive deficits in individuals with PTD, as many of these studies suggest older age as a contributing factor to PTD (Rapoport et al., 2005). Similarly, these studies do not incorporate factors like injury severity and antidepressant use into cognitive performance associations. This study demonstrate that both injury severity and antidepressant use are likely important contributors to consider in understanding associations with cognitive performance and PTD.

Severity of injury has not been consistently associated with depression development post-TBI. Many researchers work under the hypothesis that PTD is due to increased awareness of

deficits (Malec et al., 2010). In this case, some studies show increased depressive symptoms in subjects with less severe injuries (Hudak et al., 2012; Jorge et al., 2004) where there is likely a heightened awareness of TBI-related difficulties. Our study does add some support to this finding as individuals with PTD tended to have higher GCS scores. Participants with PTD also tended to be older than their non-PTD counterparts, similar to previous studies (Rapoport et al., 2005).

In this study, antidepressant use is consistently associated with worse cognitive performance on neuropsychological tests, across domains, as well as increased functional cognitive impairment as measured with the FIM-Cog. In some cases, like the CVLT at 6 months, this finding was associated with nearly a one standard deviation decline in cognitive performance. It is unclear if participants who are not depressed but are on antidepressants were previously depressed or were being treated for other common complications post-TBI like sleep disturbances (Larson and Zollman, 2010). In a study of individuals with moderate to severe TBI, sertraline did not improve cognition when administered early in recovery (first three months) and demonstrated a possible negative effect (though this was not significant) (Baños et al., 2010). Fluoxetine increases hippocampal neurogenesis in animal models of TBI, without any improvement in memory performance (Wang et al., 2011; Wilson and Hamm, 2002). While future studies will need to examine this relationship in *a priori* designed studies to examine the relationship of depression remittance on cognitive performance, one study in a much more mild TBI population suggests that antidepressant treatment, with remittance of depression, actually improves cognitive performance (Fann et al., 2001). Another small study supported this finding (Horsfield et al., 2002), but other studies have shown no effects of antidepressants on cognitive improvement (Lee et al., 2005). While treatment with antidepressants are common for PTD, there is even limited evidence of efficacy in regard to remittance of depressive symptoms (Ashman et al., 2009; Fann

et al., 2009a; Warden et al., 2006). Thus, if antidepressants are negatively impacting cognitive recovery as a side-effect, this could be a reason for the reduced efficacy in PTD. Additionally, these initial findings suggests understanding the impact of antidepressants on cognition post-TBI may be a deterrent for prescribing antidepressants

Functional cognition was influenced by multiple covariates in our study. At 6 months, covariate effects included cognitive composite scores in addition to a trend for a PTD*Antidepressant interaction. As the PTD*Antidepressant interaction also impacted cognitive composite scores, it is important to note that this suggests an effect of PTD*Antidepressant on FIM-Cog outside of its impact on cognitive deficits. At 12 months, there is no interaction present, and antidepressant use is not a significant predictor in the model.

One important caveat in interpreting functional cognition models is that individuals who are on antidepressants or who have reported depression receive an automatic reduction by one point on the Social Interaction subscale of the FIM-Cog. Thus, the FIM-Cog total is expected to be associated with antidepressant use by at least one point. At 6 months, it is difficult to assess the effect of antidepressant use alone, as it interacts with PTD to influence FIM-Cog.

This study suggests that, while functional cognition is greatly impacted by PTD, understanding a number of factors in an individual's recovery is important to understanding the relationship of PTD to outcome. This work, again, supports understanding outcome prediction in the context of Rehabilomics, where a number of considerations for covariates can be understood.

3.0 VARIANTS OF *SLC6A4* IN DEPRESSION RISK FOLLOWING SEVERE TBI

Published in Brain Injury (2012)

Michelle D. Failla, BS, Josh N. Burkhardt, BS, Megan A. Miller, BS., Joelle M. Scanlon, PhD, Yvette P. Conley, PhD, Robert E. Ferrell, PhD, Amy K. Wagner, MD

3.1 ABSTRACT

Post-traumatic depression (PTD), may be a result of several factors like secondary injury chemical cascades as well as psycho-social factors following traumatic brain injury (TBI). While the role of serotonin in the pathology and treatment of idiopathic major depression may be somewhat controversial, it is unclear what role serotonin may play in PTD following a traumatic brain injury (TBI). To assess serotonergic function and genetic risk for PTD development over one year following TBI, this study examined variation in the serotonin transporter gene [*SLC6A4* (5-HTTLPR, rs25331, and a variable number of tandem repeats variant in Intron 2)] in 109 subjects with moderate-severe injury. Depression was assessed using the Patient Health Questionnaire (PHQ-9) at 6 and/or 12 months post-injury. At 6 months post-injury, subjects with history of pre-morbid mood disorders and 5-HTTLPR L-homozygotes were at greater risk for PTD. Contrary to major depression, subjects without pre-morbid mood disorders (n=80) and S-carriers were 2.803 times *less* likely to be depressed compared to L-homozygotes. At 12 months post-injury, L_G-carriers were also less likely to experience PTD. Temporal analysis also showed 5-HTTLPR

associations in PTD development across recovery. This study suggests a unique injury- and temporally-specific interaction between TBI and genetic risk for depression.

3.2 INTRODUCTION

Post-traumatic depression (PTD) is the most common neurobehavioral complication following traumatic brain injury (TBI). Individuals with TBI are 10 times more likely than the general population to suffer from a depressive episode during their first year of recovery (Bombardier et al., 2010a). As individuals with TBI comprise a significantly understudied proportion of the yearly major depression disorder (MDD) incidence, examination of this specific population is important and novel, and begins to address an important gap in the literature. While studies have examined demographic variables [e.g. pre-morbid or family psychiatric illness, substance abuse, or socioeconomic class] (Jorge et al., 2004) in PTD development, they do not fully explain this increased risk for depression. For example, several studies suggest that pre-morbid mood disorder status is a risk factor for post-TBI depression, yet these studies also show an increased risk for subjects with no pre-injury history of mental health disorders (Bombardier et al., 2010a; Gould et al., 2011). Other factors, like injury severity, as measured by the Glasgow Coma Scale (Teasdale and Jennett, 1974a), do not typically correlate with degree/incidence of depressive symptoms post-TBI (Maller et al., 2010).

With an already compromised central neurologic system, the addition of depression following injury can further hinder recovery (Jorge et al., 1994). Depression in TBI has also been associated with aggression, anxiety, suicidality, and increased health care costs (Bombardier et al., 2010a; Fann et al., 2009a; Jorge et al., 2004). Some studies demonstrate that remittance of

depressive symptoms allows for greater recuperation of cognitive function post-TBI (Fann et al., 2001). These observations suggest that early treatment of PTD may be a key component of effective recovery, yet intervention is complicated by a lack of efficacious treatment paradigms and a poor understanding of the underlying neuropathology in PTD.

Decades of research in MDD have explored the role of serotonin (5-HT) in depression etiology and treatment (Krishnan and Nestler, 2008). The serotonin transporter (5-HTT), which modulates the duration and intensity of 5-HT action on its target receptors through reuptake, has been an important and controversial target for genetic studies in major depression. The most frequently studied variation of this 5-HTT gene *(SLC6A4,* located on chromosome 12q11.1-17q11.2) is a 44 bp insertion/deletion in the promoter region of *SLC6A4* and is known as the serotonin-transporter-linked polymorphic region (5-HTTLPR). The 5-HTTLPR has two common variants: the long (L) and short (S) allele, based on presence and absence of the insertion, respectively. The L-allele has a reported threefold higher transcriptional activity in vitro compared to the S-allele (Heils et al., 2002), possibly resulting in comparatively less synaptic 5-HT. One meta-analysis showed variation at 5-HTTLPR is implicated in major depression (MDD) risk (Karg et al., 2011), but conflicting meta-analyses exist (Risch et al., 2009), potentially due to specific methodological differences. The meta-analysis by Karg *et al* (Karg et al., 2011) included specific physical stressors (e.g. hip-dislocation) and showed the S-allele to be a significant factor in MDD risk. S-carriers also show increased emotionality, increased hypothalamic-pituitary-adrenal axis reactivity, and show greater reactivity of the amygdala in response to fearful faces (Hariri et al., 2002). However, as stated in a review by Homberg and Lesch (Homberg and Lesch, 2011), the S-allele is a conserved polymorphism across evolution (non-human primates also share this polymorphism), and likely has an efficacious role as well. Some argue that S-carriers, when

compared to L-homozygotes, show better cognitive performance, with some caveats, in areas that may be related to increased attention. There is also a single nucleotide polymorphism (SNP, rs25531, A>G), within 5-HTTLPR that is found almost exclusively within the L-allele (Wendland et al., 2006). The G-variant results in an additional AP2 transcription factor binding site; in vitro studies have shown that the L_G-allele is functionally similar to the S-allele (Zalsman et al., 2006). Also within *SLC6A4,* there is a variable number of tandem repeats (VNTR) within Intron 2 that results in three variants: 9, 10, and 12 repeats of a 17 bp repeat segment (Ogilvie et al., 1996). While it is not clear if the VNTR has a true functional effect on 5-HTT protein expression, the 9-allele has been associated with unipolar depression (Ogilvie et al., 1996) and the 12-allele with bipolar disorder (Rees et al., 1997). From this evidence, it is likely that genetic variation within *SLC6A4* plays a complex role in depression and mood disorder risk.

There is a lack of literature on the effects of TBI on the serotonergic system, and the relationship between serotonergic function to both mood and cognitive performance post-TBI has not been well studied. Several studies suggest a hypo-neurotransmission state following TBI, including monoaminergic neurotransmitter systems, that likely impacts chronic TBI pathology and cognitive dysfunction (James W Bales et al., 2009). Some studies even suggest that anatomic location of injury, specifically left orbitomedial frontal lobe lesions that directly affect serotonergic innervation (Jorge et al., 1993), is a major contributor to PTD development, but this pathology is not apparent in a large number of persons with PTD (Maller et al., 2010). One study showed that serum serotonin profiles of subjects with TBI fall below control subject levels during recovery, with even lower levels observed in PTD (Wozniak et al., 2010). While serotonergic systems have not been fully investigated in TBI, this neurotransmitter system is increasingly important to consider as antidepressants like serotonin selective reuptake inhibitors (SSRIs) are a routine part

of care for those with MDD, yet seem to have limited effectiveness in PTD (Fann et al., 2009a). Interestingly, 5-HT_{1A} receptor agonist administration confers some neuroprotective and cognitive benefits when administered acutely in animal models of TBI (Kline et al., 2001)(Kline et al., 2002), but their role in PTD has not been studied.

Considering the increased risk for depressive episodes in the first year post-TBI, and the lack of understanding regarding the PTD etiology, we aimed to investigate *SLC6A4* genetic variation in PTD development. As recovery from TBI is a dynamic and complicated process, we examined PTD across the first year of recovery and in relation to pre-morbid mood disorders. We hypothesized that genetic variation within *SLC6A4* would influence depressive symptomology in subjects with TBI. We expected that subjects with a history of mood disorders may have varied temporal onsets and genetic risk factors for PTD. We also hypothesized that genetic associations with PTD could differ over time, wherein PTD occurring at early recovery time-points may be biologically-driven and reflect a low serotonin environment compared to later time-points, where PTD may be driven more heavily by self-awareness of deficits and other environmental stressors.

To our knowledge only one other study has investigated the role of *SLC6A4* in PTD incidence at 1 year post-injury, with no significant associations identified (Chan et al., 2008). However, in this current study we present novel genetic associations for PTD across the first year post-TBI. Our results show that subjects with pre-morbid mood disorders were more likely to develop depression post-TBI, and consistent with major depression, tended to carry the S-allele. Subjects with premorbid mood disorders were also more likely to experience a persistent sub-type of PTD. Among those *without* a history of pre-morbid mood disorders, carriage of the S-allele was *protective* against PTD at 6 months post-TBI. At 12 months post-injury, the S-allele was not associated with PTD status, but the L_G-allele was protective against PTD. These data suggest a

complex and dynamic gene-injury interaction with PTD over time and suggest a need for further investigation into the role of *SLC6A4* in both PTD and general TBI recovery.

3.3 METHODS

3.3.1 Participants

Included for analysis were 109 Caucasian participants aged 17–71 (mean age 35.33±14 years) receiving care at inpatient and/or outpatient clinics within the University of Pittsburgh Medical Center (UPMC) after sustaining a moderate to severe, non-penetrating brain injury, with CT evidence of intracranial injury. Participants were a subset of a larger study investigating genetic factors and recovery in individuals with TBI. Participants were included in this analysis if they were cognitively able to report symptoms on the Patient Health Questionnaire–9 (PHQ-9) at 6 or 12 months post-injury.

Participants had a GCS score (Teasdale and Jennett, 1974a) of 3-15 (mean GCS, 7.76 ± 2.82, median=7) when using the best GCS obtained within the first 24 hours post-injury. Demographic information including age, gender and education, was collected through chart review as well as subject and caregiver interviews. Antidepressant usage at 6 and 12 months was extracted from both subject interview and chart review. Subjects were not stratified by antidepressant use for analysis as many individuals with PTD have a reduced response to SSRIs (Lanctôt et al., 2010) or are prescribed SSRIs for reasons other than depressive symptoms (Zafonte et al., 2002). However, antidepressant use was considered during multivariate analysis. A pre-morbid history

of mood disorders, including depression, bipolar disorder, and anxiety at each time point, was established by self-report and chart review.

3.3.2 Depressive Symptom Assessment

Depressive symptomology was evaluated at 6 and 12 months post-injury using the Patient Health Questionnaire-9 (PHQ9), a brief self-report symptom inventory based on the 9 DSM-IV diagnostic criteria for Major Depressive Disorder (MDD). The PHQ-9 requires participants to rate, on a scale between 0 (None) and 3 (Nearly Every Day), how often they experience symptoms of depression over a two week period. A higher total score reflects a greater number of and/or greater severity of depressive symptoms, with the maximum score being 27. The PHQ-9 can be used both as a continuous and categorical outcome measure. Participants with TBI were grouped as "depressed" vs. "non-depressed" using the DSM diagnostic criteria as they map to specific PHQ-9 questions as previously described (Fann et al., 2005). Individuals were categorized as depressed if they responded positively to at least five symptom based questions on the PHQ-9, with at least one pertaining to a cardinal symptom (anhedonia or depression). This method has been validated in a population with TBI showing a sensitivity of 93% and a specificity of 89% compared to the Structured Clinical Interview for DSM Diagnosis (SCID), which is also modeled on DSM diagnostic criteria (Fann et al., 2005). This method has also been examined for its ability to discriminate between chronic TBI and depression symptoms (Cook et al., 2011a).

Depression was also evaluated across recovery in a subset of participants (n=67) with both 6 and 12 month depression data. For this temporal analysis, each participant was categorized into a sub-type of PTD as follows: no depression (at either time point), transient depression (depressed

at 6 months, but not at 12 months), persistent depression (depressed at both time points), and late-onset depression (depressed at 12 months only).

3.3.3 Genotyping

DNA was isolated from either blood using a simple salting out procedure or from cerebrospinal fluid using the Qiamp protocol from Qiagen (Valencia, CA, USA). For genotyping serotonin transporter gene (*SLC6A4*) promoter polymorphism 5-HTTLPR, flanking primers 5′-TCCTCCGCTTTGGCGCCTCTTCC-3′ (forward) and 5′-TGGGGGTTGCAGGGGAGATCCTG-3′ (reverse) were used for DNA amplification (PCR) (Wendland et al., 2006). The rs25531 A>G single nucleotide polymorphism was concurrently detected by digesting the amplified fragments with the restriction enzyme MspI (New England Biolabs, Beverly, Massachusetts, USA), where the A>G substitution creates an additional MspI site. Amplification products were simultaneously resolved by electrophoresis on 3.5% agarose gels and visualized by ethidium bromide staining and UV transillumination (Edenberg and Reynolds, 1998). For genotyping the STin2 VNTR, flanking primers 5′-GTCAGTATCACAGGCTCGAC-3′ (forward) and 5′-TGTTCCTAGTCTTACGCCAGTG-3′ were used. Sequence confirmed controls of each genotype were run with each plate.

Genetic variant frequencies did not differ by demographics or clinical variables (data not shown). As this was a genetic association study, the potential for stratification effects was examined. All associations were analyzed in a Caucasian population (n=109). During this study, African-American subjects were also recruited and completed PHQ-9 assessments post injury, yet the sample size for the African-American population with depression data (n=10) was not sufficient for sub-analysis. There is also consistent evidence of race variation in *SLC6A4* allele

distribution [12], and consistent with the literature, our allelic frequencies for the variants analyzed differed by race (Gelernter et al., 1999; Lotrich et al., 2003; Xie et al., 2009) (data not shown). For these reasons, the analyses presented here are limited to the Caucasians sub-population described above.

3.3.4 Statistical Analysis

The Statistical Package for Social Sciences (SPSS, version 20) was used for data analysis. Descriptive analysis included mean and standard deviation for continuous and ordinal variables such as age, GCS, and education. Frequencies were calculated for categorical variables such as gender, and antidepressant use. Genetic information was analyzed and categorized based on genotype and allele. Demographic and relevant clinical information was compared between depression groups and variant/genotype groups using Student's *t*-tests or an ANOVA to compare means and Chi-Square or Fisher's Exact to compare frequencies. In order to control for demographic/clinical or injury severity information, and control for potential confounders, multivariate logistic regression was used when identifying associations with the absence/presence of PTD. Variables were entered in the logistic regression model based on their bivariate associations with depression. Groupings to assess multi-variable interaction effects were avoided to limit the possibility of having groups with small numbers in the multivariate regression. Variables with a p-value less than 0.3 in bivariate analysis were initially entered in each model. A backward selection method was then used to systematically remove non-significant variables from the model (**Table 3B/3D)**. The final regression model included variables that had a final p-value less than 0.2. However, additional regression analyses incorporated clinically relevant variables (gender, injury severity, education) that have been previously associated with TBI outcome risk,

regardless of their p-value. These variables were forced into the model in order to 1) show stability of associations across multiple modeling methods, and 2) to add clinical relevance to these models (**Table 3A/3C**).

3.4 RESULTS

3.4.1 Population Description

This study included 109 individuals with both genotype information and at least one PHQ-9 score (at 6 or 12 months). Of these 109 subjects, 35 were categorized as depressed at 6 months (38.46%, n=91), while 29 subjects were depressed at 12 months (30.53%, n=95); 46 subjects experienced depression at some point during the first year post-injury (42.20%, n=109). Fifteen subjects (13.76%) reported some history of pre-morbid mood disorders, with 14 of these subjects having some element of depression as a part of their pre-morbid psychiatric presentation. The remaining subject had a history of anxiety. Subjects with pre-morbid mood disorders did not differ in age, education, or injury severity compared to subjects without any history of mood disorders (data not shown). However, more subjects with pre-morbid mood disorders were taking an anti-depressant at 6 months (81.8%) compared to subjects with no history of mood disorders (33.8%, X =9.345, p=0.003). This effect was reduced at 12 months (53.3% compared to 30%, X=3.079, p=0.075). For those with *no history* of mood disorders, there were no statistically significant differences in demographic profiles between depressed and non-depressed groups (**Table 8**).

Table 8. Demographics and allele distribution for subjects with no history of depression (excluding pre-morbid subjects)

	6 Months (n=80)			12 Months (n=80)		
Variable	**Depressed** (n=27)	**Not Depressed** (n=53)	**p value**	**Depressed** (n=21)	**Not Depressed** (n=59)	**p value**
Age(mean ± STD)	37.8 ± 14.6	33.3 ± 14.4	0.116	37.5 ± 14.2	34.0 ± 14.0	0.338
Education (mean ± STD)	13.0 ± 2.0	13.0 ± 1.9	1.000	13.2 ± 2.4	13.0 ± 1.7	0.615
GCS (mean ± STD)	7.9 ± 2.6	7.9 ± 2.8	0.979	8.0 ± 2.5	7.5 ± 2.9	0.549
Male, # (%)	21 (77.7)	46 (86.8)	0.345	17 (81.0)	47 (79.7)	1.000
Antidepressant use, # (%)	12 (44.4)	15 (28.3)	0.213	9 (42.9)	15 (25.8)	0.172
5-HTTLPR S-carriers, # (%)	11 (40.7)	35 (66.0)	***0.035***	13 (61.9)	37 (62.7)	1.000
5-HTTLPR L_A-carriers, # (%)	23 (85.2)	43 (81.1)	0.763	19 (90.5)	47 (79.7)	0.334
5-HTTLPR L_G-carriers, # (%)	6 (22.2)	7 (13.2)	0.345	0 (0.0)	12 (20.3)	***0.019***
VNTR 9- carriers, # (%)	3 (11.1)	1 (1.9)	0.109	1 (4.8)	3 (5.1)	1.000
VNTR 10- carriers, # (%)	16 (59.3)	27 (50.9)	0.636	12 (57.1)	30 (50.8)	0.800
VNTR 12- carriers, # (%)	18 (66.6)	45 (84.9)	**0.083**	15 (71.4)	47 (79.7)	0.544
n: sample size; #: number of subjects; %: percent of total subjects; STD: Standard deviation; GCS: Glasgow Coma Scale; VNTR, variable number of tandem repeats, refers to VNTR in Intron 2 of *SLC6A4*						

3.4.2 Pre-morbidity and Post-Traumatic Depression

Table 9. Demographics and Analysis of Pre-morbid history in PTD (6 and 12 months)

N=109	**6 Months (n=91)**			**12 Months (n=95)**		
Variable	No history (n=80)	Pre-morbid (n=11)	**p value**	No history (n=80)	Pre-morbid (n=15)	**p value**
Age (mean ± STD)	34.8 ± 14.6	38.0 ± 13.6	0.495	34.9 ± 14.0	37.8 ± 12.0	0.462
Education (mean ± STD)	13.0 ± 2.0	12.1 ± 1.8	0.154	13.0 ± 1.9	12.1 ± 1.7	**0.081**
GCS (mean ± STD)	7.9 ± 2.7	6.5 ± 2.3	0.118	7.6 ± 2.8	7.2 ± 2.4	0.570
Male, # (%)	67 (83.8)	7 (63.3)	0.208	64 (80.0)	10 (66.7)	0.310
Antidepressant use, # (%)	27 (33.8)	9 (81.8)	***0.006***	24 (30.0)	8 (53.3)	**0.075**
Depressed, # (%)	27 (33.8)	8 (72.7)	***0.016***	21 (26.3)	8 (53.3)	***0.040***
5-HTTLPR S-carriers, # (%)	46 (57.5)	8 (72.7)	0.266	50 (62.5)	11 (73.3)	0.311
5-HTTLPR L_A-carriers, # (%)	66 (82.5)	8 (72.7)	0.336	66 (82.5)	12 (80.0)	0.531
5-HTTLPR L_G-carriers, # (%)	13 (16.3)	1 (9.1)	0.466	12 (15.0)	2 (13.3)	0.615
VNTR 9- carriers, # (%)	4 (5.0)	0 (0.0)	0.592	4 (5.0)	0 (0.0)	0.497
VNTR 10- carriers, # (%)	43 (53.8)	5 (45.5)	0.422	42 (52.5)	8 (53.3)	0.589
VNTR 12- carriers, # (%)	63 (78.8)	9 (81.8)	0.587	62 (77.5)	12 (80.0)	0.567
n: sample size; #: number of subjects; %: percent of total subjects; p= p value; SEM: standard error of mean; STD: Standard deviation						

Results comparing pre-morbid mood disorder status and PTD are presented in **Table 9**. Those with pre-morbid mood disorders were more likely to be depressed at 6 months post-injury compared to subjects with no history of mood disorders (p=0.006, n=91, **Figure 3A**). This greater risk for depression was also observed at 12 months post-injury (p=0.040, **Figure 3B**). Temporal analysis of pre-morbid status showed that subjects with pre-morbid mood disorders had a different distribution within temporal PTD subtypes compared to subjects with no history (p=0.036) and

were more likely to experience persistent PTD than other temporal sub-types of PTD (p=0.015)(**Figure 3C**).

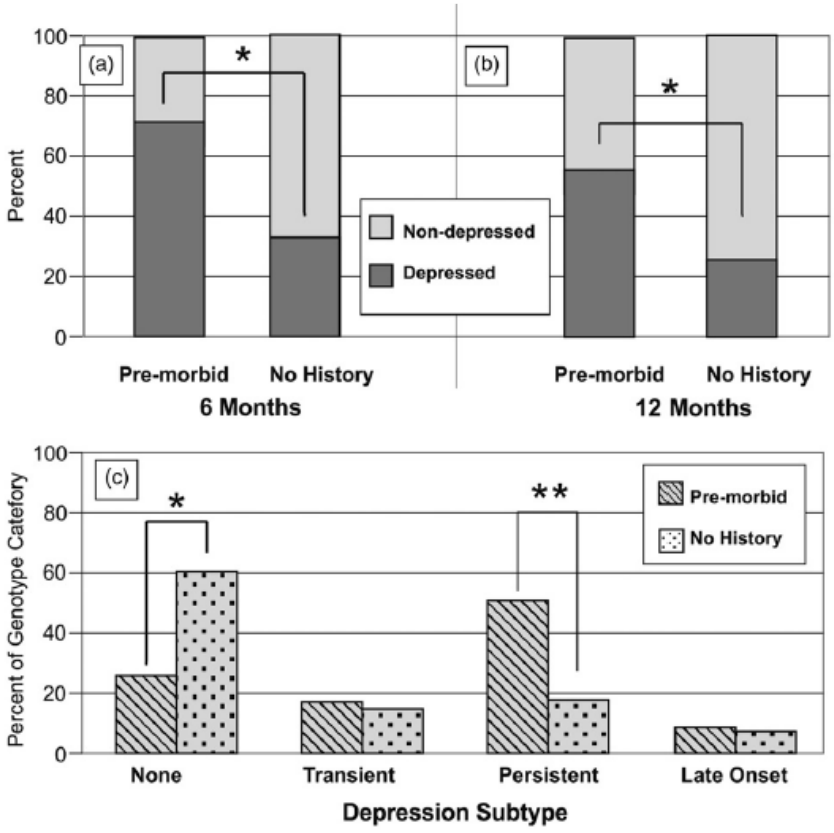


Figure 3. Pre-morbid mood disorder history in PTD risk across recovery.

(A,B) Subjects with a history of mood disorders prior to a brain injury were more likely to experience PTD at 6 months (*A, *p*=0.016, X2=6.207, n=91) and 12 months (*B, *p*=0.040, X2=4.369, n=95). (C) There was also a significant association for pre-morbid mood disorder status in prediction of PTD subtype (*p*=0.036, X2=7.308, n=81), such that subjects with pre-morbid mood disorders were more likely to have some form of PTD compared to subjects with no history (*p=0.047). This difference was primarily related to an more subjects with pre-morbid history experiencing persistent PTD (**p=0.015).

When evaluating bivariate associations performed for the entire population, there were no significant genetic associations PTD at 6 months or 12 months (**Table 8**). While not statistically significant, 16.6% of 5-HTTLPR S-carriers in our study reported a history of pre-morbid mood disorders (compared to 9.3% of non-carriers, p=0.396, data not shown). As subjects with pre-morbid mood disorders were more likely to be depressed at 6 and 12 months post-TBI, we hypothesized that an interaction between pre-morbid status and genotype might exist for PTD. Subjects were evaluated in bivariate analysis using a categorical variable comprising pre-morbid and S-carrier status; PTD rates did vary by pre-morbid history by S-carrier status (Fisher's Exact, χ^2=10.969, p=0.007**, Figure 4A**). Post-hoc analysis showed that S-carriers with *no* pre-morbid mood disorders exhibited the lowest depression frequencies (23.9% depressed), while S-carriers with pre-morbid mood disorders had the highest depression frequencies, (75.0% depressed, p=0.009). Interestingly, at 12 months, the effects of pre-morbid history by S-carrier status on PTD status in bivariate analysis was reduced (p=0.123) (**Figure 4B**).

Interactions between other variants in *SLC6A4* (rs25531, L_G, and the VNTR in Intron 2) and pre-morbid mood disorder status were evaluated for PTD risk at both time-points, but no significant results were observed (data not shown). Multivariate models including pre-morbid mood disorder status and also S-carrier status showed these variables were consistently the two most important factors in 6 month PTD risk. In a forced entry model with other covariates (age, gender, GCS, education level, and antidepressant use) entered, subjects with a history of pre-morbid mood disorders were still at the greatest risk for PTD (OR: 5.74: CI 1.133-29.092, *p*=0.035), followed by S-carrier status, where L-homozygotes had the next greatest risk (OR: 3.343, CI: 1.135-9.849, p=0.029) for PTD. In a backwards stepwise model, pre-morbid status and S-carrier status were the only variables significantly affecting PTD status. (**Tables 10 A, B**).

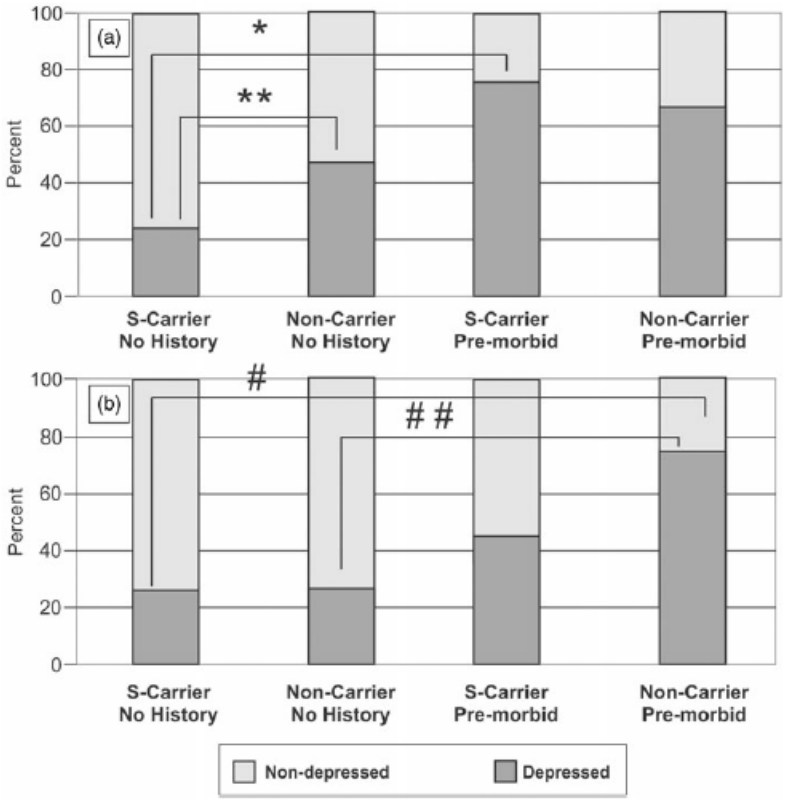


Figure 4. Pre-morbid mood disorder history and S-carrier interaction in PTD risk.

(A.) Subjects grouped by 5-HTTLPR S-carrier status and pre-morbid mood disorder history show different rates of depression at 6 months (n=91; Fisher's exact, $\chi 2=10.696$; p=0.007) based on percent depressed in each group (y-axis). Pair-wise contrasts show that subjects with pre-morbid mood disorders and who were also S-carriers, were more likely to be depressed, compared to S-carriers with no pre-morbid mood disorder (*p=0.009); within subjects with no history of mood disorders, S-carriers were less likely to be depressed (**p=0.035). (B) At 12 months, 5-HTTLPR S-carrier status and pre-morbid mood disorder history did not significantly differ in depression rates (p=0.123). Pair-wise contrasts showed trending differences such that subjects with pre-morbid mood disorders and who were non-carriers were more likely to be depressed compared to subjects who had no history of mood disorders (with both S-carriers, #p=0.089, and non-carriers, #p=0.073, in this group).

Table 10. Multivariate Analyses of Genetic Variants in PTD

A. Pre-morbid Status and 5-HTTLPR in all subjects at 6 months post-TBI, forced entry model.			
Variable (n=90*)	**Odds Ratio**	**CI (95%)**	***p* value**
Pre-morbid History	5.740	1.133-29.092	***0.035***
5-HTTLPR L-homozygotes	3.343	1.135-9.849	***0.029***
Antidepressant Use	1.450	0.478-4.400	0.512
Age	1.020	0.980-1.063	0.332
Gender	0.645	0.187-2.226	0.488
GCS	0.925	0.743-1.151	0.483
Education	1.069	0.816-1.400	0.629
B. Pre-morbid Status and 5-HTTLPR in all subjects at 6 months post-TBI, backwards conditional step-wise model.			
Variable (n=90*)	**Odds Ratio**	**CI (95%)**	***p* value**
Pre-morbid History	6.943	1.606-30.011	***0.009***
5-HTTLPR L-homozygotes	2.715	1.070-6.885	***0.035***
All other terms from Model A removed through 5 steps when p>0.2.			
C. 5-HTTLPR in subjects with no history of mood disorders, forced entry model.			
Variable (n=79*)	**Odds Ratio**	**CI (95%)**	***p* value**
5-HTTLPR L-homozygotes	2.803	1.032-7.614	***0.043***
Antidepressant Use	1.941	0.682-5.521	0.214
Age	1.014	0.977-1.051	0.466
Gender	0.537	0.144-2.006	0.355
GCS	0.983	0.808-1.196	0.865
Education	1.009	0.774-1.316	0.946
D. 5-HTTLPR in subjects with no history of mood disorders, backwards conditional step-wise model.			
Variable (n=79*)	**Odds Ratio**	**CI (95%)**	***p* value**
5-HTTLPR L-homozygotes	2.932	1.094-7.857	***0.033***
Antidepressant Use	1.965	0.716-5.391	0.190
All other terms from Model C removed through 5 steps if p>0.2.			
**One subject was missing Education information*			

3.4.3 Genetic associations with PTD among those without pre-morbid mood disorders

Among those with no history of mood disorders, **Table 9**, (n=94), S-carriers had lower PTD rates at 6 months post-injury (Fisher's exact, p=0.035), with no significant differences by S-carrier status at 12 months (p=1.00) (**see figure 5A/B**).

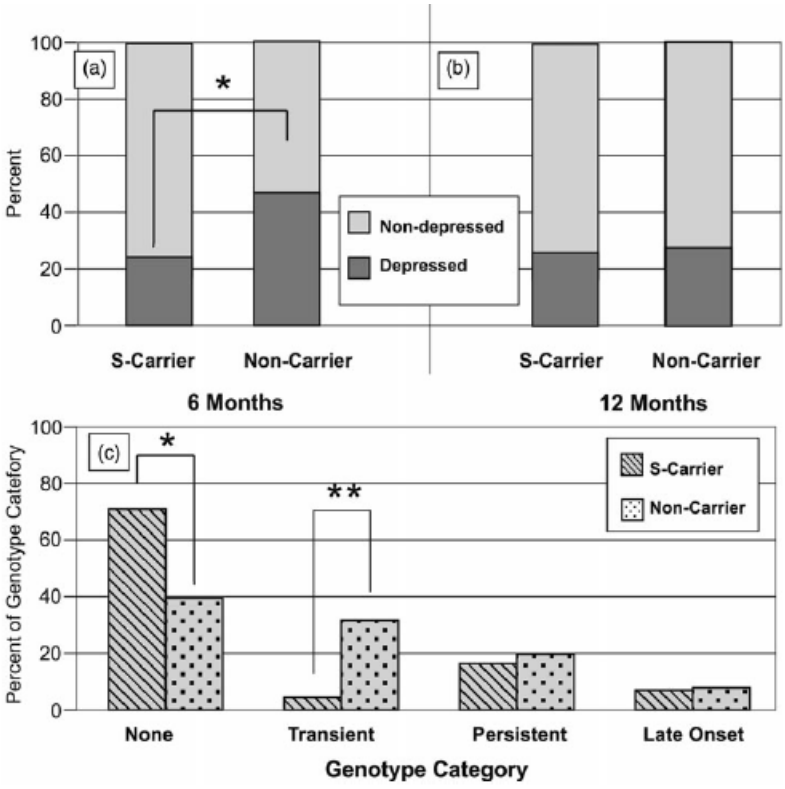


Figure 5. 5-HTTLPR S-carriers in PTD risk across recovery.

(A,B) Evaluation of the S-allele carrier frequencies in only subjects with no history of mood disorders shows S-carriers are less likely to experience PTD at 6 months compared to non-carriers (*p=0.035, A), with no significant effect of the S-allele at 12 months (p=1.00, B). (C) There was also a significant association for S-carrier status in prediction of PTD subtype (p=0.013), such that S-carriers compared to non-carriers, were less likely to experience PTD in any form (*p=0.020). This difference was primarily related to less S-carriers experiencing transient PTD (**p=0.004).

L_A- and L_G-carrier status (rs2553) (L_A/L_A vs. L_A/L_G, Fisher's exact, χ^2=0.952, p=0.457) was not associated with PTD at 6 months (**Figure 6A**). However, at 12 months post injury, no L_G-carriers were depressed (p=0.019, Fischer's exact, χ_2=5.025, n=80) (**Figure 6B**).

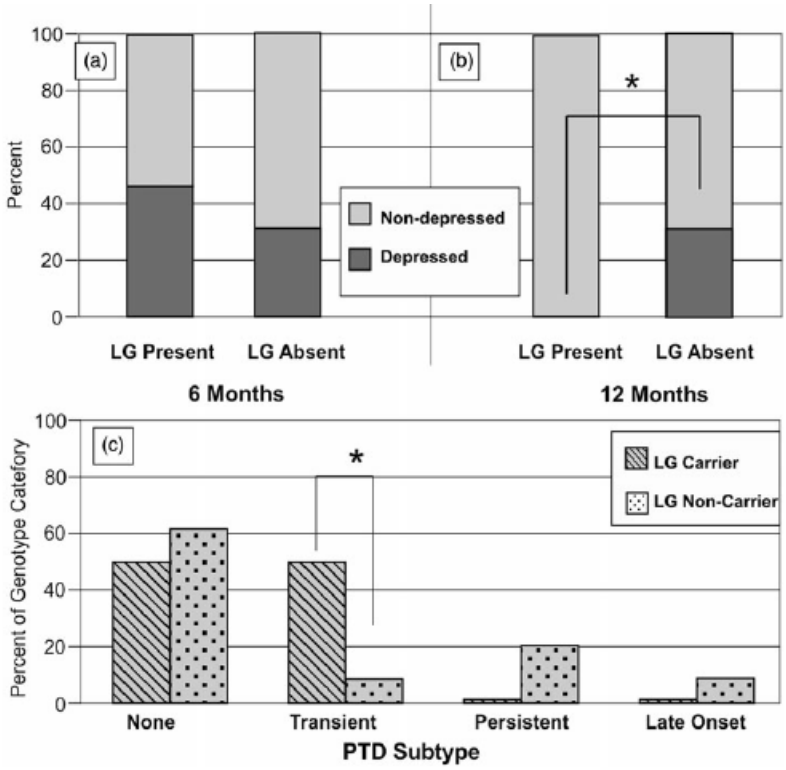


Figure 6. 5-HTTLPR LG-carriers in PTD risk across recovery.

(A,B) Evaluation of the LG-allele carrier frequencies in only subjects with no history of mood disorders shows LG-carriers status was not associated with PTD risk at 6 months (A, p=0.345, X2=6.207, n=91). However, LG-carriers were less likely to experience PTD at 12 months compared to non-carriers (**p*=0.030, B). (C) There was also a significant association for LG-carrier status in prediction of PTD subtype (p=0.011), such that LG-carriers compared to non-carriers, were more likely to experience transient PTD (*p=0.005) compared to non-carriers.

Genotype and carrier status of the VNTR in Intron 2 of *SLC6A4* was also examined in relation to PTD risk, with no significant associations (**Table 9**). All genetic variants were examined as modulators of depression severity (PHQ-9 scores) with no significant findings.

Temporal analysis of PTD showed unique genetic associations in subjects with no history of pre-morbid mood disorders. S-carrier status predicted PTD subtype (Fisher's exact, χ^2=10.496, p=0.013, **Figure 5C**), such that S-carriers were less likely to experience PTD in any form (compared to L-homozygotes, p=0.020). More specifically, S-carriers were less likely to experience a transient depression (compared to L-homozygotes, p=0.004). There was also a significant association for L_G-carrier status in prediction of PTD subtype (Fisher's exact, χ^2=12.856, p=0.011) at 12 months, such that L_G-carriers were more likely to experience transient PTD compared to non-carriers (p=0.005) **(Figure 6C)**. Temporal analysis of the VNTR in Intron 2 did not show any significant predictors of PTD subtype.

Multivariate analysis at 6 months, controlling for antidepressant use, gender, injury severity, and education, showed a robust association with 5-HTTLPR such that L-homozygotes were 2.803 (95% CI: 1.032-7.614, *p*=0.043) times more likely to be depressed compared to S-carriers. Backwards elimination of variables in this model showed that only S-carrier status and antidepressant use predicted PTD status at 6 months (**Tables 10C/D**).

3.5 DISCUSSION

This study presents evidence for a temporally-influenced, injury-specific genetic risk profile for depression following TBI. While pre-morbid mood disorder history was the most influential predictor of PTD, after controlling for this variable, L-homozygotes were at the next greatest risk

for PTD 6 months post-injury. This finding suggests a protective role for the S-allele, which was further confirmed in multivariate analysis that included only those without pre-morbid mood disorders. Interestingly, the presence of the L_G variant (compared to L_G absence) was associated with lower PTD risk at 12 months post-TBI. These PTD genetic risk profiles differ from findings reported with MDD and are dependent on time after injury, suggesting a TBI specific genetic risk relationship. These unique associations with *SLC6A4* in PTD call for further investigation into the role of the serotonergic system post-TBI, specifically in earlier recovery periods where intervention may be highly beneficial.

While this study showed a novel, *protective* association for S-carriers in PTD risk at 6 months post-TBI, particularly for subjects with no history of pre-injury mood disorders, temporal PTD analysis also supported these results by showing that S-carriers were specifically less likely to experience a transient depression, with no difference in other PTD-subtypes. Our findings also show a protective association for the L_G-allele at 12 months post-injury in these subjects, with L_G-carriers primarily experiencing either no or transient PTD.

These findings differ from 5-HTTLPR associations in MDD risk, where the S- and L_G-allele are most consistently identified as risk genotypes (Karg et al., 2011), implying a unique injury-induced neuropathology in PTD. Temporal PTD-subtype analysis of both S-carrier and L_G-carrier PTD associations show these findings are also temporally-specific during TBI recovery. Interestingly, our study found no PTD associations with the VNTR in Intron 2 of *SLC6A4*. However, it has been reported previously that the 5-HTTLPR has a dominant role over 5-HTT expression, with the VNTR acting only as a modulator (Hranilovic et al., 2004). One other study has examined S-allele 5-HTTLPR associations with PTD 12 months post-injury with no significant results (Chan et al., 2008), findings which are consistent with our results with this allele at 12

months. While our study found the L_G-allele was associated with no PTD at 12 months, this previous study did not examine the L_G-allele separate from the S-allele. Also, subjects in that study were not stratified by pre-morbid mood disorders; our analysis only showed genetic associations with PTD when pre-morbid subjects were excluded or whose effects were adjusted for in multivariate analysis (**Table 3**). Similarly, studies in post-stroke depression suggest S-homozygotes show increased rates of depression, but many of these studies did not exclude or separately analyze subjects who had a history of pre-injury depression (Mak et al., 2012).

The results of this study, with 5-HTTLPR genotype PTD risk associations that differ from MDD, suggest that depressive symptomology in TBI may arise from a unique, TBI-specific pathology. In MDD, several studies show associations between S-carriers and *increased* risk for MDD (particularly in relation to stressful life events (Karg et al., 2011)), yet these findings are inconsistent and effect sizes are small (Risch et al., 2009). Other studies show that the L-allele is associated with completed suicide, nicotine dependence, and attention deficit hyperactivity disorder (Kenna et al., 2012). While these studies all continue to suggest a role for 5-HTTLPR in psychopathology, they remain controversial, in part, due to a lack of a clear underlying mechanism for these associations. Some work suggests that cortical 5-HT levels vary by 5-HTTLPR genotype when considering both *in vitro* (Lesch et al., 1996; Little et al., 1998) and *in vivo* (Praschak-Rieder et al., 2007; Reimold et al., 2007) binding studies. However, other conflicting studies exist (Erritzoe et al., 2010; Parsey et al., 2006; van Dyck et al., 2004). More consistently, S-carriers show increased emotionality with higher amygdala reactivity, as well as a general hypervigilance towards environmental stimuli and some advantages in cognitive functioning (see Homberg and Lesch review (Homberg and Lesch, 2011)). While this behavioral adaptation could be advantageous in some aspects, it may also pre-dispose S-carriers to mood disorders (Hariri et al.,

2002; Homberg and Lesch, 2011; Risch et al., 2009). Recently, Ho et al. showed that SERT availability was reduced in subjects with major depression but that this was not associated with *SLC6A4* genotype (Ho et al., n.d.). Similarly, Kobiella and colleagues showed recently that 5-HTT binding potential did not mediate the relationship between 5-HTTLPR and amygdala reactivity, suggesting that 5-HTTLPR may not have a direct effect on 5-HTT availability (Kobiella et al., 2011). Kobiella *et al* suggest that 5-HTTLPR effects on neurodevelopment, and not 5-HT levels, may confer genotype differences in adult cognition and MDD risk.

The effects of 5-HTTLPR, either through its impact on 5-HT levels, 5-HTT functionality, or relationships with cognition and emotionality, are likely to interplay with TBI pathology to influence PTD development. In a highly stressed system like the injured brain, it is possible 5-HTTLPR may more greatly impact cortical 5-HT levels or 5-HTT availability. TBI induces a global hypo-neurotransmission state (this is especially evident in the dopaminergic system, for review see Bales *et al* (James W Bales et al., 2009)) and in experimental TBI, there is evidence of a chronic hypo-serotonergic state (Busto et al., 1997). Thus, a protective S-allele, with possibly lower 5-HT reuptake, may indicate a less severe hypo-serotonergic state post-TBI in S-carriers compared to L-homozygotes. However, it is also important to note that 5-HTTLPR can be influenced by epigenetic regulation, particularly since TBI induces a global hypo-methylation state (Gao et al., 2006). Interestingly, the human 5-HTTLPR S-allele is associated with increased methylation compared to the L-allele (Philibert et al., 2007); methylation at 5-HTTLPR has also been associated with increased depressive effects of a stressor (van IJzendoorn et al., 2010).

The effect of 5-HTTLPR on morphology of the fronto-limbic system may aid in understanding unique PTD associations, and these circuits show dysfunction in both TBI and depression (Maller et al., 2010). In the neurodegenerative state of TBI (Bendlin et al., 2008),

hippocampal atrophy frequently occurs (David F. Tate and Bigler, 2000). Hippocampal atrophy is also a common finding in MDD that is reportedly more severe in depressed L-homozygotes (Frodl et al., 2004)(Frodl et al., 2008), suggesting a potential 'advantage' for S-carriers, especially in reference to PTD. Morphological differences in the fronto-limbic system may also explain increased emotionality (increased amygdala reactivity) and cognition (better decision making, risk aversion) of S-carriers. As depression influences cognitive recovery post-TBI (Fann et al., 2001), genetic influences on cognitive recovery may impact PTD development. As attentional deficits are a hallmark of TBI, an increased hypervigilant state pre-injury (attention to emotional/environmental stimuli) in S-carriers could be beneficial post-injury; similarly, studies show persons with ADHD tend to be L-carriers (Faraone et al., 2005). Better cognitive function at early time-points (6 months) post-injury may be protective against depressive symptomology. Future studies examining the effect of 5-HTTLPR on post-TBI cognitive recovery in relation to fronto-limbic function may aid in understanding how this polymorphism influences PTD risk.

Increased PTD incidence among subjects with pre-morbid mood disorders, regardless of 5-HTTLPR status, is consistent with other studies that have reported pre-morbid status a significant predictor in PTD development (Malec et al., 2010). Our study also found that pre-morbid mood disorder history was significantly associated with a persistent PTD subtype. Other studies have shown that subjects with pre-morbid mood disorders were more likely to develop PTD at earlier time points compared to subjects with no history of mood disorders (Gould et al., 2011). As our study did not investigate depression status specifically at the time of injury, it is possible PTD at early time-points could be a continuation of depression at injury. However, Bombardier *et al* (Bombardier et al., 2010a) reported similar rates and onset of depression in subjects with depression at injury compared to subjects with a pre-injury history for depression

that were not depressed at the time of the injury. Neuropathology associated with pre-morbid mood disorders [decreased neurogenesis in the hippocampus (Warner- Schmidt and Duman, 2006), altered neuroplasticity and functionality in fronto-limbic circuits, etc.] may overlap and/or interfere with normal recovery post-TBI, resulting in increased risk for PTD. Furthermore, S-carriers with pre-morbid mood disorders were at greater risk for PTD compared to S-carriers with no history of mood disorders. It is possible that some of the suggested advantages of the S-allele in protection against PTD may be ameliorated by pre-injury neuropathology associated with pre-morbid mood disorders. Though there were no significant genetic predictors of pre-morbid status, subjects with pre-morbid mood disorders did tend to be S-carriers (**Table 2**), consistent with MDD literature.

In this study, the L_{G-} and S- alleles, believed to result in similar 5HTT expression levels based on other studies, have different temporal associations with PTD risk, particularly for those without premorbid mood disorders. It is possible that the L_G-allele's protective association has similar possible mechanisms as previously mentioned for the S-allele. Yet, it is not clear why these associations are temporally specific. While the L_G association needs to be replicated in a larger sample, a temporal difference might be explained in several ways. One possibility may be due to the regulation of the L_G allele by the transcription factor AP-2, as the L_G allele has an additional AP-2 binding site compared to the L_A allele (also, the S-allele does not have this site) (Damberg, 2005). Importantly, there is evidence to suggest that AP-2 function might highly impact injury x gene interactions (García et al., 1996). It is also not clear how allele-specific methylation might affect these associations.

While this study contains several novel and neurobiologically relevant findings, there are some caveats to consider. This study was limited by population size, and future analyses will need to include larger samples. Also, this study did not exclude subjects that were on antidepressant

treatment. Patients with TBI are given SSRIs and other antidepressants frequently for a range of issues common to TBI, including problems associated with sleep, agitation, and behavior, in addition to treatment for a depressive episode (Zafonte et al., 2002). Subjects taking antidepressants were more likely to be depressed in our study, suggesting that although subjects were taking these medications, they were still experiencing significant depressive symptoms, a finding similar to previous reports of reduced effectiveness of SSRIs in populations with TBI (Lanctôt et al., 2010). Also, serotonergic genes may modulate antidepressant efficacy in individuals with TBI as well as rates of adverse events, as one study of a population with PTD showed S-homozygotes (compared to L-carriers) experienced less adverse effects of antidepressants (Lanctôt et al., 2010). As the effect of 5-HTTLPR on pharmacotherapy has also been suggest in non-injured populations (Kenna et al., 2012), future studies are needed to examine pharmacogenetic effects of antidepressant treatment in PTD. Another caveat to consider is how gender effects may moderate 5-HTTLPR risk profiles in PTD. Here we do not report significant gender differences in PTD development nor in genetic risk profiles, but our study is likely underpowered for this analysis. Women are more at risk for MDD (Kessler et al., 2005), yet TBI is more prevalent in men (Faul et al., 2010). Thus, sex will be an important factor to consider in future and larger studies of genetic risk associations with PTD.

As serotonin signaling influences many mechanisms that impact TBI recovery (e.g. neurogenesis, regeneration, and synaptic plasticity) (Mattson et al., 2004), this study also highlights the need to understand serotonergic functioning post-TBI. While genetic associations and interactions in a specialized population like PTD are highly intriguing, understanding how these mechanisms function across the spectrum of depression and cognitive recovery post-TBI may also aid in understanding brain repair mechanisms in general.

With the increased risk for depression following TBI and the paucity of effective treatments, there is an immense need for a better understanding of underlying pathology of PTD. The results of this study, with 5-HTTLPR genotype PTD risk associations that differ from MDD and interact with pre-morbid mood disorder status, suggest that depressive symptomology in TBI likely arises from a TBI-specific pathology. This study also serves as evidence of important gene x injury interactions that may differ from previously reported associations in non-injured populations and may significantly impact recovery and treatment course for those with TBI.

4.0 POST-TBI COGNITIVE PERFORMANCE IS MODERATED BY VARIATION WITHIN *ANKK1* AND *DRD2* GENES

In Submission

Michelle D. Failla, BS, John M. Myrga, Joseph H. Ricker, PhD, C. Edward Dixon, PhD, Yvette P. Conley, PhD, Amy K. Wagner, MD

4.1 ABSTRACT

Objective: As dopamine neurotransmission impacts cognition, we hypothesized functional variants in the linked dopamine D2 receptor (*DRD2*) and ankyrin repeat and kinase domain (*ANKK1*) genes might account for some individual variability in cognitive recovery post-TBI.

Participants: Prospective cohort of 108 survivors of severe TBI, recruited consecutively from a level 1 trauma center.

Design: We examined relationships between targeted dopamine genetic variation and functional recovery at 6 and 12 months post-TBI.

Main Measures: Cognitive performance was evaluated using 8 neuropsychological tests targeting different cognitive domains. An overall cognitive composite was developed based on normative data. We also assessed functional cognition, depression status, and global outcome. Subjects were genotyped for 6 *DRD2* tagging single nucleotide polymorphisms and Taq1A within *ANKK1*.

Results: *ANKK1* Taq1A heterozygotes performed better than homozygotes across several cognitive domains at both time-points post-injury. When adjusting for age, GCS, and education,

the Taq1A (*ANKK1*) and rs6279 (*DRD2*) variants were associated with overall composite scores at 6 months post-TBI (p=0.0494, 0.0371, respectively). At 12 months, only Taq1A remained a significant genetic predictor of cognition (p=0.0081).

Conclusion: These data suggest genetic variation related to dopamine signaling mediates cognitive recovery post-TBI. Understanding genetic influences on dopamine systems post-TBI may impact current treatment paradigms.

4.2 INTRODUCTION

Among the approximately 1.7 million people treated for a traumatic brain injury (TBI) in the United States each year ("CDC - Injury - TBI - TBI in the US Report," n.d.), the majority of those individuals will experience persistent cognitive deficits following their TBI. Cognitive impairment post-TBI can impact return to work and community reintegration for patients (Jennifer Fleming, Leigh Tooth, Mary, 1999). While the resultant cognitive impairments have been well-studied (Levin et al., 1982), identifying individual patterns in recovery is still difficult. In fact, the recent international guidelines for cognitive rehabilitation post-TBI (Bayley et al., 2014), emphasize the need for individualized cognitive rehabilitation paradigms. Genetic variation in targeted systems known to affect cognition in healthy individuals may explain some variance across recovery trajectories. This study approaches the issue of poor cognitive outcomes and prognostication following TBI from a Rehabilomics (A K Wagner, 2010; Wagner and Zitelli, 2012) perspective, assessing genetic variation, in addition to other common individual factors that contribute to cognitive performance, to enhance our understanding of individual variation in cognitive recovery.

In healthy populations, dopamine (DA) neurotransmission in the brain modulates attention, processing speed, executive functioning, and working memory (Braver and Cohen, 2000). Frontal lobe regions associated with these functions have dense projections to the DA-rich striatum. In fact, positron emission tomography (PET) studies characterizing striatal D2 receptor binding demonstrate D2 binding is correlated to several aspects of cognition, such as working memory in healthy populations (Aalto et al., 2005). Dysfunctional DAergic signaling may explain many of the persistent cognitive deficits observed with TBI (see (James W Bales et al., 2009) for review). Decreased DA transporter (DAT) levels in the prefrontal cortex and striatum (Shimada et al., 2014a) have been confirmed in a rat model after TBI (H.Q. Yan et al., 2002), suggesting compensatory action by DA neurons to increase DA signaling post-TBI. In severe to very severe levels of injury associated with the controlled cortical impact (CCI) model of experimental TBI, tyrosine hydroxylase (TH), a rate-limiting enzyme in the synthesis of DA is upregulated in presynaptic terminals in the frontal cortex (Yan et al., 2001) and in the striatum (James W Bales et al., 2009; Wagner et al., 2009). These changes in DA synthesis proteins also suggest compensatory mechanisms in presynaptic DA neurons to increase DA neurotransmission post-TBI. Interestingly, real time neurotransmission studies using fast scan voltammetry with stimulated DA release in the striatum demonstrate reductions in evoked DA overflow and altered DA clearance kinetics (Wagner et al., 2005) that can be restored with daily treatment with the neurostimulant methylphenidate (Wagner et al., 2009). In clinical TBI populations, both positron emission tomography (PET) (Amy K Wagner et al., 2014) and single photon emission tomography (Donnemiller et al., 2000) studies show reduced striatal binding of the (DAT). Similarly, DAergic pharmacological treatments have shown promise in cognitive restoration post-TBI (Bleiberg et al.,

1993; Whyte et al., 2004). Together, these studies suggest that a highly dysfunctional dopaminergic state post-TBI could influence individual cognitive recovery.

Genetic variation that may affect dopaminergic signaling may elucidate some of the individual variation in cognitive recovery post-TBI. Our previous work provides mounting evidence of genetic variation on DAergic signaling post-TBI. Variation in the DAT gene (*DAT1*) moderates cerebrospinal fluid (CSF) levels of DA (Amy K Wagner et al., 2007). We have also explored *DAT1* and genetic variation associated with D2 function via the Taq1A variant in the ankyrin repeat and kinase domain (*ANKK1)* gene, just upstream from the *DRD2* gene, with PET striatal binding using DA-associated ligands for D2 and DAT (Amy K Wagner et al., 2014). In this study, the data provided evidence that *DAT1* genotype modulates DAT binding post-TBI and that both *DAT1* and Taq1A genotype interact with injury status to influence DAT binding (Amy K Wagner et al., 2014). Another study in mild TBI demonstrated that Taq1A genotype moderates performance on a measure of memory recognition at 1 month post-injury (McAllister et al., 2008). Genetic variation within the enzyme that breaks down DA in the prefrontal cortex, catechol-O-methyltransferase (COMT), also can moderate cognitive recovery post-TBI (Lipsky et al., 2005a; Willmott et al., 2014a), as can pharmacological interventions (Willmott et al., 2013). Thus, there are several lines of evidence to support our hypothesis that genetic variation in DAergic signaling may influence cognitive recovery post-TBI.

Genetic variability related to the D2 receptor has been investigated by assessing both variation within the *DRD2* gene itself and in evaluating the *ANNK1* gene immediately upstream of *DRD2*. The *DRD2* associated polymorphism rs1800497 Taq1A within the *ANNK1* gene has been studied extensively in psychiatric disorders and DA-related endophenotypes, especially addictive behaviors (Ponce et al., 2009). Also, rs1800497 has been studied in response latency and memory

recovery following mild TBI (McAllister et al., 2008, 2005). The Taq1A genotype influences striatal D2 receptor density (Thompson et al., 1997) and may impact autoreceptor mediated inhibition of DA synthesis (Laakso et al., 2005). We examined 6 SNPs within the DRD2 gene, including rs6279. Rs6279 is in linkage disequilibrium (LD) with rs6277, a synonymous mutation of C957T, which reportedly affects mRNA stability and regulation of DA-induced D2 expression (Duan et al., 2003), making this locus a likely target for associations with cognitive function.

The aim of this study was to investigate variation involved in DA function as it modulates cognitive recovery post-TBI, specifically utilizing a cognitive composite score to assess multiple cognitive performance domains. We hypothesized that genetic variation within the *ANKK1* and *DRD2* genes might influence individual cognitive recovery. In this study, we report that *DRD2* associated variation modulates cognitive recovery following TBI. Specifically, *ANKK1 Taq1A* rs1800497 heterozygotes and *DRD2* rs6279C-homozygotes demonstrate better cognitive recovery across the first year following their injury. This study further supports the hypothesis that individual variation in dopamine signaling modulates cognitive recovery post-TBI.

4.3 METHODS

4.3.1 Participants

This study was approved by the University of Pittsburgh's Institutional Review Board and consisted of 108 consecutively recruited participants receiving care at inpatient and/or outpatient clinics within the University of Pittsburgh Medical Center (UPMC). Enrollment criteria included a non-penetrating TBI, with evidence of intracranial injury on Computed Tomography (CT), an

admission GCS score ≤8 indicating severe TBI, and age, ≥16 and <75 years. All subjects included for analysis survived at least 1 year post-injury were cognitively able to perform the neuropsychological battery. Subjects were excluded for documented prolonged hypoxia prior to admission. Participants were a subset of a larger study investigating possible genetic factors related to individual recovery following TBI.

While an admission GCS ≤8 was taken as evidence of severe TBI, we used the best GCS obtained within 24 hours post-injury for analysis, as the best GCS in 24hrs shows better sensitivity in discriminating cognitive outcomes (Cifu et al., 1997a; Udekwu et al., 2004a). Best GCS in 24hrs scores ranged from 3-15 (mean GCS, 8.02 ± 3.083, median=7). Demographic information, including age, sex, and education, was collected by chart review and subject or caregiver interviews. Subjects were aged 17-71 (mean age 34.19 ± 13.75 years) and 18.5% (n=20) of participants were women.

4.3.2 Sample Collection and Genotyping

Subjects were genotyped for the dopamine receptor D2 (*DRD2*) associated polymorphism Taq1A in the *ANNK1* gene (rs1800497) and 6 tagging SNPs in the *DRD2* gene (rs6279, rs2734838, rs17529477, rs4245147, rs7131056, rs4630328). Haploview(Barrett et al., 2005) (version 4.2) was used to determine the degree of linkage disequilibrium between these 7 SNPs within this population (see **Figure 7**).

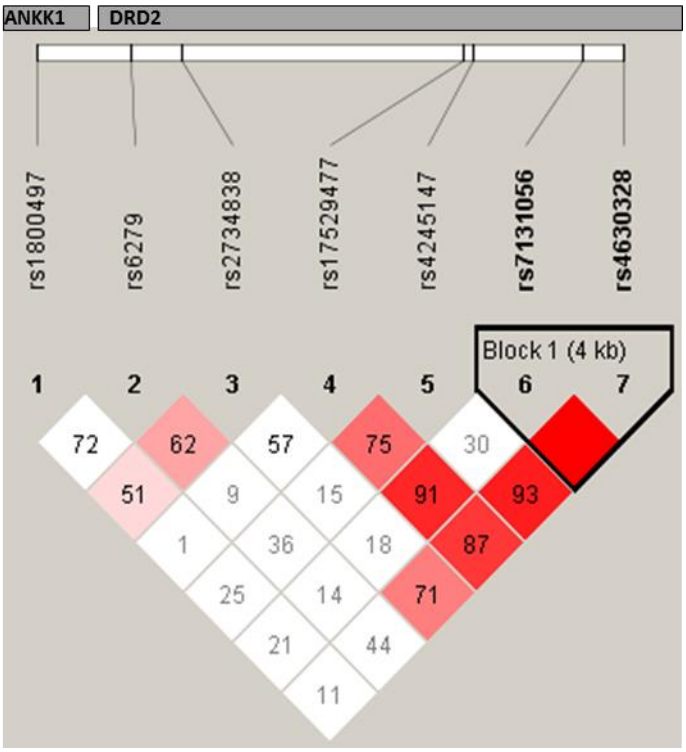


Figure 7. Targeted single nucleotide polymorphisms (SNPs) within ANKK1 and DRD2.

These SNPs lie within their respective genes as marked in the gray rectangles, and consecutively along the genome as mapped on the white rectangle. Linkage disequilibrium (LD) between the 7 SNPs examined (calculated LDs using Haploview v.4.2) is represented as the numbers in each square between each pair of SNPs (D'). Red squares indicate high LD and white squares indicate low LD based on algorithms calculated within Haploview. The bold black line around Block 1 indicates a haploblock that contains rs7131056 and rs4630328. In our sample, there is relatively low LD between rs1800497 and SNPs in DRD2.

DNA was isolated from blood, using a simple salting out procedure, or from cerebrospinal fluid, using the Qiamp protocol from Qiagen (Valencia, CA, USA). For Taq1A (rs1800497) genotyping, amplified DNA underwent 30 cycles of denaturation at 95^{o}C for 1min., annealing at 58^{o}C for 30s, and extension at 72^{o}C for 1min., to amplify the 459bp product, which was then exposed to TaqI restriction endonuclease to perform restriction fragment length polymorphism (RFLP) analysis. Digested products were electrophoresed on a 3% agarose gel, stained with ethidium bromide for

DNA band detection, and assigned a genotype based on presence/absence of original or cut DNA fragments. Primers used were 5'-CCGTCGACCCTTCCTGAGTGTCATCA-3' and 5'-CCGTCGACGGCTGGCCAAGTTGTCTA-3'. Tagging SNPs (tSNPs) covering DRD2, and 1kb flanking DNA 5' and 3', were selected using HapMap (Release 28); tSNP selection criteria was set at r^2=0.80 and minor allele frequency (MAF) ≥0.20 to allow for robust evaluation of heterozygote status (Comings and MacMurray, 2000) and potential associations with the outcomes of interest. rs2734838 was genotyped using Taqman allele discrimination and an ABI7000 (Applied Biosystems, Carlsbad, CA, USA), and the remainder were genotyped using iPLEX MassArray (Sequenom, San Diego, CA). Technical replicates were used across plates, and genotypes were double blind called for quality control.

Some participants were not successfully genotyped for each SNP (rs1800497, 1; rs6279, 2; rs2734838, 2; rs17529477, 2; rs4245147, 4; rs7131056, 1; rs4630328, 2). As this was a genetic association study, there were concerns about potential stratification effects(Freedman et al., 2004). In addition, previous studies indicate different rs1800497 allelic distributions by race that could confound association studies (Goldman et al., 1993). Thus, all reported associations were analyzed in a Caucasian sub-population (n=99). Of note, all results were also conducted in the full population showing consistent findings (data not shown). Among Caucasians, allele frequencies were in Hardy-Weinberg equilibrium. Genetic variant frequencies did not differ by demographics or clinical variables, except for associations with race (data not shown).

4.3.3 Outcome Measurements

General outcome was assessed with the Glasgow Outcome Scale (GOS) (3=severe disability, 4=moderate disability, 5=good recovery) at 6 and 12 months (Jennett and Bond, 1975). Research-

trained neuropsychometrists, blinded to genetic information, collected information used to generate GOS scores. Additionally, subjects in this cohort were evaluated, using the Functional Independent Measure subscale for Cognitive Function (FIM-Cog) (Dodds et al., 1993), at both 6 and 12 months. FIM-Cog is comprised of five component scales expression, comprehension, social interaction, problem solving, and memory. Each scale is rated from one to seven, and the sum of these five components was considered the FIM-Cog Score.

4.3.4 Cognitive Composite Score

Cognitive performance was measured at both 6 and 12 months post-injury using a battery of 8 neuropsychological tests targeting 4 domains of cognition (attention, language fluency, memory, and executive function). Trail Making Tests A and B, a test where participants draw lines between consecutive numbers (Part A) and then between alternating letters and numbers (Part B), was used to measure psychomotor processing speed and cognitive flexibility/task-switching, respectively (RM Reitan and Wolfson, 1985). Digit span, a sub-test from the Wechsler Adult Intelligence Scale-R, measures attention and memory by asking participants to repeat a sequence of numbers forward and backwards (Glenn J. Larrabee and Curtiss, 1995). Rey-Osterreith Complex Figure Test assesses visuo-spatial episodic memory by asking participants to copy an abstract line drawing from memory (PA Osterrieth, 1944). The California Verbal Learning Test-II (CVLT-II (Delis and et al., 2000)) is a list learning paradigm, with subtests measuring learning, immediate recall, interference, and recognition. Different forms of the CVLT were used at 6 and 12 months to minimize practice effects from repeated administration. The Controlled Oral Word Association (JG Borkowski et al., 1967) and Delis-Kaplan Executive Function Systems (DKEFS) Verbal Fluency assess verbal fluency. In both, participants are asked to name words beginning with a

letter (phonemic) or within a subject category (semantic); a third condition in the DKEFS assesses ability to switch between two semantic categories. The Stroop Task(J. R. Stroop, 1935) examines selective attention and cognitive flexibility by asking an individual to name the color of ink a word is printed in, suppressing a habitual response (reading the word) to produce a more effortful response (naming the color of ink).

A cognitive composite score was developed based on normed t-scores for each test (considering age, gender, race, and education where applicable) to determine a measure of overall cognitive recovery. Attention was measured using the Trails Making Test A, and the combined score of the forward and backward digit span tests. Memory was evaluated using the Rey-Osterreith Complex Figure Test and the Long Delay Free Recall Subsection of the California Verbal Learning Test. Language Fluency domain scores were calculated using Controlled Oral Word Association Animals Subsection and the Delis-Kaplan Executive Function Systems Verbal Fluency Letter Fluency subsection. Lastly, executive function was measured using the Trials Making Test B and the Stroop Task Interference Subscore. These tests were selected as the most representative measures for their associated domains. Raw scores from each test were converted into T scores using appropriate metrics (i.e education, age, gender, race) based on norms indicated by the test manufacturer. T scores were averaged within each domain to create a domain subscore. To calculate a cognitive composite score, subjects had to complete at least one test in each domain. Mean values across domain subscores were calculated, and this mean was considered the overall cognitive composite score.

4.3.5 Depression

Depression symptoms were evaluated at 6 and 12 months post-injury using the Patient Health Questionnaire-9 (PHQ9), a brief self-report inventory of depressive symptoms based on DSM-IV diagnostic criteria for Major Depressive Disorder (MDD). The PHQ-9 has been validated for assessing depression following TBI (Cook et al., 2011a; Fann et al., 2005). The PHQ-9 requires subjects to rate, on a scale between 0 (None) and 3 (Nearly Every Day), how often they experience each symptom over a two week period. A higher total score reflects a greater number of and/or greater severity of depressive symptoms, with a maximum score of 27. Individuals with TBI were grouped as "depressed" vs. "non-depressed" using the DSM diagnostic criteria as they map to specific PHQ-9 questions (Fann et al., 2005). Current DSM criteria require individuals to report at least 5 symptoms, with at least one being a cardinal symptom (anhedonia or depressive mood). In our study, individuals were categorized as depressed if they endorsed at least five questions on the PHQ-9, with endorsement of either anhedonia, depressed mood, or both. This method is validated in populations with TBI (sensitivity, 93%; specificity, 89%) compared to the Structured Clinical Interview for DSM Diagnosis (SCID), a measure modeled after DSM diagnostic criteria (Fann et al., 2005).

4.3.6 Statistical Analysis

Statistical analyses were completed using SAS (Cary, NC; version 9.3). Descriptive analyses included mean (with standard deviation or standard error of the mean) and/or median for continuous and ordinal variables including age, GCS, and education. Frequencies were calculated for categorical variables. Demographic and relevant clinical information was compared between

genotype groups using Student's *t*-tests or Mann-Whitney U to compare means and Chi-Square or Fisher's Exact to compare frequencies.

Each SNP was screened for associations with each outcome (GOS, PTD, FIM-Cog, and overall cognitive composite) at each time-point based on genotype, allele carrier status, or by heterozygote vs homozygote status, correcting for multiple comparisons using false discovery rate (FDR) (Benjamini and Hochberg, 1995). Several studies have suggested a heterozygote advantage for *DRD2* (Comings and MacMurray, 2000), warranting specific investigation of heterozygote status. Of the SNPs screened for associations with outcomes, each significant association ($p<0.05$ following FDR) with a specific outcome was further evaluated for effects on subcomponents of the associated outcome scale. Similarly, those same SNPs were then included in multivariate models examining the associated outcome in order to control for demographic/clinical or injury severity characteristics, and potential confounders. If a SNP association with an outcome was significant following FDR at only 1 time-point, it was explored in multivariate models at both time-points in order to understand the clinical implications of that SNP across recovery. A backward selection method was used to systematically remove non-significant variables from the model. The final regression model included variables that had a final p-value less than 0.2.

4.4 RESULTS

4.4.1 Genotype associations with demographics, disability, functional cognition, and global outcome

Table 11. Demographic and clinical variables by genotype.

Variant	Genotype	Age	Gender	GCS	Education
rs1800497	CC (n=55)	35.4 ± 13.8	85.5% (47)	7	12.9 ± 1.9
	CT (n=40)	34.0 ± 14.8	75.0% (30)	7	12.8 ± 2.0
	TT (n=4)	25.5 ± 7.3	75.0% (3)	8	13.8 ± 2.1
	p value	0.315	0.425	0.714	0.519
rs6279	CC (n=61)	34.4 ± 14.5	83.6% (51)	7	13.1 ± 2.0
	CG (n=30)	33.5 ± 14.0	76.7% (23)	7	12.6 ± 1.3
	GG (n=6)	37.8 ± 12.1	83.3% (5)	6	13.2 ± 3.1
	p value	0.659	0.758	0.368	0.417
rs2734838	AA (n=9)	31.4 ± 14.0	66.7% (6)	7	12.1 ± 0.8
	AG (n=45)	34.3 ± 13.1	73.3% (33)	7	12.7 ± 1.9
	GG (n=43)	34.6 ± 14.5	90.7% (39)	7	13.3 ± 2.1
	p value	0.707	0.058	0.935	0.162
rs17529477	AA (n=10)	32.6 ± 15.2	80.0% (8)	7.5	13.2 ± 2.9
	AG (n=47)	34.6 ± 15.1	80.9% (38)	7	12.9 ± 1.7
	GG (n=40)	34.5 ± 13.1	80.0% (32)	7	12.6 ± 1.9
	p value	0.815	0.994	0.945	0.804
rs4245147	CC (n=21)	34.9 ± 15.2	85.7% (18)	8	12.6 ± 1.8
	CT (n=49)	32.9 ± 13.4	77.6% (38)	7	13.2 ± 1.9
	TT (n=25)	37.4 ± 15.2	80.0% (20)	7	12.6 ± 1.9
	p value	0.485	0.725	0.380	0.436
rs7131056	AA (n=14)	38.5 ± 13.2	92.9% (13)	7	12.1 ± 1.6
	AC (n=55)	32.2 ± 13.8	80.0% (44)	7	12.9 ± 1.7
	CC (n=29)	36.4 ± 14.8	75.9% (22)	8	13.0 ± 2.4
	p value	0.186	0.352	0.975	0.532
rs4630328	AA (n=11)	34.2 ± 15.9	81.8% (9)	9	13.1 ± 2.7
	AG (n=52)	34.0 ± 14.3	78.9% (41)	7	13.1 ± 1.9
	GG (n=34)	35.2 ± 13.6	85.3% (29)	7	12.3 ± 1.7
	p value	0.872	0.748	0.368	0.244

Demographic information by genotype is presented in **Table 11**. There was a trend for significantly different distributions based on sex within *DRD2* rs2734838 genotypes (χ^2=5.6940; p=0.058). None of the other examined demographic variables were significantly different by any of the genotypes examined. **Table 12** shows a summary of associations between each SNP and outcomes measured.

Table 12. SNP Associations with measured outcomes at 6 months and 12 months post-TBI.

	GOS		PTD		FIM-Cog		Cognitive Composite	
Variant	**Raw**	**Corrected**	**Raw**	**Corrected**	**Raw**	**Corrected**	**Raw**	**Corrected**
6 Months								
rs1800497	**0.0244**[1]	0.2807	0.1369	0.9231	**0.0278**	0.1946	**0.0080**	**0.0427**
rs6279	0.1486	0.5201	0.7087	0.9670	**0.0207**	0.1946	**0.0008**	**0.0128**
rs2734838	0.9109	0.9928	0.1837	0.9231	0.2390	0.3718	0.0547	0.1250
rs17529477	0.8104	0.9928	0.6635	0.9670	0.2314	0.3718	0.1288	0.2290
rs4245147	0.8906	0.9928	0.6695	0.9670	0.2236	0.3718	0.0673	0.1346
rs7131056	0.8312	0.9928	0.8979	0.9670	0.1570	0.3663	0.4483	0.7086
rs4630328	0.6704	0.9928	0.7497	0.9670	0.5629	0.6970	0.1624	0.2362
12 Months								
rs1800497	0.7892	0.9359	0.3290	0.9746	0.4218	0.6327	**0.0294**	0.2464
rs6279	0.1088	0.5698	0.3220	0.9746	0.1772	0.5697	**0.0308**	0.2464
rs2734838	0.1311	0.5698	**0.0225**	0.3375	**0.0395**	0.5697	0.1654	0.3828
rs17529477	0.2255	0.5698	0.4696	0.9746	0.3426	0.6172	0.3577	0.5723
rs4245147	0.3139	0.5886	0.9316	0.9746	0.0908	0.5697	0.2122	0.3828
rs7131056	0.2659	0.5698	0.8967	0.9746	0.2472	0.6172	0.2100	0.3828
rs4630328	0.2587	0.5698	0.6384	0.9746	0.3703	0.6172	0.2153	0.3828

The lowest p-value for each SNP grouping (genotype, allele carrier status, or heterozygote vs homozygote) is reported. There was a significant difference in PTD incidence by *DRD2* rs2734838 genotype at 12 months post-TBI (χ^2= 7.589; p=0.023), but it did not survive correction for multiple comparisons (p=0.338). There were significant associations between *ANKK1* rs1800497 and GOS at 6 months, where a larger percentage of rs1800497 heterozygotes were categorized as a GOS=5

(45.0% compared to 20.3% for homozygotes, χ^2=7.428, p=0.024, uncorrected) but this did not survive correction (p=0.281, FDR corrected). There was no difference between GOS score and rs1800497 genotype/allele-carrier status at 12 months. FIM-Cog scores significantly differed by both rs1800497 and rs6279 at 6 months post-TBI. Both rs1800497 heterozygotes (p=0.028, uncorrected) and rs6279 C-homozygotes (p=0.021, uncorrected) had higher FIM-Cog scores at 6 months, but neither of these findings survived FDR correction (p=0.195 and p=0.194, respectively). At 12 months, rs2734838 A-carriers had lower FIM-Cog scores (p=0.040) but this comparison did not survive FDR correction (p=0.593). There were no other significant associations between genotype and FIM-Cog at 12 months.

4.4.2 Genotype associations with cognitive deficits

The two polymorphisms had significant associations with overall cognitive composite performance that survived correction for at least one time-point (**see Table 12**), and these polymorphisms were then evaluated further for effects on subcomponents of the cognitive composite. The *ANKK1* rs1800497 polymorphism had the strongest relationship to overall cognitive composite scores when comparing homozygotes vs heterozygotes (see **Table 12**). In further evaluation, Rs1800497 heterozygotes performed better than homozygotes across several cognitive domains at both 6 and 12 months post-injury (**Figure 8**).

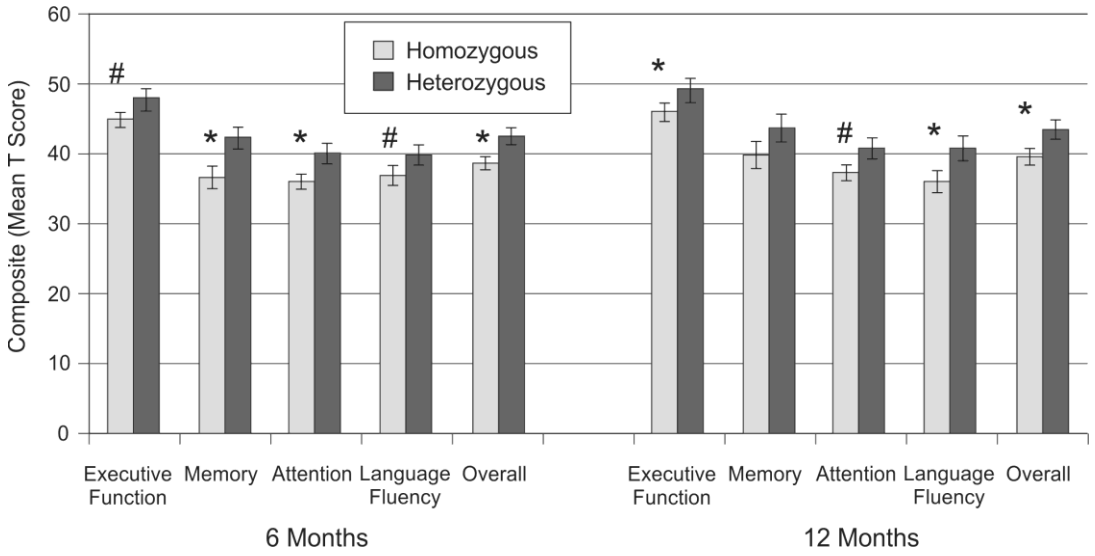


Figure 8. Overall and domain specific cognitive composite scores at 6 and 12 months post-injury show rs1800497 (Taq1A) heterozygotes exhibit better cognitive recovery compared to homozygotes.

Error bars represent SEM. *p<0.05, #p<0.10.

Rs1800497 heterozygotes performed better in attention (p=0.0086) at 6 months post-injury and with language fluency (p=0.032) at 12 months, with trends on other domains noted.

In **Table 13**, individual neuropsychological tests were examined by rs1800497 group. At 6 months, rs1800497 heterozygotes performed significantly better on Trails A, Trails B, and the CVLT Long Delay Free Recall. At 12 months, rs1800497 heterozygotes performed significantly better on Trails B and the COWA Animals category.

Table 13. T scores on neuropsychological tests utilized at 6 and 12 months post-injury.

Cognitive Test	*6 Months*			*12 Months*		
rs1800497	**CC, TT (n=59)**	**CT (n=40)**	**p value***	**CC, TT (n=37)**	**CT (n=26)**	**p value**
Rey	41.31±11.60	43.78±10.57	0.140	43.72±9.47	44.23±11.78	0.368
Digit Span	41.38±6.98	42.12±7.43	0.221	40.58±7.27	42.38±8.02	0.182
DKEFS	40.71±10.30	42.57±10.54	0.156	40.97±8.99	44.83±11.12	**0.067**
Trails A	33.12±13.18	39.21±11.94	***0.003***	34.89±13.30	40.71±12.37	**0.077**
Trails B	36.98±13.23	43.28±14.14	***0.006***	37.43±13.83	44.23±12.41	***0.045***
CVLT Long Delay Free Recall	32.94±16.55	39.74±14.80	***0.036***	37.05±16.30	41.96±16.79	0.210
Stroop	54.11±8.13	52.93±7.93	0.182	54.38±7.06	53.68±7.70	0.293
COWA Animals	32.82±12.93	34.69±12.71	0.291	30.86±12.25	37.04±12.35	***0.035***
rs6279	**CC (n=61)**	**CG, GG (n=36)**	**p value**	**CC (n=39)**	**CG, GG (n=25)**	**p value**
Rey	43.03±10.91	41.06±11.98	0.167	44.73±9.52	42.76±11.71	0.283
Digit Span	43.27±6.66	39.34±7.16	***0.008***	42.54±7.86	39.56±6.91	**0.072**
DKEFS	43.41±10.84	38.33±9.01	***0.009***	44.33±10.33	39.57±8.80	***0.037***
Trails A	38.51±12.14	30.91±13.30	***0.002***	39.51±12.01	33.17±14.18	***0.045***
Trails B	42.12±12.80	34.79±15.05	***0.001***	42.19±12.68	36.48±14.58	***0.045***
CVLT Long Delay Free Recall	37.85±16.43	32.63±14.96	**0.074**	41.03±17.23	35.83±15.16	0.137
Stroop	54.41±7.42	52.56±9.01	0.317	55.03±7.22	52.72±7.26	**0.082**
COWA Animals	36.07±12.69	29.79±12.20	***0.017***	34.12±12.49	32.36±12.92	0.362

*Bolded p values, p<0.1. Bolded and italic p values, p<0.05.

The *DRD2* rs6279 polymorphism was also significantly related to cognitive performance. At 6 months post-injury, rs6279 C-homozygotes had higher overall composite scores (**Table 12**); at 12 months, rs6279 C-homozygotes performed significantly better overall (p=0.031) but this association did not survive correction for multiple comparison (p=0.246). **Figure 9** shows that rs6279 C-homozygotes performed better in executive function (p=0.013), attention (p=0.001), and language fluency (p=0.003) domains at 6 months post-injury, and subjects this group performed better with executive function (p=0.022) and attention (p=0.039) domains at 12 months.

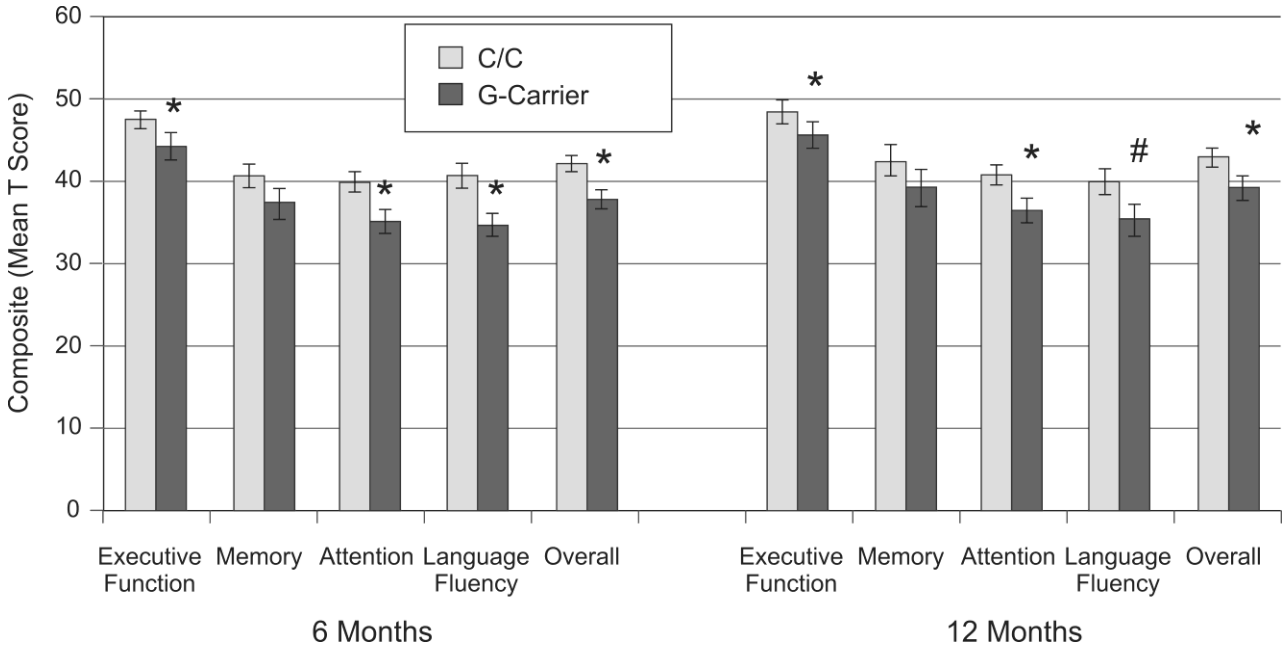


Figure 9. Overall and domain specific cognitive composite scores at 6 and 12 months post-injury show rs6279 C-homozygotes exhibit better cognitive recovery compared to G-carriers.

Error bars represent SEM. *p<0.05, #p<0.10.

In **Table 13**, individual tests were examined by rs6279 G-carrier status. At 6 months, rs6279 C-homozygotes performed significantly better on Digit Span, DKEFS, Trails A, Trails B, and COWA Animals. At 12 months, rs6279 C-homozygotes performed significantly better on DKEFS, Trails A and Trails B.

To evaluate these polymorphisms in overall cognitive performance, both SNPs were tested in backwards step-wise multivariate linear regression models predicting overall cognitive composite scores at 6 and 12 months. At 6 months, both rs1800497 and rs6279 contributed significantly to overall cognitive composite score prediction, even after adjusting for age, GCS, and education (**Table 14**). At 12 months, only rs1800497 remained in the final model.

Table 14. Multivariate model of overall cognitive composite scores at 6 and 12 Months.

Variable	Parameter Estimate	Standard Error	t value	*p* value*	95% Confidence Interval
6 Months – Overall Composite (n=99)					
Age	-0.12879	0.05196	-2.48	***0.0151***	(-0.23204 - -0.02553)
GCS	0.84796	0.24580	3.45	***0.0009***	(0.35955 - 1.33636)
Education	1.19127	0.39517	3.01	***0.0034***	(0.40608 - 1.97646)
rs1800497 Heterozygotes	2.97878	1.49539	1.99	***0.0494***	(0.00747 – 5.95010)
rs6279 G-carriers	-3.20962	1.51691	-2.12	***0.0371***	(-6.22369 - -0.19554)
12 Months – Overall Composite (n=64)					
Age	-0.12435	0.06068	-2.05	***0.0450***	(-0.24586 - -0.00284)
GCS	0.92104	0.30755	2.99	***0.0041***	(0.30518 - 1.53690)
Education	1.59569	0.50538	3.16	***0.0025***	(0.58368 - 2.60770)
rs1800497 Heterozygotes	4.70872	1.71488	2.75	***0.0081***	(1.27474 - 8.14271)

*Bolded p values, $p<0.1$. Bolded and italic p values, $p<0.05$.

4.5 DISCUSSION

While cognitive function restoration is one of the most important aspects in recovery post-TBI, there is considerable heterogeneity in cognitive recovery patterns. In this study, we utilize a Rehabilomics framework (A K Wagner, 2010; Wagner and Zitelli, 2012) to examine individual recovery patterns using genetic, clinical, and demographic factors that influence cognitive recovery, with the notion that genetics may influence other downstream outcomes like participation and quality of life. In this study, we showed that genetic polymorphisms in the *ANKK1* and *DRD2* gene were associated with cognitive recovery post-TBI. This report is one of the first studies to utilize cognitive composite scores to evaluate genetic modulators of TBI cognitive recovery (Wagner et al., 2012). By using normative data adjustments prior to analysis of neuropsychological test performance, we showed injury specific effects of age and education effects beyond that observed in uninjured populations. Furthermore, this work suggests a need for

personalized approaches to treatments like cognitive rehabilitation strategies and neurostimulant use.

While the *DRD2* gene codes for D2 receptor expression, most studies have focused on the Taq1A polymorphism in *ANKK1* as a potential functional moderator of D2 expression/function. Previously, it was believed that the relationship between rs1800497 and D2 receptors was due to high LD with variation in *DRD2*. rs1800497 is in high LD with SNPs within *DRD2* that are linked to alternative splice variants capable of moderating neuronal activity during a working memory task (Y. Zhang et al., 2007). Now, it is accepted that rs1800497 lies within the *ANKK1* gene (Neville et al., 2004), calling into question the direct genetic link to D2 function. *ANKK1* appears to code for a serine/threonine kinase that may interact with D2 receptor signal transduction. Also, rs1800497 induces an amino acid change (Glu713Lys) within a substrate binding domain, thus it is possible ANKK1 interacts with D2 or other DAergic signaling to impact cognitive recovery post-TBI, but more basic research into ANKK1 is needed to elucidate this mechanism.

Even with this superficial understanding of ANKK1's potential function, several studies have identified differences in DAergic function based on rs1800497 genotype. As D2 autoreceptors are important for regulation of striatal DA synthesis, PET studies utilizing [^{18}F]fluorodopa as a measure of DA synthesis suggest T-carriers have increased DA levels and reduced D2 receptor expression (Laakso et al., 2005). Increased DA synthesis capacity, and/or basal DA levels, could be beneficial to recovery given the functional hypo-dopaminergic state post-TBI that is postulated based on our experimental (Wagner et al., 2009) and clinical (Amy K Wagner et al., 2014) work. Similarly, if ANKK1 affects DAergic signaling, heterozygotes may express both kinase structures, exhibiting dopamine levels in an optimal range for recovery, consistent with the 'inverted U' hypothesis for DAergic function (Cools and D'Esposito, 2011a).

Many studies have examined rs1800497 Taq1A genotypes in *ANKK1* grouped by T-carrier status. We grouped rs1800497 Taq1A based on genotype, allelic carrier status, and heterozygotes vs homozygotes. Using this approach, heterozygotes for rs1800487 had the best cognitive performance post-TBI. Consistent with this finding, multiple studies have evaluated heterozygosity for rs1800497 and found that heterozygote status is a biologically relevant comparison to explore, revealing a possible heterozygote advantage (Comings and MacMurray, 2000). Consistent with much of the literature (Munafò et al., 2007), T-homozygotes are less frequent in our population (n=4). Thus, the comparison of rs1800497 heterozygotes vs homozygotes is similar to a T-carrier approach. With mean cognitive composite comparisons, T-homozygotes were more similar to C-homozygotes than C/T heterozygotes, supporting a homozygotes/heterozygote approach. However, utilizing a T-carrier approach showed similar findings; T-carriers (91.6% of which are heterozygotes) had better cognitive composite scores at 6 and 12 months (data not shown). Our study was designed a priori with a MAF criteria of >0.2 to provide robust heterozygosity within our sample size, maximizing our ability to evaluate heterozygosity with outcome prediction post-TBI. Examining heterozygosity across functional and tagging SNPs was an important study goal, given the dimerization patterns associated with the D2 receptor(Zawarynski et al., 1998). Depending on SNP function, a mixture of different translated peptides, due to genetic heterozygosity associated with this receptor, could alter dimerization characteristics and affect DA signaling.

ANKK1 rs1800497 heterozygotes (T/C) exhibited better cognitive performance in this study. Clinically relevant, in some domains, homozygotes would be considered impaired with T scores <40, where heterozygotes would not be significantly impaired. Previous work from our lab utilizing PET imaging indicates that T-carriers (A1-carriers, with the majority being

heterozygotes) have higher striatal dopamine transporter (DAT) binding following TBI. Yet, in this same study, there were no significant differences in D2 binding by rs1800497 genotype. This lack of association between rs1800497 and D2 binding post-TBI, combined with another report of higher striatal DAT binding in A1-carriers, (Laine et al., 2001) suggests there may be important distinctions in trafficking striatal DAT based on rs1800497 genotype. It is also possible protein-protein interactions between DAT and D2 (Lee et al., 2007) may significantly differ by rs1800497 genotype.

Previous studies in mild TBI show that T-carriers performed worse on the CVLT recognition measure at 1 month post-injury (McAllister et al., 2008). Our data demonstrate heterozygotes (C/T) perform better on other neuropsychological measures in our severe TBI population. These apparent differences in gene x cognition associations may be due to several factors. In mild TBI, McAllister *et al* (McAllister et al., 2008, 2005) demonstrated gene-risk relationships that mirror findings in healthy populations. Importantly, our study examines a larger, more severely injured population, and cognition was examined farther out from injury. Thus rs1800497 Taq1A may have a stratified injury severity interaction with cognitive recovery. Also, several factors (socioeconomic, access to care, spontaneous recovery) can influence cognitive recovery trajectories (Hoofien et al., 2002; León-Carrión et al., 2013). Outcome measures instruments also differed across these studies, suggesting this polymorphism may have domain-specific relationships to cognition. However, our study examines multiple cognitive domains, demonstrating that variation within *ANKK1* and *DRD2* influences multiple areas of cognition.

This study also examined 6 SNPs covering variation in the *DRD2* gene. Rs6279 was significantly associated, across domains, with cognitive recovery at 6 months post-TBI; the association was less strong with cognition at 12 months. McAllister and colleagues(McAllister et

al., 2008) found a relationship between rs6279 and CVLT recognition task performance in subjects with TBI, but no relationship in healthy controls. Rs6279 has not been well studied, but it is in high linkage disequilibrium with rs6277, a synonymous mutation C957T, that results in impaired stabilization of *DRD2* mRNA (Duan et al., 2003). This mutation may impair the ability of DA to stabilize *DRD2* mRNA, which then leads to reduced translation and D2 expression in response to increased DA concentrations. Rs6277 is implicated in risk for schizophrenia (Monakhov et al., 2008) and posttraumatic stress disorder (Voisey et al., 2009). As there is no known functional impact of rs6279, our findings with rs6279 may actually be reflective of its high LD with rs6277.

Interestingly, multivariate analysis including rs6279 and rs1800497 showed that both SNPs have independent effects at 6 months post-injury. While only rs1800497 was significantly associated with overall composites in our 12 month multivariate model, it is possible that in a larger sample size, there may be a significant influence of rs6279, in addition to rs1800497, on cognition. However, it is also possible the mechanism of rs6279's impact on cognition post-TBI may be diminished at 12 months. The temporal dynamics of these *DRD2* associations is consistent with other studies from our group that demonstrate transient genetic outcome associations across recovery post-TBI (Failla et al., 2014, 2013). Domain specific analysis supports slightly different roles for each SNP in overall cognitive recovery. For example, at 6 months post-TBI, rs6279 shows stronger associations to executive function and language fluency compared to rs1800497.

Our multivariate data also suggests there are specific injury-age interactions following TBI that differ from control populations. Our cognitive composites accounted for age, gender, and education, based on standardized norms for each neuropsychological measure. Yet age and education were still significant predictors of cognitive composite score in our multivariate models, suggesting these demographic variables have an amplified injury-specific effect on cognitive

performance. Education may be a correlate for post-TBI cognitive reserve that could likely impact an individual's cognitive recovery trajectory post-TBI (Green et al., 2008; Schneider et al., 2014). Given the detrimental effects of age on other TBI pathology/recovery domains (Kumar et al., 2013; Onyszchuk et al., 2008; Susman et al., 2002; Wagner et al., 2004), age enhanced vulnerability to cognitive dysfunction post-TBI likely also occurs. Additionally, our inclusion of the best GCS in first 24 hours post-injury confirms other studies findings where best GCS in 24 hours predicts long-term outcome (Cifu et al., 1997a; Udekwu et al., 2004a).

This study also examined *ANKK1* and *DRD2* variation in post-TBI depression (PTD), FIM-Cog, and GOS. Despite some associative trends, there were no relationships between any of the variants examined and PTD following FDR correction. While there is emerging evidence of DAergic signaling involvement in depressive symptoms (Dunlop and Nemeroff, 2007), this study may be underpowered to examine DAergic relationships to PTD. Rs1800497 and rs6279 were associated with GOS and FIM-Cog, though none survived correction for multiple corrections. However, consistent with the Rehabilomics Model (A K Wagner, 2010; Wagner and Zitelli, 2012), the data suggest a need for larger studies to examine how genetic relationships associated cognitive performance and may moderate modest effects on other outcomes like GOS and FIM-Cog.

One important caveat in this study is the reported findings were conducted in a population of Caucasians-only. *DRD2* SNP distributions were significantly different by race (data not shown), highlighting the need to limit our analysis to Caucasians to avoid any potential stratification effects. This study was not powered to examine genotypic associations by race in post-TBI cognitive outcomes, and future studies are needed to examine more racially-diverse populations. While this study is limited by its overall sample size, it is one of the larger studies examining genetic factors in cognitive recovery post-TBI, similar to our previous studies (Wagner et al.,

2012). Investigating DA signaling effects of these gene variants post-TBI may inform relationships to cognitive recovery. Similarly, understanding how these genetic variants impacts DAergic pharmacological intervention (Mi et al., 2011) post-TBI may improve and personalize our current treatment paradigms.

5.0 BRAIN-DERIVED NEUROTROPHIC FACTOR IN TBI-RELATED MORTALITY: INTERRELATIONSHIPS BETWEEN GENETICS AND ACUTE SYSTEMIC AND CNS BDNF PROFILES

In Submission

Michelle D. Failla, BS, Yvette P. Conley, PhD, Amy K. Wagner, MD

5.1 ABSTRACT

While brain derived neurotrophic factor (BDNF) is relevant to traumatic brain injury pathology due to its role in neuronal survival, neurogenesis, and plasticity, BDNF also has strong effects on hypothalamic metabolic regulation and brainstem control of the cardiovascular system. Reduced BDNF production occurs immediately following experimental models of traumatic brain injury (TBI), with transient upregulation of BDNF's pro-apoptotic receptors. Our recent work shows variation in the BDNF gene (expressed as a gene risk score including rs6265 and rs7124442) interacts with age to influence mortality. Evidence supporting dynamic, temporal balances of pro-survival/pro-apoptotic target receptors may explain injury-specific and age-related gene associations. Thus, we investigated BDNF as a genetically-modulated biomarker of mortality following severe TBI. BDNF levels were examined in CSF and serum for 203 prospectively recruited subjects during the first week following severe TBI. Interrelationships between BDNF gene risk score and BDNF levels were assessed as mortality predictors, and compared to levels in a cohort of healthy controls (n=10). Average CSF BDNF levels tended to be higher following

injury compared to healthy controls (0.20±0.01ng/mL versus 0.14±0.02ng/mL, p=0.061), with similar trends noted for d1-5 ($p<0.10$). Average serum BDNF levels (150.05±4.90ng/mL) were reduced compared to healthy controls (277.86±28.11ng/mL, $p<0.0001$) beginning d0 (p=0.007) and remained low for all time-points ($p<0.0001$ for remaining comparisons). Following injury, CSF and serum BDNF levels tended to be negatively correlated (r=-0.209, p=0.070, n=76), but importantly, this correlation was likely modulated by age (age<45yrs: r=-0.307, p=0.029, n=51; age>45yrs: r=-0.113, p=0.590, n=25). Serum BDNF levels interacted with the BDNF gene risk score to influence mortality beyond that predicted by previously reported gene*age interactions alone. CSF BDNF levels directly impacted mortality predictions, where CSF BDNF levels predicted time until death (p=0.042, hazard ratio=10.973). BDNF is likely a genetically-modulated, physiologically relevant prognostic biomarker for mortality following TBI. Compared to positive correlations in controls, the negative correlation between CSF and serum BDNF suggest blood-brain barrier transit alterations following injury. Yet, this work suggests a role for BDNF pathophysiology in contributing to mortality following TBI that may encompass neuronal survival as well as modulation of autonomic function. BDNF pathology is likely an important new target in relationship to individual variation in mortality predictions post-TBI, and understanding BDNF signaling in relation to other secondary injury cascades, age, and receptor regulation following TBI may inform new treatment paradigms.

5.2 INTRODUCTION

The World Health Organization suggests that traumatic brain injury (TBI) will be one of the major leading causes of death and disability by 2020, with about 10 million people affected

each year (Hyder et al., 2007). Advanced age is a consistent determinant of TBI survival (Brooks et al., 2013). Older adults comprise a large segment of the population sustaining TBI with comparatively worse outcomes and higher mortality rates despite similar injury parameters (Susman et al., 2002). Beyond the acute mortality associated with moderate/severe TBI, recent literature also indicates that individuals initially surviving their TBI go on to have an increased risk for mortality during the post-acute phases of recovery and, on average, have a shorter life-span (Brooks et al., 2013; Harrison-Felix et al., 2006). Several systemic and CNS biomarkers can inform acute mortality predictions after moderate/severe TBI (Goyal et al., 2013; Wagner et al., 2011b). Biomarkers associated with acute mortality may also elucidate TBI-specific pathology relevant to the molecular pathways they represent, identifying new avenues to target for neuroprotective therapeutics or indicating important injury-specific phenomena in well-studied pathways. While some studies have evaluated early biomarker patterns when predicting later mortality and/or global outcomes (Goyal et al., 2013; Santarsieri et al., 2013; Wagner et al., 2011a, 2011b), less is known about how innate biological factors, like genetics, interact with acute biomarkers to influence mortality.

While current studies suggest demographic and clinical variables like age, injury severity, and pupillary reactivity influence mortality prediction (Roozenbeek et al., 2012), our previous work demonstrates that genetic variation within the brain derived neurotrophic factor (BDNF) gene improves mortality prognostication(Failla et al., 2014). BDNF is a neurotrophin involved in neuronal survival and synaptic plasticity in hippocampus and cortex (Martinowich and Lu, 2008). While BDNF is relevant to TBI pathology with neuronal survival, neurogenesis, and plasticity(Z.-Y. Chen et al., 2004), BDNF also has strong effects on hypothalamic metabolic regulation (Kernie et al., 2000; Pelleymounter et al., 1995) and brainstem control of the cardiovascular system (Brady

et al., 1999; Wang and Zhou, 2002; Wan et al., 2014). Similarly, variation within the *BDNF* gene has been associated with hypothalamus-pituitary-adrenal (HPA) axis reactivity (Alexander et al., 2010; Shalev et al., 2009) and autonomic regulation of heart rate (A. C. Yang et al., 2010). Given the role of BDNF in autonomic function and neuronal survival, BDNF likely has multiple actions that could impact TBI mortality, making BDNF an attractive biomarker to explore, across the recovery spectrum, where these actions may have dynamic influences.

We have previously shown associations between TBI mortality and two single nucleotide polymorphisms (SNP) within the *BDNF* gene (Failla et al., 2014) that may affect BDNF secretion, thus influencing BDNF levels in serum and CSF. The current study targets a functional SNP, Val66Met (rs6265), which alters activity-dependent BDNF secretion BDNF *in vitro* (Egan et al., 2003), as well as rs7124442, which is in linkage disequilibrium (LD) with rs6265, and affects neuronal BDNF mRNA trafficking (Orefice et al., 2013). The relationship between Val66Met and BDNF serum levels is controversial (Terracciano et al., 2013), but some studies suggest lower serum BDNF levels in Met allele-carriers. Thus, BDNF post-TBI may be a physiologically relevant, genetically-influenced biomarker.

The relationship between BDNF and TBI-related mortality is also moderated by age. Advanced age likely adversely effects secondary injury cascades. Older age can lead to greater susceptibility during glutamate-mediated oxidative stress and damage (Wagner et al., 2004). In experimental models of injury, older animals show decreased neuronal survival (Onyszchuk et al., 2008) and altered immune states (Kumar et al., 2013). Previous work from our lab demonstrates that age interacts with *BDNF* associations to predict mortality risk up to one year post-injury. We suggest that this interaction between age and *BDNF* variation in TBI-related mortality may be due to age-related changes in BDNF target receptor milieu (Romanczyk et al., 2002; Webster et al.,

2006). Understanding associations between BDNF levels, aging, and mortality will aid our understanding of this complex association.

BDNF has been extensively evaluated as a biomarker in affective disorders, where lower serum levels are associated with active depressive episodes (Brunoni et al., 2008). One TBI study by Kalish and Phillips (Kalish and Phillips, 2010) reported that serum BDNF levels are acutely decreased, correlating with injury severity. While BDNF levels have not been examined in connection with TBI-related mortality, studies show that decreases in serum BDNF levels are associated with mortality in uninjured populations (Halldén et al., 2013; Krabbe et al., 2009; Ritter et al., 2012). Based on previous studies involving uninjured humans and rodent models, serum BDNF levels may correlate with CSF and brain BDNF (Pan et al., 1998; Pillai et al., 2010).

In this study, we measured BDNF levels in serum and CSF across the first seven days after severe TBI in 203 subjects. We evaluated BDNF levels for associations with mortality across the first year post-TBI, in relation to *BDNF* genetic mortality risk. Both CSF and serum demonstrated significant capacity for predicting mortality across a 1-year recovery period, in addition to genetic risk, suggesting BDNF is a novel and informative TBI biomarker. Additionally, this work supports further study into BDNF pathophysiology as a contributing factor in recovery and mortality following TBI.

5.3 METHODS

5.3.1 Participants

This prospective cohort study was approved by the University of Pittsburgh Institutional Review Board. Enrollment criteria for this study included age, ≥16 and <75 years, and an admission Glasgow Coma Scale (GCS) score ≤8 indicating severe TBI. Exclusion criteria included documented prolonged hypoxia prior to admission or penetrating head injury. Participants were consecutively recruited while receiving inpatient care within the University of Pittsburgh Medical Center (UPMC). Consent was obtained from next-of-kin. All subjects sustained a non-penetrating TBI, and had evidence of intracranial injury on Computed Tomography (CT). Participants included had at least one sample measurement (serum or CSF, at any time-point). These participants were a subset of a larger study investigating possible biomarkers and genetic factors related to individual recovery following TBI.

Injury severity was described by the best GCS obtained for each subject within the first 24 hours post-injury. Demographics, including age, sex, and education, were collected by chart review and subject or caregiver interviews.

5.3.2 Sample Collection and Processing

BDNF levels were measured in both CSF and serum samples collected over the first week post-injury. When possible, CSF samples were collected passively up to twice daily via an external ventricular drain placed for clinical care. Serum was collected daily. BDNF values derived from CSF and serum samples were binned by day, and an average was determined for each day post-

injury for each subject. Of 203 participants, 149 had CSF samples (n=583), and 141 had serum samples (n=406). A subset had both CSF and serum samples (n=87). For biomarker comparisons, healthy adult control subjects were separately recruited to establish reference BDNF levels. Control participants providing samples [CSF (n=10) and serum (n=7)] for BDNF measurements were 18-70 years old, had no current/past history of brain injury, neurological disease, bleeding disorder or endocrine disorder. Women were excluded if pregnant, taking oral contraceptives or hormone replacement therapy, or had history of reproductive disorder.

CSF and serum samples were stored at -80ºC before BDNF analysis using an ELISA kit (RayBiotech). Briefly, standards and samples were pipetted onto a 96-well plate pre-coated with human BDNF antibody. Following shaking for 2.5hrs at room-temperature, plates were washed and incubated with biotinylated BDNF antibody for 1hr. HRP-conjugated streptavidin was then added for an incubation of 45 minutes. The addition of a tetramethylbenzidine substrate allowed for a color reaction. Concentrations were calculated using mean absorbance of each sample at 460 nm to correlate with sample BDNF concentrations present. Samples were diluted within the range of the ELISA kit (no dilution for CSF, 1:250 for serum samples), with a kit sensitivity of 80pg/ml, an intra-assay variation of <10%, and an inter-assay variation of <12%.

5.3.3 Mortality and Outcome

Time until death (TUD) was recorded in days post-injury, up to 1yr post-injury, using the Social Security Death Index ("Social Security Death Index," n.d.). For consistency with previous work (Failla et al., 2014), mortality was evaluated over two time-epochs, 0-7d post-injury (*acute*) and 8d-365d post-injury (*post-acute*), and then across the entire recovery span of 0-365. For 0-7d, survivorship was right censored at 7d post-injury. For 8-365d, participants were included if they

survived >7d, and survivorship was right censored at 365d. Mortality was also examined as a binary outcome at 365d post-injury. Acutely, 11.62% of participants died (TUD: median=3d, min=0d, max=7d, Q1=2d, Q3=6d). An additional 14.16% died post-acutely (TUD: median=19d, min=8d, max=301d, Q1=11.5d, Q3=31d).

We evaluated whether BDNF levels are indicative of mortality or graded across a range of outcomes using the Glasgow Outcome Scale (GOS) (1= dead, 2= vegetative state, 3=severe disability, 4=moderate disability, 5=good recovery) at 6 and 12 months (Jennett and Bond, 1975). Research-trained neuropsychometrists, blinded to genetic and biomarker information, obtained GOS scores.

5.3.4 Genotyping and SNP Selection

DNA was isolated from blood using a simple salting out procedure (S.A. Miller et al., 1988) or from CSF using the Qiamp protocol from Qiagen. *BDNF* rs6269 and rs7124442 were genotyped by TaqMan allele discrimination assay using Assay on Demand reagents (Applied Biosystems). This assay utilized fluorescent labeled probes to detect allele(s) present for DNA sample.

Selected SNPs (rs6265, rs7124442) had a minor allele frequency >20%. Each SNP represents a different haplotype block of *BDNF* covering variation corresponding to "isoform a". A cumulative *BDNF* GRS was used as previously published(Failla et al., 2014), where rs6265 Met (Val/Met or Met/Met) and rs7124442 C (T/C or C/C) carrier status hypothesized as hypothesized risk alleles due to reduced BDNF secretion reported in the literature(Egan et al., 2003; Orefice et al., 2013). Thus, a GRS=0 was the hypothesized no risk group (Val/Val, T/T); GRS=1 included carriers for 1 risk allele (Val/Val, C-carriers or Met-carriers, T/T); GRS=2 included carriers of

both risk alleles (Met-carriers, C-carriers). Due to possible genetic stratification effects (Freedman et al., 2004), genetic associations are reported in Caucasians only (n=181).

5.3.5 Statistical Analysis

Data analysis was conducted using Statistical Analysis Software (SAS) 9.3. Descriptive analyses included mean and standard deviation and/or median for continuous and ordinal variables, and analyses included frequencies for categorical variables. Demographic and clinical information was examined for variation with BDNF levels using Mann-Whitney and Kruskal-Wallis tests, due to skewedness of BDNF level data. Genetic analysis utilized categorizations based on allele carrier status using Chi-square or Fishers Exact test where appropriate, and the *BDNF* GRS was used to examine cumulative genetic risk associations with mortality as previously described (Failla et al., 2014). Age was dichotomized at 45yrs to emulate previous TBI mortality analyses (Failla et al., 2014).

Mortality was examined across two time-frames for consistency with previous work (Failla et al., 2014), 0-7d post-injury (*acute*) and 8d-365d post-injury (*post-acute*), and then across the entire recovery span of 0-365d. BDNF levels were examined for mortality associations post-TBI using TUD in Cox proportional hazards models (Cox, 1972). The proportionality of hazards assumption was tested and confirmed for all final Cox model variables. Interaction terms were identified by previous studies (in the case of age*GRS) or existing hypotheses (in the case of GRS*serum BDNF) and tested in Cox models. All models were developed using a backwards-stepwise approach, with variables remaining in the final models if $p \leq 0.2$. Final models were then tested to determine the effect of removal of the BDNF levels variable on the remaining associations in the model. A p-value<0.05 was considered statistically significant.

5.4 RESULTS

5.4.1 Description of CSF and serum BDNF levels 0-6d post-TBI

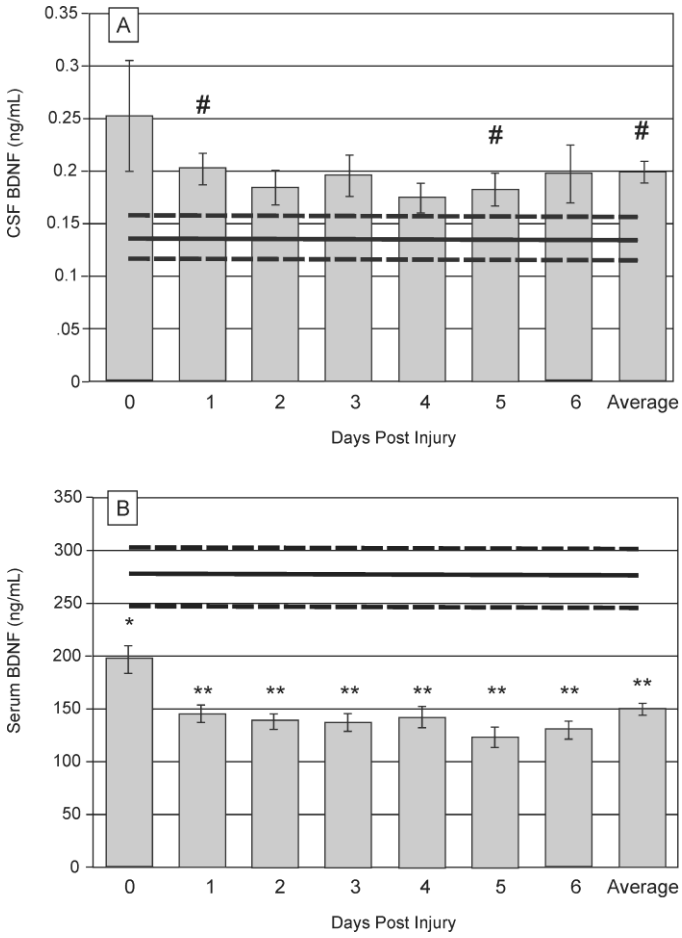


Figure 10. Daily brain derived neurotrophic factor (BDNF) levels over the first 7 days post-injury, compared to healthy controls.

(A) Daily mean CSF BDNF levels tend to be higher than control levels (trending at days 1 and 5, $p<0.1$ for both). Average CSF levels across the first week post-injury are show trends elevated from control levels ($p=0.061$). (B) Daily mean serum BDNF levels fall below control levels immediately following injury (day 0, $p=0.007$) and remain reduced through day 6 ($p<0.0001$ for all comparisons). (# $p<0.1$; *$p<0.05$, **$p<0.001$) (mean represented by gray horizontal line, ±standard error in dashed gray horizontal lines)

As depicted in **Figure 10A**, average CSF BDNF levels tended to be higher for participants with TBI compared to healthy controls (0.20±0.01ng/mL versus 0.14±0.02ng/mL, p=0.061), with similar trends noted for d1-5 (p<0.10). Average serum BDNF levels in TBI participants (150.05±4.90ng/mL) were reduced versus healthy controls (277.86±28.11ng/mL, p<0.0001) beginning d0 (p=0.007) and remained below healthy controls for all time-points (p<0.0001 for remaining comparisons, **Figure 10B**). For participants with TBI, average CSF and serum BDNF levels tended to be negatively correlated (r=-0.209, p=0.070, n=76). In healthy controls, BDNF levels suggested a positive correlation between CSF and serum but this was not significant likely due to sample size (r=0.67, p=0.219, n=5).

5.4.2 Associations of CSF & serum BDNF levels with demographic and clinical variables

Average levels (d0-6) for both CSF and serum were examined for associations with demographic and clinical variables. **Table 15** reports relationships between serum BDNF levels, CSF BDNF levels, and demographics, clinical variables, and BDNF genetic variants (rs6265 and rs7124442). All allele/genotype frequencies were in Hardy-Weinberg equilibrium. There were no significant associations between genetic variants and BDNF levels. Average CSF levels positively correlated with age (r=0.17, p=0.045), while serum levels did not correlate with age. Correlations between serum and CSF BDNF levels were examined by age. There was a negative correlation in participants <45 yrs. (r=-0.307, p=0.029, n=51), but this was not significant for participants >45yrs. (r=-0.113, p=0.590, n=25).

Table 15. Demographic associations with BDNF CSF and serum levels.

Demographic Variable		CSF Weekly Average, ng/mL (n=149)	Serum Weekly Average, ng/mL, (n=141)
rs6265	Val/Val (n=96)	0.20±0.11	159.05 ±106.59
	Met-carrier (n=61)	0.19±0.13	159.47±60.57
	p value	0.145	0.155
rs7124442	T/T (n=84)	0.19±0.10	163.13 ±113.62
	C-carrier (n=54)	0.19±0.14	153.35 ±65.49
	p value	0.238	0.311
Age	R, Mean	0.17	-0.11
	p value	*0.045*	0.244
Sex	Males	0.20±0.13	147.28±51.81
	Females	0.19±0.10	162.09±64.25
	p value	0.438	*0.099*
GCS	R, Mean	-0.08	-0.01
	p value	0.343	0.927

5.4.3 CSF and serum BDNF Associations with Mortality

Consistent with previous work (Failla et al., 2014), CSF and serum BDNF were examined for associations within two mortality time-epochs. Associations with mortality outcomes are presented in **Table 16**. Age and GCS were associated with mortality during both time epochs and across the total survival period. Average CSF BDNF levels were higher in those participants who died within the post-acute time epoch. Serum BDNF levels tended to be associated with acute mortality; participants who died 0-7d post-injury had lower serum levels (130.75±17.26ng/mL) versus survivors (159.47±60.57ng/mL).

Table 16. Demographic associations with mortality time epochs.

	Acute Mortality (0-7 days)			Post-Acute Mortality (8-365 days)			Total Mortality (0-365 days)		
	Died (n=23)	**Survived (n=157)**	***p* value**	**Died (n=32)**	**Survived (n=125)**	***p* value**	**Died (n=55)**	**Survived (n=125)**	***p* value**
Age, mean±STD	45.3 ±17.2	36.4 ±15.6	***0.010***	49.8 ±16.1	32.9 ±13.6	***<0.001***	47 ±16.6	32.9 ±13.6	***<0.001***
GCS, median	6	7	***0.004***	6	7	***0.007***	6	7	***<0.001***
Male, # (%)	15 (65.2)	129 (82.2)	*0.073*	26 (81.3)	103 (82.4)	0.880	41 (74.6)	103 (82.4)	0.232
CSF Weekly Average, ng/mL (n=133)	0.25 ±0.17	0.19 ±0.12	0.214	0.26 ±0.17	0.18 ±0.10	***0.013***	0.25 ±0.17	0.18 ±0.10	***0.012***
Serum Weekly Average, ng/mL (n=124)	130.75 ±17.26	159.47 ±60.57	*0.054*	147.04 ±44.46	155.23 ±51.84	0.353	140.06 ±58.30	155.23 ±51.84	0.107

STD=standard deviation; GCS=Glasgow Coma Scale; CSF=cerebrospinal fluid

Associations between CSF BDNF levels and TUD within each mortality epoch were examined (**Supplementary Tables 17A/B**). Given that CSF BDNF levels warranted inclusion into mortality models in both acute and post-acute models ($p<0.2$), a final model of TUD across the first year (0-365) was evaluated (**Table 18A**). In this model, average CSF BDNF levels predicted TUD (p=0.042, HR=10.973) with an age*GRS interaction (p=0.0561, HR=0.968) and GCS (p=0.004, HR=0.728) as covariates. Importantly, when CSF BDNF levels were excluded from this model, age*GRS was significant (p=0.034, HR=0.971), where similar to our previous report, older individuals were at higher risk for mortality if they carried fewer of the hypothesized risk (i.e. low BDNF secretion) alleles.

Table 17. Associations between BDNF levels and TUD within each mortality epoch.

Parameter	Parameter Estimate	Standard Error	Chi-Square	*p* value	Hazard Ratio	95% Hazard Ratio Confidence Limits
A. Acute CSF Model, (0-7 days)						
Age	0.38433	0.16365	5.5155	0.0188	1.469	(1.066-2.024)
GCS	-0.33281	0.18838	3.1212	0.0773	0.717	(0.496-1.037)
GRS=1*	12.22476	5.60575	4.7557	0.0292	203772.6	(3.448-1.20x10^{10})
GRS=2	17.51545	7.74785	5.1107	0.0238	40445053	(10.277-1.59x10^{14})
Weekly average CSF (ng/mL)	3.90419	2.50418	2.4307	0.1190	49.610	(0.366-6716.365)
GRS x Age	-0.16442	0.07791	4.4533	0.0348	0.848	(0.728-0.988)
B. Post-acute CSF Model, (8-365 days)						
Age	0.07337	0.01516	23.4196	***<.0001***	1.076	(1.045-1.109)
GCS	-0.37071	0.13638	7.3889	***0.0066***	0.690	(0.528-0.902)
Weekly average CSF (ng/mL)	3.06877	1.37887	4.9532	***0.0260***	21.515	(1.442-320.955)
C. Acute Serum Model, (0-7 days)						
Age	0.21986	0.10383	4.4838	***0.0342***	1.246	(1.016 - 1.527)
GCS	-0.31092	0.18350	2.8711	*0.0902*	0.733	(0.511 - 1.050)
GRS=1*	7.21823	3.38859	4.5376	***0.0332***	1364.075	(1.780 – 1045232)
GRS=2	10.62033	4.79042	4.9151	***0.0266***	40959.26	(3.426 – 4.8974x10^{8})
Weekly average Serum (ng/mL)	-0.01039	0.00591	3.0906	*0.0787*	0.990	(0.978 – 1.001)
GRS x Age	-0.08914	0.04739	3.5376	*0.0600*	0.915	(0.834 – 1.004)
D. Post-acute Serum Model, (8-365 days)						
Age	0.16839	0.05939	8.0396	***0.0046***	1.183	(1.053 – 1.329)
GCS	-0.25839	0.11724	4.8574	***0.0275***	0.772	(0.614 – 0.972)
GRS=1*	5.63009	2.35580	5.7116	***0.0169***	278.686	(2.753 – 28207.98)
GRS=2	11.50550	4.36513	6.9473	***0.0084***	99259.92	(19.106 – 5.1568x10^{8})
Weekly average Serum (ng/mL)	0.03425	0.01797	3.6307	*0.0567*	1.035	(0.999 – 1.072)
GRS x Age	-0.05337	0.02728	3.8283	*0.0504*	0.948	(0.899 – 1.000)
GRS x Weekly average Serum	-0.01918	0.00875	4.8026	***0.0284***	0.981	(0.964 – 0.998)

*Reference Group, GCS=0; GCS=Glasgow Coma Score, GRS=Gene Risk Score

Associations between average serum BDNF and TUD were examined in each mortality epoch. Given similarities of acute and post-acute models (**Supplementary Table 17C/D**), a final model of TUD across the first year (d0-365) was evaluated. There was a serum BDNF*GRS interaction (p=0.047, HR=0.987), with an age*GRS interaction (p=0.007, HR=0.943) and GCS (p=0.010, HR=0.780) as covariates (**Table 18B**).

Table 18. Modeling time until death across recovery (0-365 days).

Parameter	Parameter Estimate	Standard Error	Chi-Square	*p* value	Hazard Ratio	95% Hazard Ratio Confidence Limits
A. CSF Model						
Age	0.11680	0.03343	12.2106	***0.0005***	1.124	(1.053 - 1.200)
GCS	-0.31807	0.11010	8.3462	***0.0039***	0.728	(0.586 - 0.903)
GRS=1*	1.69271	1.04421	2.6278	0.1050	5.434	(0.702 - 42.069)
GRS=2	3.29990	1.74153	3.5904	*0.0581*	27.110	(0.893 - 823.231)
Weekly average CSF (ng/mL)	2.39545	1.17526	4.1544	***0.0415***	10.973	(1.096 - 109.828)
GRS x Age	-0.03228	0.01690	3.6485	*0.0561*	0.968	(0.937 - 1.001)
B. Serum Model						
Age	0.16690	0.04778	12.2032	***0.0005***	1.182	(1.076-1.298)
GCS	-0.24855	0.09676	6.5977	***0.0102***	0.780	(0.645-0.943)
GRS=1	5.38067	1.74053	9.5567	***0.0020***	217.168	(7.166-6581.739)
GRS=2	9.97054	3.06393	10.5896	***0.0011***	21387.04	(52.739-8673014)
Weekly Average Serum (ng/mL)	0.02089	0.01435	2.1189	0.1455	1.021	(0.993-1.050)
GRS x Age	-0.05840	0.02180	7.1755	***0.0074***	0.943	(0.904-0.984)
GRS x Weekly Average Serum (ng/mL)	-0.01261	0.00634	3.9496	***0.0469***	0.987	(0.975-1.000)

*Reference Group, GRS=0; GCS=Glasgow Coma Score, GRS=Gene Risk Score

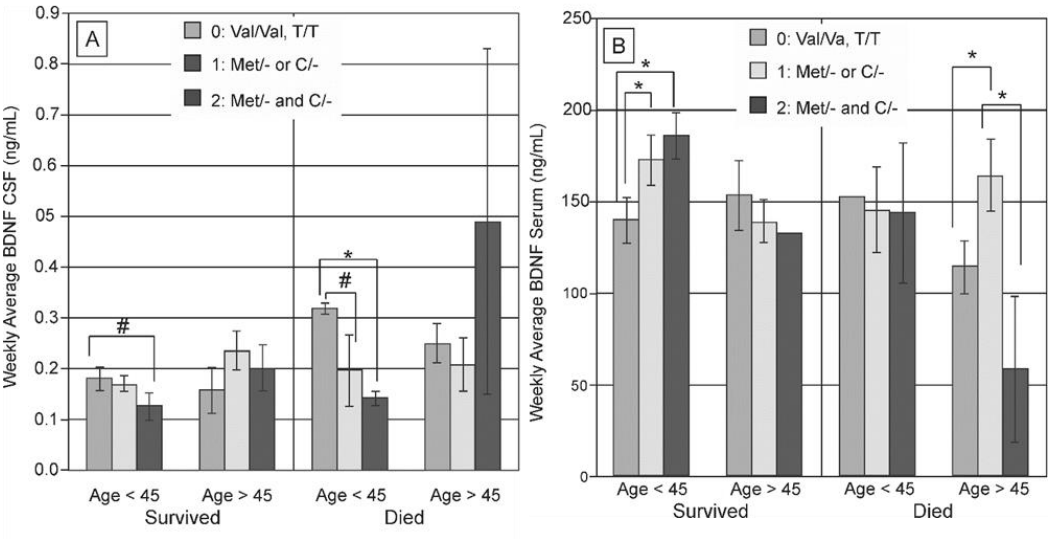


Figure 11. BDNF serum levels and BDNF gene variation show relationships to mortality at 1 year post-TBI.

The cohort is separated by BDNF GRS and age (below and above 45 yrs.). (A) CSF averaged serum levels by 0-365 day survival. Within the age <45 group who died by day 365, subjects with a GRS=0 had significantly higher CSF levels compared to GRS=2 (p=0.024), a similar trend existed compared to GRS=1 (p=0.076). Within survivors under the age of 45, there was a trend such that subjects with a GRS=0 showed higher levels compared to GRS=3 (p=0.092). (B) Weekly averaged serum levels by 0-365 day survival. Within survivors under the age of 45, subjects with a GRS=0 had significantly lower serum levels compared to GRS=1 (p=0.037) and GRS=2 (p=0.018). In subjects over the age of 45 who died by day 365, GRS=1 levels were significantly higher compared to GRS=0 (p=0.041) and GRS=2 (p=0.025).

To interpret interactions between BDNF levels and GRS in the context of age in mortality predictions, **Figure 11** shows averaged CSF and serum levels by 0-365 day survival. Participants were separated by GRS and age (split at 45 yrs.). While there were no significant interactions between CSF BDNF and GRS, BDNF CSF levels graphed by age, mortality, and GRS, are shown in **Figure 11A.** Within participants <45yrs who died, subjects with a GRS=0 (Val/Val, T/T) had higher CSF levels compared to GRS=2 (Met-carriers and C-carriers, p=0.024); a similar trend

existed versus GRS=1 (Met-carrier or C-carriers, p=0.076). Within survivors <45yrs, there were no associations with GRS, though similar trends were observed for the hypothesized high secretion and neuroprotection GRS (Val/Val, T/T, GRS=0) to have more CSF BDNF. For participants >45yrs. there were no differences in CSF BDNF levels by GRS, regardless of mortality. **Figure 11B** shows averaged serum BDNF by age, GRS, and mortality. Among survivors <45yrs, participants with a GRS=0 (Val/Val, T/T) had lower serum levels compared to GRS=1 (Met-carrier or C-carriers, p=0.037) and GRS=2 (Met-carriers and C-carriers, p=0.018). There was no relationship of GRS to serum levels in participants <45yrs. who died by d365. Similarly, within survivors >45yrs., there was no relationship between GRS and serum BDNF. Yet, for subjects >45yrs. who died, GRS=1 levels were higher versus GRS=0 (p=0.041) and GRS=2 (p=0.025).

5.4.4 CSF and serum BDNF Associations with GOS

In a secondary analysis, we evaluated CSF and serum BDNF level associations with global outcome (GOS) to determine if BDNF levels are indicative of mortality or graded across a range of outcomes. Average CSF BDNF levels differed at 6 months between individuals who died versus survived (with both favorable and unfavorable outcome groups, **Figure 12A**). At 12 months, individuals with unfavorable outcome had CSF levels between those with favorable outcomes and those that died, but were not significantly different from either group. However, CSF BDNF levels were higher in those who died by 12 months vs. those with favorable outcome. Serum BDNF was not associated with 6-month GOS scores, though qualitatively higher serum BDNF occurred in those with favorable outcomes. Serum BDNF levels tended to be lower in participants who died by 12 months (140.06±8.99 ng/mL) versus those with favorable outcomes (167.97±8.23 ng/mL, p=0.051, **Figure 12B**).

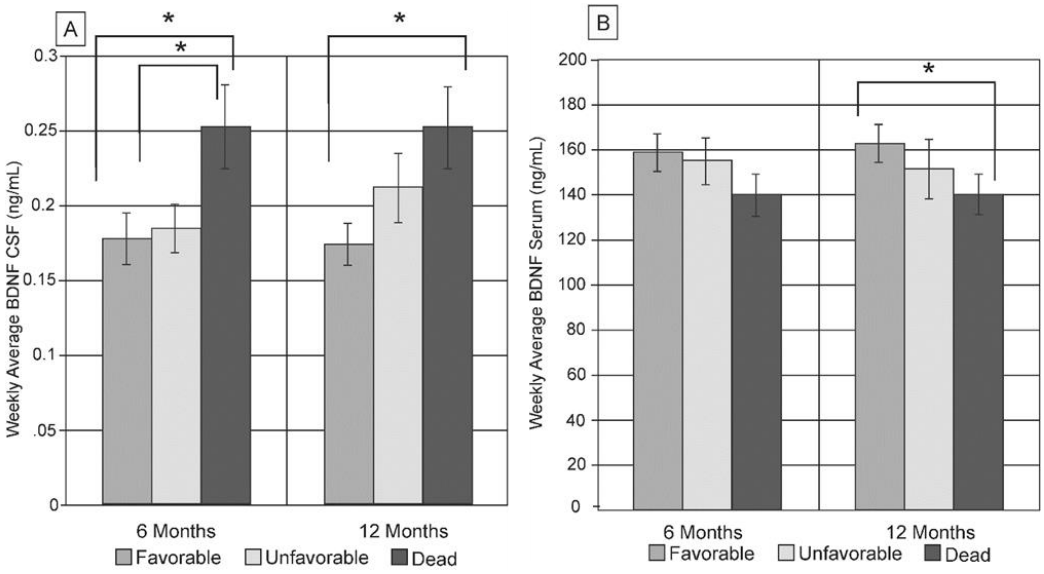


Figure 12. BDNF levels associations with Glasgow Outcome Scale.

(A) At 6 months, weekly averages of CSF were significantly higher in subjects who died compared to subjects who survived (both favorable and unfavorable outcomes, $p<0.05$). At 12 months, GOS levels were significantly different between subjects who died and subjects with favorable outcomes ($p<0.05$). (B) Weekly serum levels tended to be lower in subjects who died by 12 months compared to subjects who had favorable outcomes ($p=0.051$), but there was no significant association at 6 months.

5.5 DISCUSSION

The work presented here suggests unique relationships between neurotrophic signaling, injury, genetics, and age. Importantly, this work furthers experimental studies that indicate BDNF signaling may be highly dependent on age and injury specific alterations in target receptor milieu, allowing for the possibility of both beneficial and detrimental effects following neurological injury. Additionally, this work suggests that BDNF profiles immediately following injury may impact both neuronal survival/apoptotic signaling as well as interplay with the autonomic nervous

system to affect outcome. With the growing elderly population globally, the implications of altered BDNF signaling in the context of advanced age could inform geriatric care. Similarly, this work may also have applications in acquired brain injuries like stroke, where BDNF signaling may be altered under ischemic conditions (Gomes et al., 2012; Vidaurre et al., 2012). Understanding BDNF signaling in relation to other secondary injury cascades, age, and receptor regulation in neurological injury may inform new and more tailored treatment paradigms.

This study reports reduced serum BDNF levels immediately following TBI versus controls, and modestly higher CSF BDNF in individuals with TBI versus controls. Importantly, results show that serum and CSF BDNF levels aid mortality predictions post-TBI, in concert with *BDNF* genetic variation, age, and injury severity. Contrary to hypothesized relationships, elevated CSF BDNF may not be neuroprotective as it was associated with higher mortality risk. This finding is consistent with previous experimental work showing elevated pro-apoptotic CNS BDNF target receptors in the context of injury (Rostami et al., 2013) and our clinical work suggesting that age*GRS interactions for *BDNF* may represent age and injury specific increases in pro-apoptotic BDNF target receptors (Failla et al., 2014). Also, serum BDNF, when considered in an interaction with *BDNF* genotypes, was associated with mortality. The data also suggest that serum BDNF contributes, at least in part, to CSF BDNF levels via BBB disruption. However, reduced serum BDNF may also have other relationships (e.g. autonomic function) that could contribute to mortality.

Clinical TBI biomarker studies that focus on BDNF are limited, and to our knowledge, have not investigated BDNF levels and TBI outcome, especially in the context of genetic variation. One study in pediatric TBI showed rapid increases in CSF BDNF levels immediately following the injury (at 2hrs post-injury) that remained higher than controls throughout the first 24hrs

(Chiaretti et al., 2003). Kalish and Phillips (Kalish and Phillips, 2010) found serum BDNF levels were correlated with injury severity post-TBI, such that subjects with mild injuries had high serum BDNF levels. Yet, BDNF levels have never been examined as a marker for TBI-related mortality.

Our previous research showed that *BDNF* variation interacts with age to influence mortality predictions post-TBI(Failla et al., 2014). In this study, we utilized our previous *BDNF* GRS to account for genetic variation at both loci within the *BDNF* gene. Previously, *BDNF* GRS was associated with acute mortality, regardless of age. Contrary to expectations, participants in the hypothesized "no-risk" (higher proposed BDNF secretion) group had the *lowest* survival probability. Post-acutely, *BDNF*-GRS interacted with age to support the neuroprotection hypothesis such that younger participants in the "no-risk" group had the *highest* survival probabilities, but older participants in the "no-risk" group had the *lowest* survival probabilities.

Previously, we suggested that age-specific risk profiles are due to a balance shift in BDNF receptor ratios with aging, from pro-survival to pro-apoptotic, diminishing recovery post-TBI (Croll et al., 1998; Romanczyk et al., 2002; Tapia-Arancibia et al., 2008; Webster et al., 2006). BDNF's signaling capabilities depend on a balance of tissue receptor milieu. In brain, BDNF is synthesized as pro-BDNF then cleaved to mature BDNF (Barker, 2009). Mature BDNF has pro-survival signaling capabilities via the full length TrkB receptor (TrkB.FL), while pro-BDNF can be pro-apoptotic via the $p75^{NTR}$ receptor (Barrett, 2000). There are two truncated TrkB isoforms lacking intracellular tyrosine signaling capacity, with physiological implications in other pathways (Gomes et al., 2012; Vidaurre et al., 2012). Experimental TBI induces transient increases in hippocampal TrkB.FL followed by delayed increases in TrkB.T with regionally-specific $p75^{NTR}$ increases up to 8 weeks post-TBI (Rostami et al., 2013). Relevant to acute TBI mortality, the ratio of TrkB.FL/Trk.T expression may support cell survival during excitotoxic injury(Gomes et al.,

2012; Vidaurre et al., 2012). With a shift in BDNF receptor ratios occurring in both injury and aging, understanding the effects of both is likely necessary for TBI-related mortality predictions.

To build on our previous hypothesis, we examined how serum and CSF BDNF levels influenced mortality after adjusting for age*GRS interaction. Given our strategy examining age*GRS mortality effects with/without adjusting for CSF BDNF levels, the age*GRS interaction likely captures elements of aging effects on BDNF secretion in addition to aging influences on target receptor milieu in the context of TBI. Notably, BDNF serum levels are reduced in older individuals in studies without TBI (Erickson et al., 2010; Gunstad et al., 2008; Lommatzsch et al., 2005), although age*BDNF levels interaction with mortality were not observed.

BDNF biomarker profiles suggest an important TBI-specific neurotrophic pathology. Previously, evidence of BDNF upregulation immediately following experimental TBI (Hicks et al., 1997; Rostami et al., 2013) was thought to aid neuroprotection (Almeida et al., 2005; Lindvall et al., 1994). However, in our study, elevated CSF levels are associated with mortality. This may be due to age or injury related changes in BDNF target receptor expression that favor apoptotic (p75) pathways, effectively making BDNF exposure detrimental. Yet, there tended to be a negative correlation between CSF and serum in our study that differs from studies in healthy populations (Pillai et al., 2010) and rodent models (Pan et al., 1998). The negative correlation between CSF and serum following TBI suggests BBB disruption with serum BDNF transit into the CNS acutely after TBI and may also reflect platelet dumping BDNF near vascular injury sites. Thus, higher CSF BDNF levels associations with mortality may represent both receptor expression profiles and BBB disruption.

Serum BDNF levels are thought to reflect brain BDNF levels in uninjured populations where BDNF is release into the blood stream from brain (Dawood et al., 2007; Rasmussen et al.,

2009). However, in addition to a possible negative correlation between CSF and serum, there are also additional BDNF sources. BDNF is also synthesized and secreted from vascular endothelial cells and may have a peripheral action (see review, Caporali *et al* (Caporali and Emanueli, 2009)). In blood, BDNF is sequestered in platelets, and is synthesized in the periphery (Nakahashi et al., 2000). Some reports suggest that platelets can release BDNF near injured tissue (Fujimura et al., 2002), but it is unclear if platelet release is altered in TBI. Similarly, serum BDNF could also be attributed to increased autonomic function, as HPA axis activation can diminish BDNF levels (Rothman et al., 2012). Thus, lower serum BDNF association with mortality may reflect platelets dumping BDNF into the CNS in the case of BBB disruption and/or reflect autonomic changes in BDNF levels related to HPA axis reactivity.

In uninjured populations, studies are mixed on the relationship between the *BDNF* gene and serum BDNF levels. While some studies show a relationship between rs6265 and serum BDNF levels such that Met-carriers have reduced serum BDNF, a meta-analysis did not support this relationship (Terracciano et al., 2013). Some have examined relationships between rs7124442 and serum BDNF levels, with mixed results (Hohenadel et al., 2014; Mercader et al., 2007). Yet, in our work post-TBI, relationships between genetic variation and serum BDNF levels are integral in mortality predictions, possibly reflecting injury-specific genetic effects on BDNF secretion and/or transit into the CNS.

Given the relationships between age, *BDNF* gene, and BDNF levels in our mortality predictions post-TBI, we examined these relationships descriptively for interpretation purposes. Our final model includes age*GRS and GRS*serum BDNF interactions that may actually be reflective of dual BDNF actions in both autonomic function and neuronal support; alternatively there may also be a three-variable interaction for which this study is likely under-powered (**Figure**

11). Given the hypothesized effects on secretion associated with the BDNF variants examinede, we expected subjects with a GRS=0 would have the highest serum BDNF levels, yet **Figure 11A** shows this is not the case when considering age. Age related increases in BBB permeability (Cernak et al., 2010; Lee et al., 2012; Oakley and Tharakan, 2014) may contribute to a possible 3 factor interaction. For example, among survivors where age<45, the hypothesized low secretion GRS=2 had significantly higher serum BDNF versus the 'high secretion' genotypes (GRS=0). This finding may be indicative of a compensatory BDNF response among younger individuals with GRS=2 but could also be due to less BBB disruption and transit into CSF as CSF levels are also lower in this group. CSF BDNF levels did not interact with GRS in mortality predictions. Regardless of GRS, CSF BDNF levels were higher among those who died, suggesting that other factors like BBB transport and neuronal production contribute to the CSF levels observed and their associations with mortality. Among participants >45yrs, there was no difference between serum or CSF levels by GRS in subjects who survive. Among participants in this older group who died, those with the low secretion genotype (GRS=2) not only had the lowest serum levels but also the highest CSF levels, again possibly suggestive of greater age-related BBB disruption and transport affecting serum levels in addition to genotype.

In evaluating BDNF utility as a biomarker, we examined CSF and serum relationships to 6 and 12 month GOS. As GOS includes mortality as an outcome, CSF relationships were driven by higher CSF levels predicting mortality. Serum levels showed no relationship to 6 month GOS, but there was some capacity for mortality prediction with 12 month GOS. However the primary significant comparison at 12 months was between those who died versus those with favorable outcomes. This finding suggests acute serum BDNF levels are more reflective of mortality status,

lacking discrimination for outcome among survivors. Future BDNF evaluation in the context of other survivor-specific outcome measures should be explored.

There are some limitations to consider. Our BDNF ELISA assay does not differentiate between proBDNF and mature BDNF. Future studies may investigate differences in pro versus mature BDNF. Serum BDNF levels can be affected by circadian rhythms (Piccinni et al., 2008b). However, it is not clear if/how BDNF levels vary daily in the context of acute TBI as studies report a loss of normal circadian rhythms acutely after TBI (Wagner et al., 2011b). As BDNF levels are associated with depression, participants depressed at the time of injury may have lower serum BDNF initially. Examining pre-injury function and pre-morbidity may help delineate more about these issues. One study suggests plasma BDNF levels predict mortality in ICU patients without TBI. While associated mechanisms are unclear (Ritter et al., 2012), with altered metabolic homeostasis and autonomic dysfunction immediately following TBI, there may be vascular BDNF actions that influence systemic contributors to mortality. This study suggests that BDNF pathology is likely an important new target in relationship to individual variation in mortality predictions post-TBI.

6.0 INTERRELATIONSHIPS BETWEEN MEMORY DEFICITS, FUNCTIONAL COGNITION, AND DEPRESSIVE SYMPTOMS FOLLOWING SEVERE TBI: TOWARD A BDNF HYPOTHESIS.

Michelle D. Failla, BS, Shannon B. Juengst, PhD, Patricia Arenth, PhD, Amy K. Wagner, MD

6.1 ABSTRACT

Background: Traumatic brain injury (TBI) results in mood and cognitive complications that impact functional recovery. Understanding common signaling pathways between post-TBI depression (PTD) and cognition may elucidate underlying neuropathology. Brain-derived neurotrophic factor (BDNF) is a likely target as BDNF reductions occur in both experimental TBI and depression models.

Objective: To evaluate BDNF as a biomarker for PTD, cognitive impairment, and functional cognition in a prospective cohort with severe TBI.

Methods: Participants with TBI (n=170) were evaluated for PTD (Patient Health Questionnaire-9), cognitive deficits (cognitive composite score) and functional cognition (Functional Independence Measure–Cognition, FIM-Cog). BDNF levels were measured in cerebrospinal fluid (CSF) and serum 0-6 days post-injury. Serum was also measured at 6 and 12 months post-injury.

Results: Serum BDNF was reduced compared to controls at all time-points. Acute serum BDNF were positively associated with Memory composite and FIM-Memory component scores

at 6 and 12 months. Chronic BDNF serum tended to be lower in participants with PTD, however, chronic BDNF serum levels correlated with depressive symptom severity at 12 months post-TBI

Conclusions: PTD is associated with functional cognitive deficits, but not cognitive performance, with apathy/motivation as likely etiological factors. Acute BDNF associations with memory performance may implicate hippocampal damage/degeneration, though acute and chronic BDNF associations with PTD were not as strong. Further investigation may delineate longitudinal BDNF profiles, and BDNF responsive treatments, as they reflect mood and cognitive recovery following TBI.

6.2 INTRODUCTION

In recent years, traumatic brain injury (TBI) has been recognized as a chronic medical condition with accompanying mood and cognitive complications. Alterations in cognition and mood can negatively affect quality of life and influence return to work or school following TBI (Cifu et al., 1997a; Fleming et al., 1999; Juengst et al., 2013; Yasuda et al., 2001). Identifying sensitive TBI biomarkers may be useful to manage these two major clinical issues and to measure responses to pharmacological and/or behavioral interventions. In fact, post-traumatic depression (PTD) is the most common neurobehavioral complication following TBI. Individuals with TBI are 10 times more likely than the general population to experience a depressive episode during their first year of recovery (53% (Bombardier et al., 2010a) compared to 6% (Kessler et al., 2005) per 12 months in the general population). The identification of an early biomarker for PTD development would aid in screening and early intervention, by identifying those at greatest risk and in need of close

tracking and frequent follow-up. Additionally, a biomarker that is reflective of PTD symptoms may be useful in improving treatment effectiveness, by informing dose or timing of interventions.

In non-brain injured populations, individuals with depression often have comorbid cognitive deficits, likely due to common pathology (Levin et al., 2007). While individuals with depression report a number of cognitive difficulties (Gotlib and Joormann, 2010), memory deficits are consistently problematic (Burt et al., 1995). Similar to individuals with depression, individuals with TBI commonly exhibit significant memory, executive function, and attentional difficulties after their injury (Brooks et al., 1999; Sumit N. Niogi et al., 2008; Perlstein et al., 2006; Vakil, 2005). We have recently shown individuals with PTD have no significant cognitive performance deficits compared to individuals with no PTD, even after correcting for a number of demographic and clinical variables. However, individuals with PTD still have significant functional cognition impairment (**Chapter 2**). Yet, some studies suggest that, for individuals with TBI, remittance of depressive symptoms often leads to increases in cognitive performance (Fann et al., 2001), suggesting overlapping biological pathways or interacting symptomology.

One promising biomarker for common pathology in mood and cognitive complications post-TBI is brain-derived neurotrophic factor (BDNF). BDNF, a neurotrophin involved in neuronal survival and synaptic plasticity, has been implicated in depression (Martinowich et al., 2007), memory and learning (Alonso et al., 2002), and TBI pathology (Chen et al., 2005; Failla et al., 2014; Griesbach et al., 2009). In the hippocampus, BDNF affects synaptogenesis and maintenance, particularly through long-term potentiation associated with activity-dependent secretion of BDNF (Kovalchuk et al., 2002). BDNF is also reportedly an underlying substrate for persistent long-term memory storage (Bekinschtein et al., 2008a, 2008b).

Reduced BDNF is known to be associated with depression, and serum BDNF levels are a consistent marker for depressive symptomology (Sen et al., 2008). Serum BDNF levels are decreased in untreated depression but increase with antidepressant treatment, indicating the viability for BDNF serum levels as a biomarker of depression (Hashimoto, 2010; Karege et al., 2002; Sen et al., 2008). In TBI, serum BDNF is acutely decreased, correlating with injury severity (Kalish and Phillips, 2010). Hippocampal BDNF is chronically decreased in experimental TBI (Chen et al., 2005). Importantly, therapies that increase brain BDNF expression, like environmental enrichment (Chen et al., 2005) and exercise (Griesbach et al., 2009; Hoffman et al., 2010; Wise et al., 2012), are promising therapies for mood and cognitive recovery post-TBI. Similarly, hippocampal BDNF expression has been linked to spatial memory in experimental TBI studies (Griesbach et al., 2009). These studies suggest that BDNF may be a viable biomarker for long-term complications like depression and memory deficits that impact TBI recovery.

In this study, we assessed BDNF as a viable biomarker for interrelationships between cognitive deficits, functional cognitive impairments, and PTD in the first year following TBI, with specific attention to memory domains. As BDNF serum levels have never been examined in TBI beyond the first 24hrs or in relation to PTD or cognition, BDNF serum levels may be a novel biomarker for these complications and may help elucidate convergent pathways in cognitive and depressive symptomology post-TBI.

6.3 METHODS

6.3.1 Participants

Participants in this study, approved by the University of Pittsburgh's Institutional Review Board, were recruited while receiving care at inpatient and/or outpatient clinics within the University of Pittsburgh Medical Center (UPMC). All participants sustained a non-penetrating traumatic brain injury (TBI), with evidence of intracranial injury on Computed Tomography (CT). Exclusion criteria included: cardiac arrest prior to admission, documented prolonged hypoxia or hypotension prior to admission, or penetrating TBI. All participants survived for at least one year post-injury and were a subset of a larger study investigating possible biomarkers and genetic factors related to individual recovery following TBI.

Healthy adult controls were also recruited for comparison in biomarker analysis (n=9). Control CSF was obtained via lumbar puncture for research purposes, and was not a part of a clinical work-up. Criteria for enrollment of controls included: (i) 18-70 years old; and (ii) no current or past history of brain injury, neurological disease, or bleeding disorder. Women were excluded if they were pregnant, were taking oral contraceptives or hormone replacement therapy, or had any history of reproductive or endocrine disorder.

Injury severity was described using the best GCS obtained within the first 24 hours post-injury. Demographic information, including age, sex, and education, was collected by chart review as well as through participant or caregiver interviews. Similarly, anti-depressant use at 6 and 12 months was extracted from both participant interview and chart review. A pre-injury history of mood disorders, including depression, bipolar disorder, and anxiety, was established by self-report and chart review.

6.3.2 Cognitive Assessment

Participants' functional cognitive impairment was assessed with the FIM-Cog(Dodds et al., 1993) at both 6 and 12 months. FIM-Cog has five component scales: expression, comprehension, social interaction, problem solving, and memory. Each scale is rated from one to seven, with a 5 or lower indicative of need for caregiver assistance. The sum of these five components was considered the FIM-Cog Score.

Similar to previous studies (**Chapter 4**), cognitive deficits were measured at both 6 and 12 months post-injury using a cognitive composite score developed with a battery of 8 neuropsychological tests targeting 4 domains of cognition (attention, language fluency, memory, and executive function). Attention was measured using the Trails Making Test A (RM Reitan and Wolfson, 1985), and the combined score of the forward and backward digit span tests from the Wechsler Adult Intelligence Scale-R (Glenn J. Larrabee and Curtiss, 1995). Memory was evaluated using the Rey-Osterreith Complex Figure Test (PA Osterrieth, 1944) and the Long Delay Free Recall Subsection of the California Verbal Learning Test (Delis and et al., 2000) (CVLT-II). Language Fluency domain scores were calculated using Controlled Oral Word Association (JG Borkowski et al., 1967) Animals Subsection and the Delis-Kaplan Executive Function Systems Verbal Fluency Letter Fluency subsection. Lastly, executive function was measured using the Trails Making Test B (RM Reitan and Wolfson, 1985) and the Stroop Task (J. R. Stroop, 1935) Interference Sub-score. These tests were selected as representative measures for their associated domains. Raw scores from each test were converted into T-scores using appropriate metrics (i.e education, age, sex, race) based on norms indicated by the test manufacturer. T-scores were averaged within each domain to create a domain sub-score. To calculate a cognitive composite

score, participants had to complete at least one test in each domain. Mean values across domain sub-scores were calculated for the overall cognitive composite score.

6.3.3 Depressive Symptom Assessment

At 6 and 12 months, depressive symptoms were evaluated using the Patient Health Questionnaire-9 (PHQ-9), a brief self-report symptom inventory based on the 9 DSM-IV diagnostic criteria for Major Depressive Disorder (MDD). The PHQ-9 asks participants to rate how often they have experienced symptoms of depression, on a scale between 0 (None) and 3 (Nearly Every Day), over a two-week period. Higher total scores (PHQ-9 Total) reflect greater number of and/or greater severity of depressive symptoms, with the maximum score being 27. Participants were grouped as "depressed" vs. "non-depressed" using the PHQ-9 questions as they map to DSM diagnostic criteria (previously described) (Fann et al., 2005). For a categorization of depression (PTD), individuals responded positively to at least five symptom questions on the PHQ-9, with at least one pertaining to a cardinal symptom (anhedonia or depression). Compared to the Structured Clinical Interview for DSM Diagnosis (SCID) (Fann et al., 2005), this method has been validated in populations with TBI showing a sensitivity of 93% and a specificity of 89%. Importantly, the PHQ-9 is reliably able to discriminate between chronic TBI and depression symptoms (Cook et al., 2011a).

6.3.4 BDNF Sample Cohort, Collection, and Processing

BDNF levels were measured in both CSF and serum. When possible, CSF samples were collected via passive drainage up to twice daily for six days post-injury by an external ventricular drain

(EVD) placed for clinical care. Serum was collected acutely, daily for the first six days and chronically, at 6 months and 12 months post-injury, via venipuncture. Acute CSF and serum samples were binned by day, and an average was calculated for each day post-injury for each participant.

CSF and serum samples were stored at -80ºC before measuring BDNF levels with an enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech, Georgia, USA). Briefly, standards (of varying BDNF concentrations) and samples were pipetted onto a 96-well plate pre-coated with human BDNF antibody. Following shaking for 2.5 hours at room-temp, the plate was washed and then incubated with biotinylated BDNF antibody for 1 hour. HRP-conjugated streptavidin was then added for an incubation of 45 minutes. The addition of a tetramethylbenzidine substrate allows for a color reaction. Concentrations were calculated using mean absorbance of each sample at 460 nm, as it correlates to amount of BDNF present in the sample, as plotted on a per-assay standard curve. Samples were diluted within the range of the ELISA kit (no dilution for CSF, 1:250 for serum samples), with a sensitivity of 80 pg/ml, a kit supplied intra-assay variation of <10%, and an inter-assay variation of <12%.

For biomarker analysis, participants were assayed for BDNF levels in CSF or serum samples (n=170). As BDNF levels have been shown to be moderated by racial background (Nettiksimmons et al., 2014), our BDNF levels cohort associations were limited to Caucasians only. As the study population was 91.9% Caucasians, there was not enough power to detect racial differences between BDNF level associations and outcome. There were 117 participants with CSF samples (n=446) and 98 with acute serum samples (n=335). One acute serum sample measurement and 4 CSF sample measurements were removed as outliers (based on ±1.5*interquartile range). Chronic serum samples were collected at 6 and 12 months (±1 month) post-injury and averaged

for each time-point for each participant. At 6 months, 54 participants had at least 1 serum sample (n=112 samples); at 12 month, 36 participants had at least 1 serum sample (n=36 samples). A subset of participants had both acute and chronic samples (n=44).

6.3.5 Statistical Analysis

Data analysis was conducted using Statistical Analysis Software (version 9.4; SAS Institute). Descriptive analysis included mean and standard deviation and/or median for continuous and ordinal variables such as age, GCS, and education. Frequencies were calculated for categorical variables such as sex and antidepressant use. Demographic and relevant clinical information was assessed for relationships with BDNF levels using Student's t-tests or ANOVA to compare means. Non-parametric tests (Mann-Whitney and Kruskal-Wallis) were used when appropriate. Outliers were assessed using ±1.5 * interquartile range. Pearson's or Spearman's rho (r) correlations were used to assess relationships between two continuous variables.

6.4 RESULTS

Specific cohort demographics are shown in **Table 19**. Overall, participants had a GCS (best in 24hrs) of 3-15 (mean GCS, 7.7 ± 2.8, median=7). Participants were aged 16-72 (mean age 33.2±13.8 years) and 16.1% of participants were women. At 6 months post-injury, 36.5% of participants with TBI had PTD, while 28.6% had PTD at 12 months. Participants with PTD tended to have a higher mean age compared to those without PTD. There was a higher percentage of women in the group with PTD at 6 months (32.3% compared to 11.1%, p=0.018). Participants

with PTD at both 6 (22.6 versus 5.6%, p=0.022) and 12 months (33.4 versus 8.3%, p=0.007) were more likely to have had premorbid mood disorders. Importantly, there was no difference in antidepressant use between PTD groups at 6 or 12 months.

Table 19. Demographic description of study population.

	Total Population (n=170)	**6 Months**			**12 Months**		
		None (n=54)	PTD (n=31)	p value	None (n=60)	PTD (n=24)	p value
Age, mean±STD	33.2±13.8	31.5±12.9	36.1±14.1	0.094	32.7±13.2	35.9±13.5	0.098
GCS, median	7	7	7.5	0.431	7	7.5	0.426
Sex, # (%) Males	141 (83.9)	48 (88.9)	21 (67.7)	*0.018*	50 (83.3)	16 (66.7)	0.102
Race, # (%) Caucasian	154 (91.7)	51 (94.4)	29 (93.6)	0.867	56 (93.3)	21 (87.5)	0.398
Education, mean±STD	12.8±1.7	12.9±1.9	12.4±1.5	0.261	12.9±1.7	12.1±1.5	0.137
Premorbid Mood Disorders, # (%)		3 (5.6)	7 (22.6)	*0.022*	5 (8.3)	8 (33.4)	*0.007*
Antidepressant Use, # (%)		18 (34.0)	16 (51.6)	0.113	19 (32.2)	10 (41.6)	0.416

STD, Standard Deviation; PTD, Post-TBI Depression; GCS, Glasgow Coma Scale

As shown in **Figure 13A**, daily mean CSF BDNF levels among TBI participants did not differ from healthy control levels. Conversely, daily mean serum BDNF levels in participants with TBI were consistently reduced compared to healthy control levels starting at day 0 (206.27±16.4ng/mL compared to 277.86±28.1ng/mL for healthy controls, p=0.024) and remained below healthy controls for all days (all comparisons $p<0.01$, n=49, **Figure 13B**). Serum BDNF levels at 6 (205.57±10.2ng/mL, p=0.011) and 12 (188.67±13.5ng/mL, p=0.008) months were also below healthy control levels.

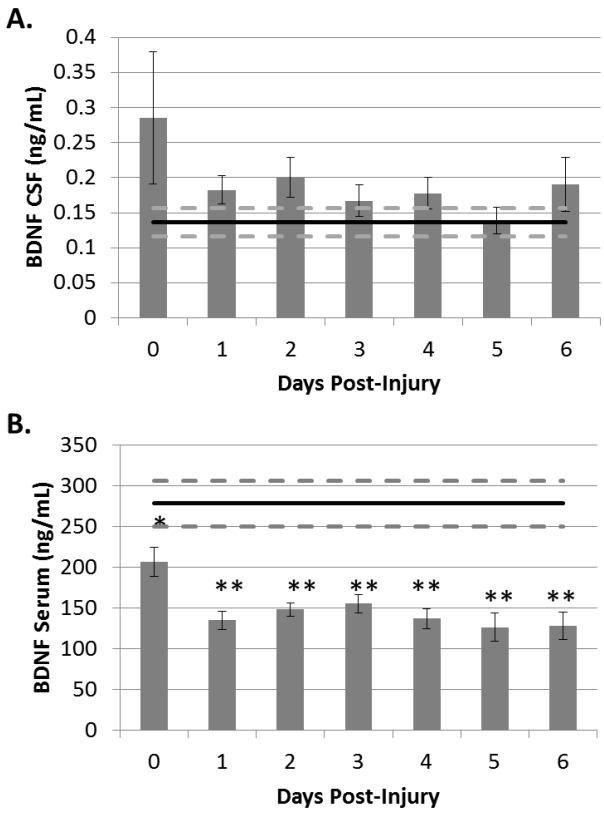


Figure 13. Daily brain derived neurotrophic factor (BDNF) levels over the first 7 days post-injury, compared to healthy controls.

Means in black line, standard error in light gray dashed lines. (A) Daily mean CSF BDNF levels do not differ significantly from control levels. (B) Daily mean serum BDNF levels fall below control levels at day 0 post-injury and remain reduced through day 6 (day 0, $p<0.05$, day 1-6, $p<0.001$).

BDNF levels were examined for associations to PTD. CSF BDNF levels were higher in participants with PTD at 6 months, though this was not significant (p=0.089). Acute serum BDNF levels in participants with PTD tended to be reduced compared to those with no PTD at 6 months (p=0.074) and were negatively correlated with PHQ-9 Total at 6 months (*r*=-0.38, p=0.044, n=29, **Table 20**).

Table 20. Bivariate Correlations between BDNF Levels and Mood and Cognitive Outcome.

	Acute Serum			Acute CSF			Chronic		
	r*	**p value**	**n**	**r**	**p value**	**n**	**r**	**p value**	**n**
6 Months									
PHQ-9 Total	-0.13	0.490	30	0.16	0.334	39	-0.20	0.242	37
Cognitive Composites									
Overall	0.14	0.474	30	0.13	0.424	41	0.09	0.625	35
Memory	0.43	***0.019***	30	0.11	0.490	42	0.04	0.803	35
Executive Function	-0.18	0.332	31	0.01	0.964	42	0.13	0.448	36
Attention	-0.11	0.532	32	-0.02	0.900	43	-0.08	0.626	37
Language Fluency	0.10	0.599	30	-0.09	0.589	42	-0.01	0.953	35
Functional Independence Measure									
Cognitive Total	0.31	***0.041***	45	-0.24	**0.090**	53	0.16	0.271	47
Memory	0.35	***0.019***	45	-0.24	**0.080**	53	0.13	0.369	47
Problem Solving	0.33	***0.029***	45	-0.33	***0.015***	53	0.11	0.463	47
Social Interaction	0.30	***0.044***	45	-0.22	0.116	53	0.05	0.738	47
Expression	0.34	***0.024***	45	-0.12	0.390	53	0.19	0.190	47
Comprehension	0.20	0.186	45	-0.06	0.648	53	0.27	**0.068**	47
12 Months									
PHQ-9 Total	-0.38	***0.044***	29	0.10	0.551	37	-0.41	***0.019***	32
Cognitive Composites									
Overall	0.39	0.101	19	0.06	0.746	32	-0.04	0.856	28
Memory	0.53	***0.005***	26	0.17	0.339	34	0.11	0.580	28
Executive Function	-0.15	0.502	22	0.01	0.962	33	-0.14	0.478	29
Attention	-0.08	0.756	19	0.04	0.840	32	0.09	0.641	29
Language Fluency	0.18	0.372	26	0.10	0.584	34	0.00	0.984	29
Functional Independence Measure									
Cognitive Total	0.31	**0.063**	38	0.00	0.991	47	0.19	0.271	35
Memory	0.38	***0.018***	38	-0.15	0.327	47	0.20	0.251	34
Problem Solving	0.30	**0.068**	38	-0.05	0.762	47	0.04	0.805	34
Social Interaction	0.25	0.133	38	-0.02	0.893	47	0.08	0.672	34
Expression	0.27	**0.098**	38	0.03	0.817	47	0.07	0.692	34
Comprehension	0.18	0.282	38	0.24	0.101	47	0.32	**0.062**	34

*Spearman's r; PHQ-9, Patient Health Questionnaire-9.

Figure 2 shows chronic serum BDNF levels by PTD status. At 6 months, there were no significant differences in chronic serum BDNF by PTD status (p=0.174). Yet, participants with PTD had significantly lower serum BDNF compared to controls (p=0.012), while those without PTD did not differ significantly from controls (p=0.070). At 12 months, participants with PTD

tended to have lower serum BDNF levels than those without PTD (p=0.066). Participants with and without PTD showed lower chronic serum BDNF levels compared to controls (p=0.037, 0.004, respectively). At 12 months, chronic BDNF serum levels negatively correlated with PHQ-9 Total (*r*=-0.41, p=0.019, n=32, **Table 20**).

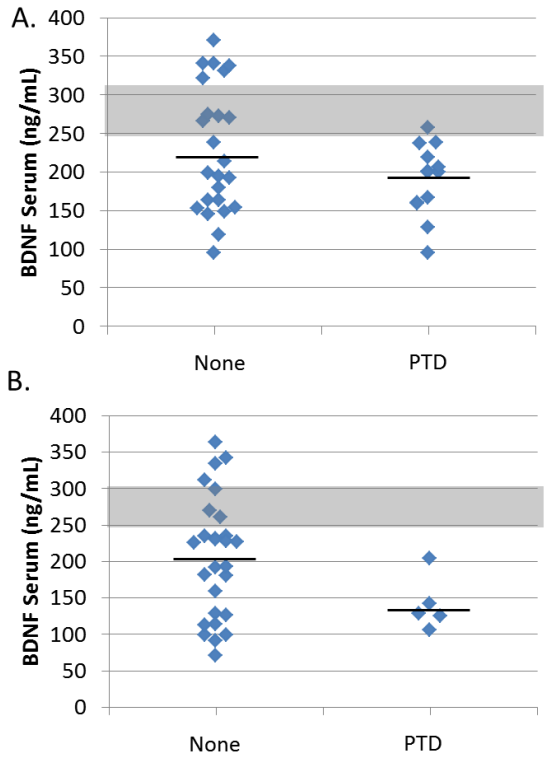


Figure 14. Serum BDNF levels during chronic recovery at 6 and 12 months by PTD status.

At 6 months (A), there were no significant differences in serum BDNF by PTD status (p=0.174). At 12 months (B), participants with PTD had lower serum BDNF levels compared to participants with no PTD, though this was not significant (p=0.066). Levels in participants with PTD were reduced compared to control levels at 6 months and 12 months, similarly levels of participants with no PTD were reduced compared to controls (p<0.05 for all comparisons).

BDNF levels were examined for associations to cognitive deficits and functional impairments (**Table 20**). Acute serum BDNF levels were positively correlated with memory composite scores at 6 (r=0.43, p=0.019, n=30) and 12 months (r=0.53, p=0.005, n=26). CSF BDNF and chronic serum BDNF levels were not associated with any cognitive composite components. Acute serum BDNF levels were positively correlated with FIM-Cog at 6 months (r=0.31, p=0.041, n=45). CSF BDNF levels tended to show a negative correlation with FIM-Cog at 6 months (r=-0.24, p=0.090, n=53), with a significant association with FIM-Problem Solving (r=-0.33, p=0.015). At 12 months, acute serum BDNF levels were positively correlated to FIM-Memory (p=0.018); CSF BDNF did not show any significant correlations to 12 month FIM-Cog or FIM-Cog components. There were no significant relationships between chronic BDNF and functional cognition at either 6 or 12 months.

6.5 DISCUSSION

This study aimed to investigate the relationship between mood and cognitive complications post-TBI by identifying a biomarker (BDNF) that may elucidate common mechanisms of PTD and cognitive deficits and functional cognitive impairment. In our previous study (**Chapter 2**) there was no significant evidence that cognitive deficits were associated with PTD, yet individuals with PTD had significantly more functional impairments. In this current study, we examined BDNF acute and chronic associations with PTD, cognitive deficits, and functional cognition. Acute serum BDNF levels were associated with chronic memory deficits, global functional cognitive impairment, and depressive symptom severity. Chronic serum BDNF levels were not associated

with any cognitive outcomes, but did tend be lower in individuals with PTD. This work suggests a common underlying BDNF-related pathology in both mood and cognitive recovery post-TBI.

This study supports BDNF as a biomarker for cognitive and mood complications post-TBI. Serum BDNF levels were low immediately after injury, consistent with previous work (Kalish and Phillips, 2010). However, inconsistent with previous studies (Kalish and Phillips, 2010), there was no association between GCS (best in 24hrs) and serum BDNF. This may be due to differences in severity measures or specific timing of measurements between studies. We also show serum BDNF levels remain reduced chronically, adding evidence to the chronicity of TBI pathology. We show a trend for a negative correlation between CSF and serum BDNF (consistent with previous work, **Chapter 4**). While it has been suggested that BDNF can cross the blood brain barrier (BBB) in both directions under normal conditions (Pan et al., 1998; Poduslo and Curran, 1996), periods of BBB disruption following TBI could allow for increased movement of BDNF into the brain, especially acutely (Başkaya et al., 1997; Pan et al., 1998). This increased movement into the CSF from the blood could reflect a possible restorative process, as platelets dump BDNF in response to vascular injury (Nakahashi et al., 2000). Therefore, lower BDNF levels in serum acutely could be suggestive of more extensive injury that could correlate with later chronic complications.

In uninjured populations, BDNF serum levels have been shown to be consistently reduced in individuals experiencing depressive symptoms (Karege et al., 2005). Under normal circumstances, serum BDNF levels likely reflect CNS functioning, as BDNF is primarily synthesized in the brain and secreted in an activity dependent manner (Z.-Y. Chen et al., 2004). A number of studies suggest serum increases in BDNF are due to brain level changes (Dawood et al., 2007; Rasmussen et al., 2009). It is suggested that serum BDNF is likely reflective of BDNF function in the hippocampus. In the hippocampus, BDNF expression levels are decreased in

correlation with stress and depression (Pittenger and Duman, 2007). BDNF signaling in the hippocampus is also implicated in mechanisms of antidepressant treatment (D'Sa and Duman, 2002). In rat models, intracerebral BDNF infusions have antidepressant effects, while decreased BDNF signaling results in decreased hippocampal neurogenesis (Siuciak et al., 1997).

There is evidence that BDNF could be indicative of a depressive state following neurological insult. Following stroke, BDNF serum levels were increased during periods of no depressive symptoms, but decreased when individuals presented with depression (L. Yang et al., 2010). However, our study did not show concrete evidence for associations between PTD and BDNF levels at the time of the depressive state. However, acute serum BDNF levels did correlate with depressive symptom severity (PHQ-9 Total) at 12 months. Similarly, chronic BDNF levels at 12 months correlated with depressive symptom severity. These data suggest BDNF may be more reflective of depressive symptomology later in recovery. In our previous studies (Failla et al., 2013), we have shown transient risk factors for depression across the first year, thus, BDNF may be more representative of stable risk factors and/or depressive state once other risk factors have resolved. It is also possible that this study was underpowered to detect reductions in chronic serum BDNF associated with current depressive state given the possible effects of demographics like age and gender on BDNF levels (Lommatzsch et al., 2005).

The data from this study suggest acute serum levels of BDNF are highly associated with memory deficits post-TBI. Other studies have demonstrated similar relationships between serum BDNF levels and cognitive function (Griffin et al., 2011). Specifically, in patient populations (schizophrenia, bipolar disorder, mild cognitive impairment) and healthy controls (Gunstad et al., 2008), there are relationships between BDNF levels and memory performance (Dias et al., 2009). Animal studies with conditional BDNF knock-out mice show impairment in

hippocampal-dependent cognition and behavior (Bath and Lee, 2006). Thus, in injury, reduced serum BDNF acutely may be indicative of more damage to the hippocampus. Similarly, it may also be indicative of an acute injury state that is conducive to neuronal death and atrophy that would predict later memory performance. Low BDNF signaling in the hippocampus may diminish synaptic plasticity and neurogenesis, negatively affecting these chronic recovery endpoints. Future studies are needed to examine the relationship between acute BDNF levels and chronic hippocampal volume, as this may aid interpretation of these findings. As experimental models of TBI show that BDNF expression in the hippocampus is correlated with cognitive recovery (Griesbach et al., 2009), it will be critical to understand if this could be relevant to early interventions to improve chronic memory problems. Participants with lower BDNF levels acutely do tend to have lower levels chronically, thus, understanding the trajectory of these profiles, and their relationship to memory performance, could impact treatment and intervention strategies.

Acute BDNF serum levels did not correlate with any other measures of cognitive performance, but were significantly associated with multiple components of the FIM-Cog. This may be due to the role of memory deficits in other aspects of functional cognition (Lewis and Horn, 2013). Similarly, BDNF CSF levels did not show consistent associations with any aspects of mood or cognitive recovery. For CSF BDNF levels, higher CSF levels tended to be associated with worse outcomes on the FIM-Cog subcomponents. This is consistent with our previous work (**Chapter 4**) showing high levels of CSF were associated with greater mortality post-TBI. CSF levels may not be as indicative of functional outcome post-TBI as there are competing sources of BDNF (brain versus platelets in blood) and the relationship between brain levels, CSF, and serum is unclear immediately following TBI.

Importantly, BDNF levels may be influenced by *BDNF* genetic variation (Terracciano et al., 2013). Future studies in larger cohorts are needed to determine the effect *BDNF* variation on post-TBI BDNF levels, especially chronically. It will also be important to evaluate BDNF serum post-TBI in relation to therapies that stimulate BDNF signaling (e.g. exercise, SSRIs). In uninjured populations, serum BDNF levels are decreased in untreated depression but increase with antidepressant treatment, indicating the viability for BDNF serum levels as a biomarker of depression (Hashimoto, 2010; Karege et al., 2002; Sen et al., 2008). Unfortunately, this study was not designed to evaluate the usefulness of BDNF as a biomarker of PTD remittance following antidepressant use. A limitation in this study is the inclusion of individuals on antidepressants, however, BDNF levels are thought to be more indicative of depressive symptomology and remittance than antidepressant use (Piccinni et al., 2008a), thus, future studies in PTD will need to examine these relationships carefully.

There are some important limitations to consider in this study. In consideration of BDNF levels, larger sample sizes are needed to address covariates that may affect CSF and serum levels, namely genetic variants and other demographics (age, sex, race). Another limitation is the assumption that serum BDNF levels represent brain levels, as there is a substantial peripheral production of BDNF in vascular endothelial cells (Nakahashi et al., 2000) and stored in platelets (Gass and Hellweg, 2010). Literature suggests platelet release is not altered in depression (Karege et al., 2005), but it is unclear if platelet release could be altered in PTD or TBI or how this store of BDNF might influence our findings. This study suggests that, while functional cognition is impacted by PTD (**Chapter 2**), BDNF's usefulness as a biomarker may be more linked to cognitive deficits post-TBI. Importantly, overlapping symptoms can also make identification of PTD difficult, though the PHQ-9 has been shown to differentiate between cognitive symptoms and PTD

(Fann et al., 2005). Identifying biomarkers like BDNF for mood and cognitive difficulties may also help to delineate risk for PTD with or without cognitive deficits. Future studies may examine BDNF levels across recovery as PTD develops and/or resolves for a better understanding of its relationship to mood post-TBI. Similarly, it will be important to understand the relationship between BDNF levels in participants with PTD, with and without cognitive deficits.

7.0 POST-TBI ALTERATIONS IN FRONTO-LIMBIC MORPHOMETRY ARE ASSOCIATED WITH DEPRESSION

Michelle D. Failla, BS, Anne C. Ritter, MPH, John M. Myrga, Patricia M. Arenth, PhD, Joseph H. Ricker, PhD, Amy K. Wagner, MD

7.1 ABSTRACT

Post-traumatic depression (PTD) is a common neurobehavioral complication following traumatic brain injury (TBI). Studies shows volumetric alterations in fronto-limbic brain regions are associated with depressive symptomology. This study examined fronto-limbic brain morphometry in TBI as a possible biomarker of PTD susceptibility, implicating regional reductions post-TBI. Structural MRI scans were acquired 1-3 years post-TBI in 40 adults with moderate/severe TBI and 33 age-matched controls. Regional brain volumes were calculated using automatic segmentation software (FreeSurfer). A subset of individuals with TBI (cases, n=21) were assessed for depressive symptomology using the Patient Health Questionnaire (PHQ-9). Mann-Whitney and Spearman correlations were used as appropriate. Data were corrected for multiple comparisons. Within cases, amygdala volume (right, $p=0.002$) and hippocampus (bilaterally, $p<0.005$) were reduced versus controls. Correlations between regional volumes also differed among cases versus controls. Among cases, left amygdala volume was positively correlated with left hippocampus ($r=0.428$, $p=0.023$) and left caudal anterior cingulate volume ($r=0.461$, $p=0.014$), while these correlations

were non-significant among controls. However, bilateral amygdala volumes were more correlated with medial orbitomedial cortex volumes in controls versus cases. Interestingly, cases with PTD had larger left amygdala volumes compared to cases with no depression (p=0.0003). We suggest relative regional atrophy within fronto-limbic circuits following TBI may result in altered volume correlations. This relative TBI-related fronto-limbic regional atrophy may contribute to PTD susceptibility following TBI. Larger amygdala volumes in individuals with PTD could indicate increased emotional reactivity. Future studies need to validate these findings in larger populations, examining how fronto-limbic regional volumes may reflect fronto-limbic circuit function and relate to PTD susceptibility.

7.2 INTRODUCTION

Approximately 1.7 million individuals sustain a TBI in the United States every year ("CDC - Injury - TBI - TBI in the US Report," n.d.). The most common neurobehavioral complication is post-TBI depression (PTD), with the prevalence reported at 53% over the first year post-injury (Bombardier et al., 2010b). PTD is associated with poorer psychosocial function (Hibbard et al., 2004), functional outcome (Hudak et al., 2012), and especially functional cognition (**Chapter 2**). Recent studies report that treatment with selective serotonin reuptake inhibitors (SSRI) in PTD have both limited effectiveness and increased incidence of adverse events (Fann et al., 2009a; Lanctôt et al., 2010; Lee et al., 2005), emphasizing the need for a greater understanding of the underlying mechanisms of PTD in order to refine treatment and intervention.

In uninjured populations, significant alterations in fronto-limbic circuit function are hypothesized to contribute to depression pathology (Mayberg, 2003). Consistent with this hypothesis, individuals with major depressive disorder have reduced fronto-limbic volumes in structures like the hippocampus (Sheline et al., 2003), amygdala, anterior cingulate, and orbitomedial frontal cortex (Koolschijn et al., 2009). Recent reviews show overlapping regions of vulnerability between depression and TBI, including frontal-limbic regions, with decreased white matter integrity implicated in the uncinate fasciculus, cingulum gyrus, and fimbria-fornix (Maller et al., 2010). These tracts are also implicated in fronto-limbic circuits associated with depression (Hamani et al., 2011; Mayberg, 2003; Mettenburg et al., 2012) due to their connectivity with the hippocampus, amygdala, and anterior cingulate.

As TBI is a clinically heterogeneous disorder, making personalized and early treatment interventions difficult, we have proposed a Rehabilomics approach to understanding how demographic and clinical variables interact with risk for complications (like PTD) to impact functional recovery and rehabilitation post-TBI (Wagner, 2011; A K Wagner, 2010). In the field of brain injury, neuroimaging has the potential to inform clinical decisions and rehabilitation as well as to serve as a biomarker for successful treatment paradigms (Irimia et al., 2014). While the potential ability of imaging to identify patients at risk for complications like PTD remains to be seen, the susceptibility of patients with TBI to mood regulation deficits suggests a common pathology that may be more nuanced than would be currently available on clinical MRI. As such, there has not been conclusive evidence to suggest location of injury predicts PTD development. In experimental TBI models, there is evidence of depressive-like behavior following frontal lobe damage (Moritz et al., 2014). Some clinical studies support the notion that injury foci in left frontal/medial regions (as well as regional brain volume relationships in chronic TBI) are relevant

to PTD development (Chan et al., 2008; Jorge et al., 2004); though this susceptibility pattern does not explain pathology mechanisms for a large portion of PTD sufferers (Maller et al., 2010).

One common pathology that may impact PTD susceptibility is diffuse axonal injury (DAI). Angular rotation occurs even during linear blows to the head as basilar brain structures, including the pituitary stalk) are tethered to the base of the skull (Bayly et al., 2005). This rotational force around the brain's fulcrum regions often results in shearing of long axonal tracts that connect fronto-limbic areas (Sabet et al., 2008). Compared to penetrating TBI, closed head injuries often result in wide-spread DAI. DAI is an inherent biomechanical component of the TBI complex, and DAI relationships to PTD have been suggested (Maller et al., 2010). DAI is due to microstructural axonal damage resulting in axonal disruptions and disconnections that, when severe enough, result in diaschisis (Carrera and Tononi, 2014; Hernández, 2006). While DAI cannot always be imaged using standard magnetic resonance imaging (MRI) techniques, there is evidence that white matter integrity measures associated with techniques like diffusion tensor imaging (DTI) are highly related to gray matter volume of fronto-limbic regions in PTD(Warner et al., 2010), suggesting overall reductions in fronto-limbic structural integrity. In fact, atrophy is a widespread radiographic feature characterizing TBI (MacKenzie et al., 2002). Previous PTD studies provide evidence of atrophy in the hippocampus (D. F Tate and Bigler, 2000) and anterior cingulate (Chen et al., 2008b; Maller et al., 2014). Recent work involving populations with moderate-severe TBI suggests that the degree of fronto-limbic structure atrophy in the hippocampus and other regions, was associated with PTD severity (Hudak et al., 2011).

Based on this literature, we hypothesized that reduced structural integrity in fronto-limbic regions post-TBI may underlie alterations in functional connectivity of these circuits, leading to PTD susceptibility. Importantly, we suggest there are consistent patterns of fronto-limbic atrophy

that are not uniform across all areas, and thus, investigated volumetric associations between fronto-limbic regions in healthy controls and subjects with TBI. We report non-uniform atrophy post-TBI and propose that these regional volumetric patterns that occur within fronto-limbic circuits after TBI, may be a neural correlate of PTD susceptibility and development. Similarly, for cases with TBI, we show relatively higher amygdala volumes in subjects with PTD that correlate with depressive severity.

7.3 MATERIALS AND METHODS

7.3.1 Participants

This study consisted of 40 subjects recruited consecutively while receiving care at inpatient and/or outpatient clinics within the University of Pittsburgh Medical Center (UPMC) and this study was approved by the University of Pittsburgh's Institutional Review Board. Enrollment criteria for this study included age (≥16 and <75 years), right-hand dominance, and a non-penetrating TBI. This study included severe (≤8), moderate (9-12), and complicated mild (≥13) injures as defined by an admission GCS (Teasdale and Jennett, 1974b) score. Complicated mild injuries were defined by both GCS and positive neuroradiological findings attributable to TBI. Subjects were excluded for documented prolonged hypoxia prior to admission or for metal in body preventing research MRI scans. We also examined the best GCS obtained within 24 hours post-injury. Participants had a best GCS in 24hrs score of 3-15, mean GCS of 9.9 ± 3.6 with a median of 10. Demographic information including age, sex, and education was collected by chart review as well as through

subject or caregiver interviews. Subjects were aged 18-54 (mean age 29.0 ± 10.3 yrs) and 9 participants were female.

Healthy adult control subjects were separately recruited as age and sex matched to subjects with TBI to minimize effects of age and sex on brain volumes. Control subjects included 29 men and 8 women, with a mean age of 28.2±10.0yrs and education 15.3±2.7yrs. Control subjects self-reported no current or past history of brain injury or neurological disease, and had no contradictions to MRI (ie. no metallic implants), and were all right-hand dominant.

7.3.2 PTD Symptom Assessment

PTD symptom assessment was done at 6, 12, and 24 months. Depressive symptoms were evaluated using the Patient Health Questionnaire-9 (PHQ9), a brief self-report symptom inventory based on the nine DSM-IV diagnostic criteria for Major Depressive Disorder (MDD). The PHQ-9 asks participants to rate how often they have experienced symptoms of depression, on a scale between 0 (None) and 3 (Nearly Every Day), over a two-week period. Higher total scores reflect greater number of and/or greater severity of depressive symptoms, with the maximum score being 27. Participants were grouped as "depressed" vs. "non-depressed" using the PHQ-9 questions as they map to DSM diagnostic criteria (previously described (Fann et al., 2005)). For a categorization of depression (PTD), individuals responded positively to at least five symptom questions on the PHQ-9, with at least one pertaining to a cardinal symptom (anhedonia or depression). Depressive symptoms were examined as a continuous variable using PHQ-9 total score to represent depressive symptom severity. The PHQ-9 has been validated in populations with TBI showing a sensitivity of 93% and a specificity of 89% when compared to the Structured Clinical Interview for DSM Diagnosis (SCID) (Fann et al., 2005),. Importantly, the use of the PHQ-9 in this manner is reliably

able to discriminate between chronic TBI and depression symptoms(Cook et al., 2011a). Pre-morbid history of mood disorders (depression, bipolar disorder, and anxiety) was extracted from subject interview or chart review for those subjects with depressive symptomology assessments. Only one individual had a history of premorbid mood disorders. For each individual, depression assessment for association with imaging was taken at the closest time-point to imaging acquisition.

7.3.3 Magnetic Resonance Imaging (MRI) Acquisition and Analysis

MRI scans were acquired between 6 months and 3 years post-TBI (mean: 1.6±0.1yrs; min: 0.7; max: 3.1). For all participants, T1-weighted images were acquired on either a 1.5 or 3T GE Signa or a 3T Siemens Trio system, similar to published protocols(Arenth et al., 2012; Amy K Wagner et al., 2014). For the GE Signa, SPGR sequences were collected with slice thickness=1.5 mm/0mm interslice, TR=25ms, TE=5, 256x192 matrix, FOV=240mm and a flip angle=40°. For the 3T Siemens Trio, sagittal 3D MPRAGE sequences were collects with 224 contiguous 0.78mm slices, TR = 1680ms, TE = 2.48ms, 256x256 matrix, FOV = 200mm, flip angle = 8°.

Fronto-limbic volumes were obtained using FreeSurfer (v5.3.0, Martinos Center for Biomedical Imaging, Laboratory for Computational Neuroimaging) whole-brain automatic segmentation (Fischl et al., 2004, 2002). This automatic segmentation method uses probabilistic estimations of 40 anatomical regions of interest based on a manually labeled dataset from 40 individuals. Several comparative studies have shown subcortical and cortical segmentation with FreeSurfer is comparable to manual labeling (Morey et al., 2009; Pardoe et al., 2009; Sánchez-Benavides et al., 2010). FreeSurfer provides automatic segmentation of cortical regions and is highly adept at parcellation of particularly heterogeneous structures (Fischl, 2012).

We examined the right and left of the following regions: amygdala, hippocampus, caudal anterior cingulate cortex (ACC), rostral ACC, and medial orbitofrontal cortex (mOFC). After visual inspection, two subjects with TBI had one region each (right hippocampus and left rostral ACC) that could not be measured due to errors within the FreeSurfer process. To control for individual brain size, regional volumes were examined as ratios to total intracranial volume (ICV, estimated with FreeSurfer). Because published work shows minimal differences across different scanners in regard to volumetric measurements (Nugent et al., 2013), volumes were collapsed across scanners.

7.3.4 Statistical Analysis

Data management and analysis was performed using Statistical Analysis Software (SAS) version 9.4 (Cary, NC). Descriptive analyses included mean (with standard deviation or standard error of the mean) and/or median for continuous and ordinal variables including age, best in 24hr GCS, and education. Frequencies were calculated for categorical variables. Demographic information was compared between healthy controls and cases with TBI using Student's *t*-tests or Mann-Whitney U to compare means and Chi-Square or Fisher's Exact to compare frequencies. Spearman's r correlations were calculated to examine relative brain atrophy across regions (region to region correlations among healthy controls and subjects with TBI) and relationships between continuous demographic or outcome variables with regional volumes. Multiple comparison correction was performed using false discovery rate (FDR) (Benjamini and Hochberg, 1995).

7.4 RESULTS

Table 21. Population Descriptions.

A. Description of overall population.

Demographic Variable	TBI (n=40)	Controls (n=37)	p value
Age (mean±STD)	29.01±10.32	28.15±10.01	0.395
Sex (Male, n (%))	31 (77.5)	29 (78.4)	0.926
Race (Caucasian, n (%))	38 (95.0)	30 (81.1)	0.080
Education (mean±STD)	14.30±2.17	15.30±2.72	0.096
GCS (median)	10	-	-

B. Subpopulation of participants with TBI and PTD assessment.

Demographic Variable	No Depressed (n=14)	Depressed (n=8)	p value
Age (mean±STD)	29.95±12.55	32.64±8.23	0.103
Sex (Male, n (%))	13 (92.9)	7 (87.5)	0.485
Race (Caucasian, n (%))	14 (100.0)	8 (100.0)	-
Education (mean±STD)	14.29±1.82	14.13±2.95	0.999
GCS (median)	7	9.5	0.198

Table 21 describes demographic information for the total population (A) and for the population assessed for depressive symptomology (B). Within healthy controls, there were no significant associations between any regional volumes with age or education. For cases with TBI, there were no significant associations between any of the regions and age, education, GCS, or time from injury. GCS tended to have a positive association between left hippocampus (r=0.28135, p=0.0786) and left caudal ACC, (r=0.27414, p=0.0869). Education tended to have a positive correlation with left hippocampus (r=0.29056, p=0.0689). Out of the 22 subjects with TBI screened for depressive symptomology, 8 (36.3%) were depressed on the PHQ-9 at the time of MR acquisition.

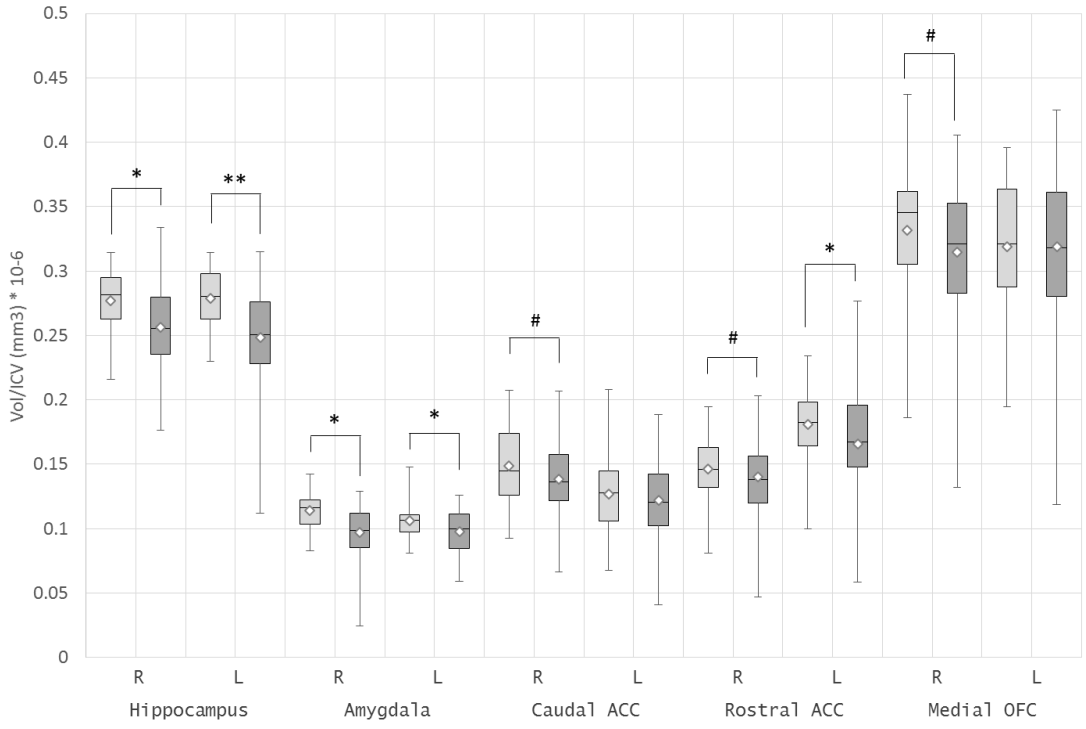


Figure 15. Fronto-limbic gray matter volumes show relative reductions following TBI (dark gray boxes), compared to healthy controls (light gray boxes).

Significant reductions are observed in the right and left amygdala, right and left hippocampus, and the left rostral anterior cingulate (ACC). There were trends for similar associations in the right caudal ACC, right rostral ACC, and right medial orbitofrontal cortex (OFC). All volumes expressed as a ratio to estimated total intracranial volume (ICV). (**p<0.01, *p<0.05, #p<0.1)

In **Figure 15**, fronto-limbic gray matter volumes were compared in subjects with TBI versus healthy controls. Overall, there were relative reductions in multiple regions following TBI. Significantly reduced volumes were observed in the right (p=0.005; FDR corrected, p=0.016) and left hippocampus (p<0.0001; FDR corrected, p=0.001) and the right amygdala (p=0.0002; FDR

corrected, p=0.001). There were similar reductions in the left amygdala (p=0.039) and the left rostral ACC (p=0.035) that did not remain significant following FDR correction (p=0.077, 0.077 respectively). **Table 22** shows correlations between fronto-limbic regional volumes within healthy controls and cases with TBI. Several regional volumes are correlated in healthy controls, including bilateral medial OFC with bilateral amygdala volume. However, in cases with TBI, only right medial OFC correlated with left amygdala volume. Multiple region-to-region volumes are more correlated in TBI cases compared to healthy controls. Both rostral and caudal ACC volumes are more positively correlated with medial OFC and amygdala in subjects with TBI compared to control subjects.

Table 22. Region to region correlations in healthy controls and subjects with TBI.

Controls	R Hipp	L Hipp	R Amyg-dala	L Amyg-dala	R caudal ACC	L caudal ACC	R rostral ACC	L rostral ACC	R medial OFC	L medial OFC
R Hipp	-	0.803* (0.001)	0.479 (0.014)	0.448 (0.017)	0.280 (0.223)	0.018 (0.980)	0.075 (0.875)	-0.049 (0.941)	0.425 (0.029)	0.307 (0.172)
L Hipp		-	0.352 (0.099)	0.318 (0.155)	0.135 (0.661)	0.007 (0.991)	-0.005 (0.991)	-0.069 (0.881)	0.125 (0.679)	0.201 (0.475)
R Amygdala			-	0.789 (0.001)	0.112 (0.719)	0.049 (0.941)	0.148 (0.614)	-0.002 (0.991)	0.528 (0.005)	0.554 (<0.001)
L Amygdala				-	0.299 (0.180)	-0.031 (0.941)	0.237 (0.341)	0.103 (0.742)	0.571 (<0.001)	0.557 (<0.001)
R caudal ACC					-	-0.169 (0.550)	0.597 (0.001)	0.031 (0.941)	0.464 (0.016)	0.188 (0.495)
L caudal ACC						-	-0.044 (0.941)	0.537 (0.005)	0.123 (0.679)	0.157 (0.587)
R rostral ACC							-	-0.031 (0.941)	0.271 (0.236)	0.184 (0.495)
L rostral ACC								-	0.452 (0.017)	0.197 (0.475)
R medial OFC									-	0.596 (0.001)
L medial OFC										-

TBI	R Hipp	L Hipp	R Amyg dala	L Amygdala	R caudal ACC	L caudal ACC	R rostral ACC	L rostral ACC	R medial OFC	L medial OFC
R Hipp	-	0.766 (0.001)	0.516 (0.006)	0.373 (0.047)	0.113 (0.611)	0.356 (0.059)	-0.069 (0.706)	-0.106 (0.611)	0.173[#] (0.412)	0.101 (0.611)
L Hipp		-	0.194 (0.357)	0.428 (0.023)	-0.063 (0.716)	0.184 (0.377)	-0.259 (0.207)	-0.080 (0.675)	0.099 (0.611)	0.030 (0.854)
R Amygdala			-	0.530 (<0.001)	0.232 (0.268)	0.547 (<.0001)	0.085 (0.661)	0.213 (0.310)	0.211 (0.310)	0.322 (0.092)
L Amygdala				-	0.314 (0.098)	0.461 (0.014)	0.104 (0.611)	0.378 (0.047)	0.410 (0.027)	0.145 (0.480)
R caudal ACC					-	0.149 (0.474)	0.552 (<0.001)	0.163 (0.436)	0.424 (0.024)	0.213 (0.310)
L caudal ACC						-	0.182 (0.377)	0.527 (0.006)	0.243 (0.248)	0.459 (0.014)
R rostral ACC							-	0.436 (0.023)	0.369 (0.047)	0.487 (0.006)
L rostral ACC								-	0.404 (0.031)	0.418 (0.026)
R medial OFC									-	0.559 (0.001)
L medial OFC										-

*Correlations are reported as Spearman's r (p value). All p values are FDR corrected.

[#]Correlations in subjects with TBI are highlighted if the correlation is stronger (dark gray) or weaker (light gray) compared to the corresponding correlation in healthy controls.

Fronto-limbic gray matter volumes were then examined for differences by depressive symptomology within cases with TBI (**Figure 16)**. Cases with depression had significantly higher left amygdala volumes compared to those with no depression (p=0.0003; FDR corrected, p=0.003). Several other regions had significantly higher volumes in cases with depression versus those with no depression (right hippocampus, p=0.029; right amygdala, p=0.026; left caudal ACC, p=0.022; and right medial OFC p=0.022) but did not survive FDR correction (p=0.057 for all comparisons). When compared to healthy controls, cases with PTD showed comparatively reduced left hippocampal volumes (p=0.009) and increased left amygdala (p=0.003) and caudal ACC volumes (p=0.047).

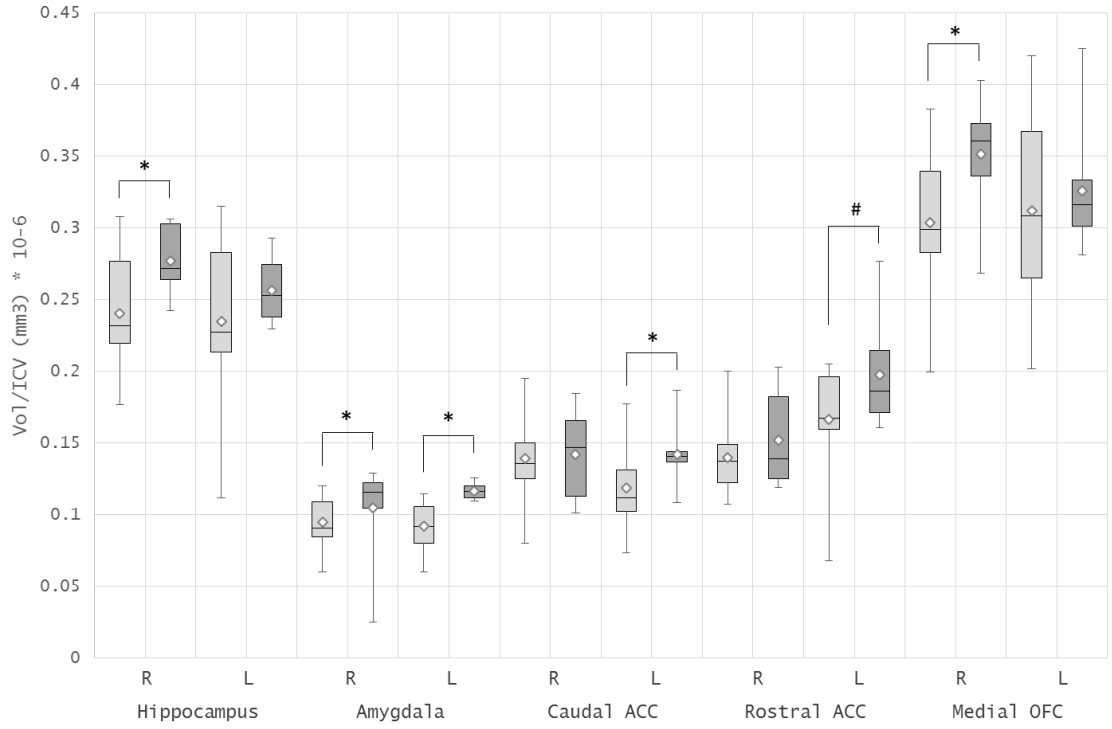


Figure 16. Fronto-limbic gray matter volumes show relative sparing in subjects with PTD (dark gray boxes), compared to subjects with TBI and no depressive symptoms (light gray boxes).

Subjects with PTD show higher right hippocampus, right and left amygdala, left caudal anterior cingulate (ACC), and right medial orbitofrontal cortex (OFC) volumes compared to subjects with no depressive symptoms (**p<0.01, *p<0.05, #p<0.1). All volumes expressed as a ratio to estimated total intracranial volume (ICV).

All regional volumes were examined for significant correlations to PHQ-9 total values. Left amygdala volume correlated with PHQ-9 total (r=0.645, p=0.0012; FDR corrected, p=0.012, **Figure 17**) regardless of PTD status. Similarly, right amygdala volume correlated with PHQ-9 total score regardless of PTD status (r=0.424, p=0.049) regardless of PTD status, but this did not survive FDR correction. However, sensitivity analysis showed that with removal of a visual outlier (empty circle data point in **Figure 17**), right amygdala volumes correlated significantly with PHQ-9 total scores (r=0.613, p=0.003, FDR corrected, p=0.016). There were no other significant correlations between PHQ-9 total and fronto-limbic regional volumes.

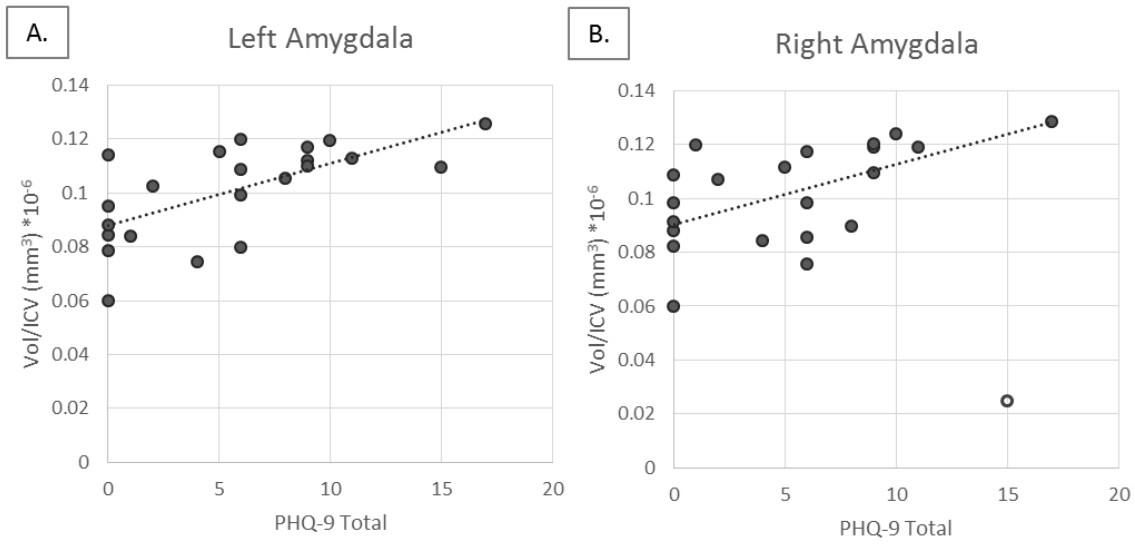


Figure 17. Amygdala gray matter volumes are correlated with PHQ-9 total scores.

(A) Left amygdala volumes show a strong correlation (r=0.645, p=0.0012; FDR corrected, p=0.012) with PHQ-9 total scores. Dashed line is representative of best-fit. (B) Right amygdala volumes (filled circles) were correlated with PHQ-9 total score with removal of a visual outlier (empty circle) (r=0.613, p=0.003, FDR corrected, p=0.016). Dashed line is representative of best-fit line when outlier (empty circle) is removed. All volumes corrected by estimated total intracranial volume (ICV).

7.5 DISCUSSION

The study examined fronto-limbic brain regional volumes following TBI, showing atrophy in multiple regions compared to healthy controls. These findings are consistent with similar studies showing reduced hippocampal and ACC volumes post-TBI (Chen et al., 2008b; D. F Tate and Bigler, 2000). This study applies a unique approach to regional volume analysis by examining

correlations between regions. Multiple region-to-region correlations are altered post-TBI suggesting specific regional atrophy that is not uniform across regions.

Overall, cases with TBI had reduced gray matter volumes in several fronto-limbic regions when compared to healthy controls. This is consistent with previous studies showing atrophy across a number of cortical and subcortical regions (Fujiwara et al., 2008; Gale et al., 2005; Hudak et al., 2011; Kim et al., 2008; Salmond et al., 2005). In this study, we show the most significant reductions in the hippocampus and amygdala volumes. Previous studies have shown reduced hippocampal volumes post-TBI (Arciniegas et al., 2001; D. F Tate and Bigler, 2000; Tomaiuolo et al., 2004) and one study has suggested reduced amygdala volumes in mild blast TBI(Depue et al., 2014). The unique patterns of reduced atrophy post-TBI, as illustrated by altered region-to-region correlations, may be related to reduced white matter tract integrity between regions due to DAI. The work here showing altered volumetric patterns in PTD and evidence for structural connectivity alterations in PTD as seen with DTI (Hudak et al., 2011) suggest structural susceptibility of fronto-limbic circuits in PTD. These structural changes may underlie hypothesized alterations in functional connectivity of limbic circuits, leading to PTD.

In both healthy controls and cases with TBI, right and left hemispheres correlate well across fronto-limbic regions, with the exception of ACC regions. In healthy controls, mOFC volumes were well correlated with amygdala volumes, but this pattern was not evident in cases with TBI. If these volumetric changes led to altered circuit function, the findings could be indicative of susceptibility of reduced top-down control (higher-order regions modulation of lower-order, subcortical regions (Pessoa et al., 2003)) from the mOFC over the amygdala as theorized in uninjured populations with depression (Disner et al., 2011). Overall, cases with TBI show more significant correlations between fronto-limbic regions compared to healthy controls. Specifically,

the left caudal ACC was more correlated with the amygdala and the right rostral ACC was more correlated with mOFC. These patterns suggests atrophy within these three regions is not uniform and may be functionally altering fronto-limbic circuit function. Computational modeling studies of TBI (Bayly et al., 2005; Kim et al., 2008), and studies of axonal force during angular rotation of the head in humans (Sabet et al., 2008), demonstrate that fronto-limbic regions may be at greater risk for TBI-related shearing, suggesting there may be a common pattern of non-uniform fronto-limbic degeneration/atrophy post-TBI.

Overall, cases with PTD showed higher fronto-limbic volumes in a number of regions when compared to subjects with TBI and no depressive symptoms. Cases with PTD show larger amygdala volumes compared to cases with no PTD. In uninjured populations, individuals with depression have greater amygdala reactivity in response to negative stimuli(Bylsma et al., 2008). Less relative amygdala atrophy may be a susceptibility marker for greater amygdala reactivity and PTD. Importantly, amygdala volumes in subjects with PTD were actually higher than healthy controls. Thus, this finding could be indicative of pre-injury susceptibility to mood disorders or maladaptive compensatory mechanisms post-injury. Yet, a meta-analyses of studies involving amygdala volumes in uninjured populations with depression found inconsistent results, perhaps due to modulation of amygdala volume by medication (Hamilton et al., 2008). In fact, larger amygdala volumes are reported following antidepressant treatment in individuals with depression (Hamilton et al., 2008), which could be due to increased neurogenesis following antidepressant treatment (Jiang et al., 2014). As subjects with TBI tend to be less responsive to antidepressant treatment (Fann et al., 2009a), larger amygdala volumes could reflect a treatment-resistant depression phenotype post-TBI. Future studies will need to examine this effect in medicated subjects with PTD. Amygdala volume positively correlated with total PHQ-9, suggesting it may

also be a biomarker for PTD severity. Interestingly, following TBI, smaller amygdala volumes are associated with post-traumatic stress disorder and a loss of inhibitory cognitive control following TBI (Depue et al., 2014). Animal TBI studies show evidence of increased excitation within the basolateral amygdala following injury, with an increase in fear conditioning and generalization (Reger et al., 2012). Studies show that the mOFC and ACC may influence emotional regulation, particularly in a top-down fashion, modulating amygdalar responses (Etkin et al., 2011). If these regulatory regions are comparatively more susceptible to injury or atrophy, the result could functionally alter the circuit.

Our PTD rates in this study (36.3% depressed) were similar to our previous reports (Failla et al., 2013) and within published ranges (Bombardier et al., 2010b). Multiple studies suggest an even greater susceptibility to depression following TBI with rates of ~53% within the first year post-TBI (Bombardier et al., 2010b), though this finding was reflective of depression over the first year post-injury. There were no significant demographic characteristics associated with PTD development in this study. Many studies do suggest associations with injury severity and PTD, though this finding has not been noted consistently (Maller et al., 2010). Importantly, in this study there was only one individual with a reported history of mood disorders, which likely did impact our findings significantly. It will be important in future studies to understand how pre-injury mood disorders impact fronto-limbic volumes. Similarly, understanding treatment effects on these measures following TBI will be important for use of morphometry as a neural correlate of PTD.

There are several important caveats to morphometric studies to consider. Regional volume may not always reflect function within healthy populations, yet, following TBI, where there is potential for considerable atrophy, volumetric analysis may be more closely related to functionality. However, atrophy may still serve as a susceptibility marker, and thus, fronto-limbic

circuit function may be able to compensate for injury-related volumetric reductions. Recent work following TBI showed regional grey matter atrophy is closely linked to damaged white matter tracts post-TBI (Warner et al., 2010), indicating that volumetric loss following TBI may also reflect structural connectivity changes, possibly reducing the ability for circuit-level functional compensation. Another limitation with morphometric studies is reliance on manual-tracing versus automatic segmentation software. While manual tracing of regions can be more reliable, expert raters are not always available, and for large scale studies, reliable metrics obtained using automatic segmentation paradigms are practical. Several studies have suggested FreeSurfer automatic segmentations correlate reliably with manually-labeled segmentation. On average, errors from automatic segmentation are likely to result in false negatives (Pardoe et al., 2009), finding that then support the robustness of our findings, given the small sample size for this study. While this study, and others (Hudak et al., 2011), demonstrate the utility of automatic segmentation tools in cases with TBI, there have not been definitive comparative studies.

Future studies are needed to validate these findings in larger populations, understanding what injury parameters might lead to these specific volumetric alterations. It will be important to investigate why specific fronto-limbic regions may be more susceptible to TBI-related atrophy, possibly providing insight into mechanisms of PTD. Similarly, understanding exactly what volumetric measurements reflect with regard to neuronal loss, neural pill, or glial support changes will inform treatment paradigms. However, this study suggests that structural changes in fronto-limbic regions is related to depressive symptomology. Similarly, this study suggests underlying structural patterns of fronto-limbic atrophy that emerge after TBI and are related to PTD but, it will be imperative to examine how fronto-limbic regional volumes may reflect functional changes, in the areas of both depression and cognition.

8.0 DISCUSSION

8.1 REHABILOMICS: TAKING ON THE COMPLEXITY OF POST-TBI DEPRESSION

When studying depression in a population with another primary disease or disorder, like traumatic brain injury, a natural approach is to evaluate the etiology of idiopathic depression to identify similarities. In some diseases or disorders, where the central nervous system is not directly impacted, the research into increased incidence of depression may center on psychological impacts of the diagnosis, such as employment status, increased care burden on family, loss of independence, or changes in life roles. However, depression involving systemic disorders may also have a biologically induced component such as sickness behavior (Dantzer et al., 2008). Sickness behavior is a concerted strategy of the immune, endocrine, and nervous systems to work in concert to fight an infection. In many chronic disorders like TBI, with associated chronic inflammation (Kumar et al., 2014), this physiological interplay may lead to a pathological cytokine induced depression, with accompanying neuroendocrine dysfunction. In the context of brain injury, where there is the possibility of direct neurological injury to the brain circuits involved in emotional regulation, there is an additional layer of complexity in understanding the depressive symptomology that often accompanies TBI. In post-TBI depression (PTD), it is likely that psychogenic reactions to injury effects interact heavily with neurological damage to brain regions involved in emotional regulation and sickness behavior (due to increased inflammation and neuroendocrine dysregulation), as well as pre-injury predispositions to depression, either in the form of pre-morbid mood disorders, environmental risk factors, or genetic risk. In severe TBI,

understanding survival from the injury and resultant cognitive deficits can add another layer to investigation of post-TBI depression. Given this level of complexity, we utilized a Rehabilomics (A K Wagner, 2010) approach to understanding how monoaminergic and neurotrophic signaling may impact survival, depression, and cognition following TBI. **Figure 18** highlights the findings described here in a comprehensive model.

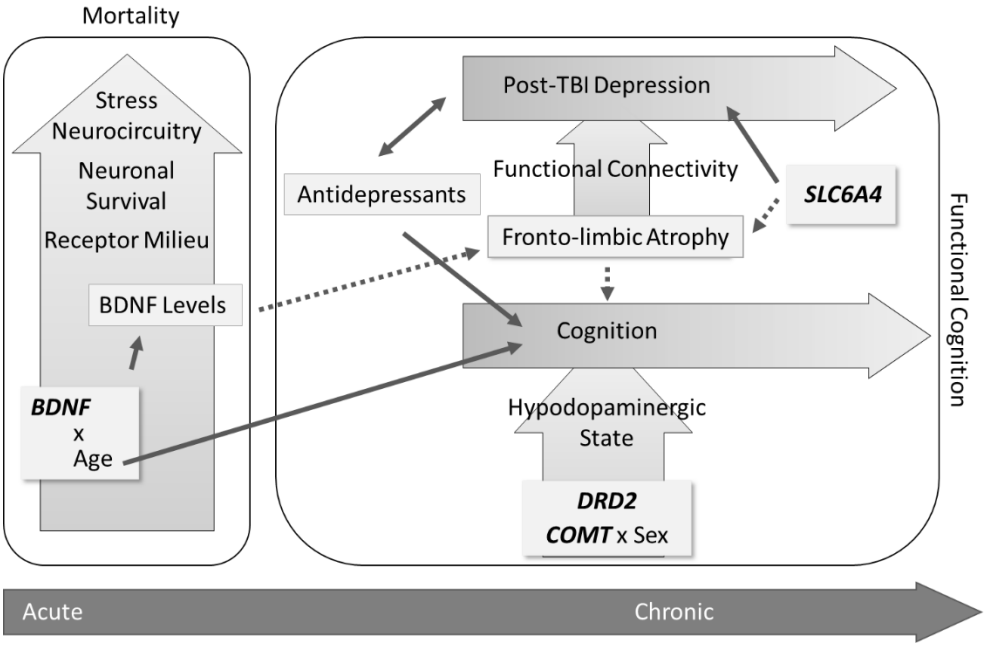


Figure 18. Summary of investigation into monoaminergic-neurotrophic signaling in mortality, depression and cognition following TBI.

In survival studies, we have discovered that *BDNF* genetic variation interacts with age (**Appendix A**), in concert with BDNF levels (**Chapter 5**), to influence mortality predictions. This work is discussed in greater detail in **section 8.3.1**. Briefly, we suggest these findings may be due to injury- and age-induced alterations in receptor milieu, altering the signaling capacity of BDNF immediately following injury. However, in **Chapter 6**, we also highlight relationships between

acute BDNF levels that predict chronic cognitive deficits and depression risk after TBI. In survivors, acute BDNF levels maybe reflective of early neurotrophic support, such that reduced neurotrophic support may result in less neuroplasticity, synaptogenesis, or neuronal survival. We suggest reduced neurotrophic support in survivors, early in recovery, may lead to non-uniform fronto-limbic atrophy (**Chapter 7**) and susceptibility to depression and cognitive deficits at chronic time-points. Future studies are needed to examine the relationship between acute BDNF levels and chronic fronto-limbic atrophy. Similarly, we aim to understand the relationship between fronto-limbic atrophy and specific cognitive deficits. While we demonstrated no depression effects on cognitive deficits within our sample, there may be nuanced differences in individuals with PTD in how fronto-limbic atrophy correlates with cognitive functioning.

In **Chapter 2**, we demonstrated that while there was no predictable pattern of cognitive deficits in individuals with PTD, both cognitive performance and PTD contribute to functional cognition. Antidepressant use, of which was more common in individuals with PTD, was also a contributing negative factor on cognitive performance and functional cognition. This was surprising, as antidepressants are thought to improve cognition (in uninjured populations). However, the literature following brain injury suggests antidepressants have no real effect on cognition post-TBI (Wang et al., 2011; Wilson and Hamm, 2002). Future studies may examine the relationship between serotonin and BDNF during treatment with to determine if variable SSRI efficacy may, in part, be due to neurotrophic mechanisms post-TBI.

In studying genetic risk for depression post-TBI, we show in **Chapter 3** important dynamic risk relationships for *SLC6A4* in PTD that differ from the literature reporting on uninjured populations with depression. We suggest this risk may be exhibited through the developmental effects of the serotonin transporter on fronto-limbic circuitry, particularly for those with a pre-

injury history of depression, in addition to a possible role of *SLC6A4* in serotonergic signaling post-injury where there may be a hypo-monoaminergric state. We also demonstrate important relationships between dopaminergic genetic variation in DRD2 (**Chapter 4**) and COMT (interacting with sex, **Appendix B**) on cognitive function. We suggest that variation in these genes have such impactful relationships on cognitive function post-TBI due to a hypodopaminergic state post-TBI.

In the remaining sections of this Chapter, we discuss a number of caveats to this work, both inherent to studying clinical populations, as well as limitations specific to studying individuals with traumatic brain injury. We also present comments on future studies that would support and increase the weight of the work presented thus far.

8.2 MONOAMINERGIC-NEUROTROPHIC GENETIC RISK IN SURVIVAL, DEPRESSION, AND COGNITION POST-TBI

In the work presented here, one of our main tools in understanding implications of monoaminergic-neurotrophic signaling in survival, depression, and cognition is studying functional genetic variants as proxies for individual differences in signaling within these pathways. Several human studies have begun to study the impact of genetic variation in TBI recovery and biosusceptibility to complications, both in the acute care (Failla et al., 2014; Wagner et al., 2010; Amy K Wagner et al., 2007) and chronic rehabilitation phases (Darrah et al., 2012; Failla et al., 2013; Hoh et al., 2010; Krueger et al., 2011; Wagner et al., 2012, 2010). Many of these studies have focused on genetic variation within our targeted pathways in addition to studies in inflammatory pathways.

Table 23 highlights to findings from the work presented here in monoaminergic and neurotrophic gene variation.

Table 23. Summary of observed monoaminergic and neurotrophic genetic risk in mortality, PTD, and cognition post-TBI.

Gene	Variation	Function; Implications	Mortality	PTD	Cognition
SLC6A4	5-HTTLPR	5-HTT expression, fronto-limbic circuitry; depression risk, TBI	Not examined	S-carriers show reduced PTD risk at 6 months post-injury (**Chapter 3**)	Trend interaction with sex (data not shown)
DAT1	10/9 VNTR	Reduced DAT expression(VanNess et al., 2005); TBI	Not examined	No significant effect	No significant effect
COMT	Val158Met	Reduced COMT activity(Lotta et al., 1995); depression(Baune et al., 2007), TBI(Lipsky et al., 2005a)	Not examined	Interacts with behavioral disinhibition to influence PTD (in preparation)	Interacts with sex to impact cognition (**Appendix 2**)
DRD2	TaqIA1	Related to D2 binding; depression(Opmeer et al., 2010), TBI(McAllister et al., 2008)	Not examined	No significant effect	A1/A2 show better overall cognitive performance (**Chapter 4**)
BDNF	Val66Met	Reduced BDNFsecretion(Egan et al., 2003); depression(Martinowich et al., 2007), TBI(Krueger et al., 2011)	Acutely, Met-alleles have increased survival; Chronically, interacts with age in mortality. (**Chapter 5**)	BDNF interacts with 5-HTTLPR to modulate PTD risk* (data not shown)	Trend interaction with *DRD2* (data not shown)

8.2.1 Development of a gene risk score

As shown in Table 23, we have observed genetic risk factors in a number of our targeted genes in PTD and cognitive deficits post-TBI. Given the overlapping nature of these genes, in both interactive signaling and effects on similar outcomes, we aim to develop a comprehensive monoaminergic-neurotrophic gene risk score as a future direction arising from our work. We are

especially interested in this as many genetic risk effects can be quite small, perhaps even undetectable alone, but may contribute to risk associations in an incremental way. In this mechanism, we may be able to understand genetic risk within genes that we are not powered to observe in individual candidate gene studies. There is also evidence our targeted genes may interact in an epistatic fashion, where having both risk alleles may result in a greater than additive effect. Thus, understanding how a battery of genetic risk influences depression or cognition post-TBI may provide greater benefit than individual candidate gene studies.

Future work will develop a gene risk score, encompassing all of our tagerted genes. In these score, there is an incorporated genetic "load" assumption, suggesting there is a positive relationship between increased risk allelic load risk for the targeted disorder or disease. The recent guidelines published in the GRIPS (Genetic RIsk Prediction Studies) statement do not recommend methodological approaches, but note the lack of discrimination between count (unweighted) and weighted GRS approaches (H. Chen et al., 2011). Weighted approaches draw from individual candidate gene studies, utilizing effect sizes as weighting mechanisms. In our preliminary data, we have noted no significant differences between unweighted and weighted approaches. However, as we have noted a number of interactions in our outcomes (ie. COMT x sex on cognitive outcomes), our genetic risk scores must embody the Rehabilomics approach and incorporate effect size differences among different demographic groups. We believe gene risk scores are a promising strategy for post-TBI rehabilitation studies in assessing complication risk, with the caveat of understanding important relationships to demographic variables within each proposed candidate gene that would be considered in a GRS.

8.2.2 Considerations for candidate gene studies in TBI

8.2.2.1 Divergent Risk Relationships from Uninjured Populations

Unsurprisingly, many studies in clinical TBI set out to identify candidate genes that may influence recovery by examining gene risk associations in the uninjured literature. For example, when considering genetic risk factors for PTD, understanding candidate genes from literature on major depressive disorder (MDD) is invaluable of the previous literature available outlining possible genetic risk factors. However, as evidenced by our work described in **Chapter 3** (Failla et al., 2013), there can be risk relationships following TBI that are opposite in direction (risk versus protective) when compared to uninjured populations, even when studying similar outcomes like depressive symptomology. A number of studies within our lab (Wagner et al., 2012) (and see **Chapters 3, 4, 5**) and others (Krueger et al., 2011) have begun to demonstrate gene risk relationships following TBI that differ substantially from risk relationships in uninjured populations, which may be logical genetic predictions when understanding the pathology accompanying TBI within these pathways. Similarly, it is important to understand evolving genetic risk relationships following TBI where there is a specific temporal recovery within many aspects of recovery (secondary cascades, evolving neurodegeneration) that may impact genetic risk relationships of TBI recovery.

There are likely a few reasons for divergent genetic risk profiles post-TBI. While not well-understood, TBI induces a number of epigenetic alterations(Z.-Y. Zhang et al., 2007) that may differ wildly from patterns in uninjured populations, making interpretation of gene-candidate studies difficult. Another layer of complexity stems from a lack of or rudimentary understanding of TBI-induced signaling or receptor changes with certain pathways. A prime example of this phenomenon is apparent in our work with the BDNF gene in mortality predictions (**Appendix A**

and **Chapter 5**). Still further, candidate gene studies (as well as genome wide association studies) in uninjured populations may differ from relationships observed with TBI because of a lack of well-defined outcomes or endophenotypes. As we show in **Chapter 2**, the expected relationships between cognitive deficits and depression differ following TBI compared to idiopathic depression, thus, the outcome of PTD may be inherently, biologically different from MDD. With the distinct possibility of a unique pathology profile for PTD, it is then unsurprising that we find 'divergent' or 'opposite' risk profiles in PTD (**Chapter 3**) when compared to idiopathic depression.

8.2.2.2 Impact of Mortality Associations on Candidate Gene Associations with Recovery

When studies aim to identify candidate genes for recovery post-TBI, one important caveat within these studies in disease populations, and especially TBI, is to be cognizant of mortality effects. If the gene under evaluation is associated with mortality, this association can result in a disproportionate amount of individuals with a particular allele, altering observed frequencies of alleles among survivors. Even aside from maintaining Hardy-Weinberg equilibrium, this issue can have effects on how powered studies are for genetic variants with lower minor-allele frequencies. Additionally, understanding how variants seemingly unrelated to the cause of mortality under investigation (e.g. BDNF and TBI) may interact in other body systems *outside* of the CNS to influence mortality, may add insight in to work with chronic outcome endpoints like depression and cognition.

8.2.2.3 Limitations of candidate gene studies

There are a number of limitations in candidate gene studies that are inherent to the approach. Importantly, there is the risk of stratification effects, where unintentionally, variation in racial background can be contributing to candidate gene associations. To combat this, we have used self-

reported race categorizations and examined all genetic findings in Caucasians-only as well as the total population. Using self-reported race categorization is not ideal, yet genotyping racial background using commercially available microchips with upwards of 10,000 markers carries its own complexities. Importantly, our studies tend to draw from a fairly racially homogenous population. While this abates some concerns about possible stratification effects, the issue also results in a lack of generalizability of our candidate gene studies to racially diverse TBI population that exists nationally ("CDC - Injury - TBI - TBI in the US Report," n.d.). Future studies will need to examine these associations in larger, more diverse populations with TBI to aid in the generalizability of our findings.

8.3 BIOMARKERS FOR DEPRESSION AND COGNITION POST-TBI

8.3.1 BDNF Serum and CSF as a biomarker in TBI

The work presented here suggests BDNF signaling post-TBI may have a number of different effects based on age, time from injury, and the outcome of interest. For example, in **Chapter 5**, we demonstrate acute BDNF levels are associated with mortality when BDNF genetic variation is taken into account. Yet, acute serum BDNF levels predicted chronic memory problems independent of genetic risk among survivors. These complex relationships between genetics and BDNF levels in CSF and serum, across recovery, may be due to the diverse actions of BDNF.

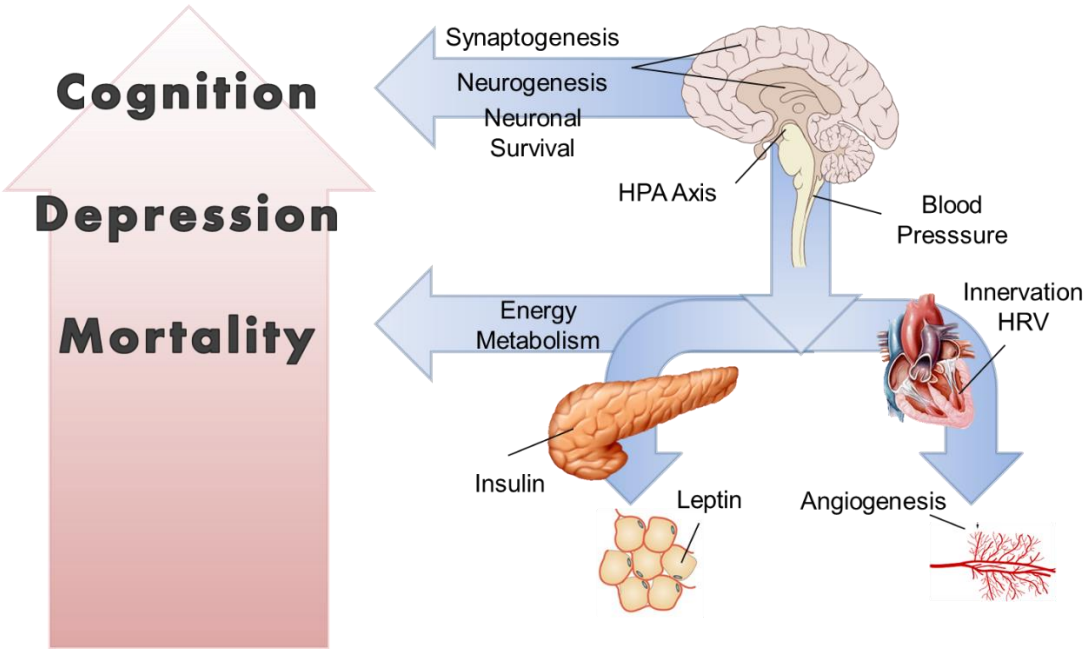


Figure 19. Schematic of BDNF's actions relevant to mortality, depression or cognition following TBI.

Figure 19 summarizes a several areas of BDNF action that may be relevant to survival, depression, and cognition post-TBI. Traditionally, BDNF has been studied due to its role in synaptic plasticity, neurogenesis and neuronal survival (Z.-Y. Chen et al., 2004), making it an interesting target for chronic TBI. Yet, newer lines of investigation show BDNF can regulate energy metabolism and autonomic function. Evidence suggests that BDNF may be involved in brainstem regulation of cardiovascular function (Brady et al., 1999; Wang and Zhou, 2002; Wan et al., 2014). Similarly, BDNF modulates the sympathetic/parasympathetic balance in cardiovascular function (Yang et al., 2002). Interestingly, rs6265 is associated with innate differences in heart rate variability (A. C. Yang et al., 2010) and acute stress heart rate reactivity in healthy populations (Alexander et al., 2010). In fact, one study showed that local BDNF administration following surgical sympathectomy induced hippocampal vascular changes and

edema (Kasselman et al., 2006). This study suggests BDNF effects during a state of compromised autonomic function (e.g. immediately following TBI (Goldstein et al., 1998)) could impact TBI pathology, particularly with regard to acute survival. There is a dearth of research about BDNF function outside of cognition or plasticity post-TBI, limiting speculation about how BDNF and CNS-peripheral modulation of autonomic function post-TBI might occur. Thus, BDNF may affect autonomic regulation and reflect the current energy state (Rothman et al., 2012), suggesting multiple mechanisms through which BDNF signaling could influence TBI mortality and, later, recovery.

As far as a biomarker for mortality, **Figure 20** depicts our overall model of BDNF as an acute biomarker following TBI based on our findings in **Chapter 5** and **Appendix A**.

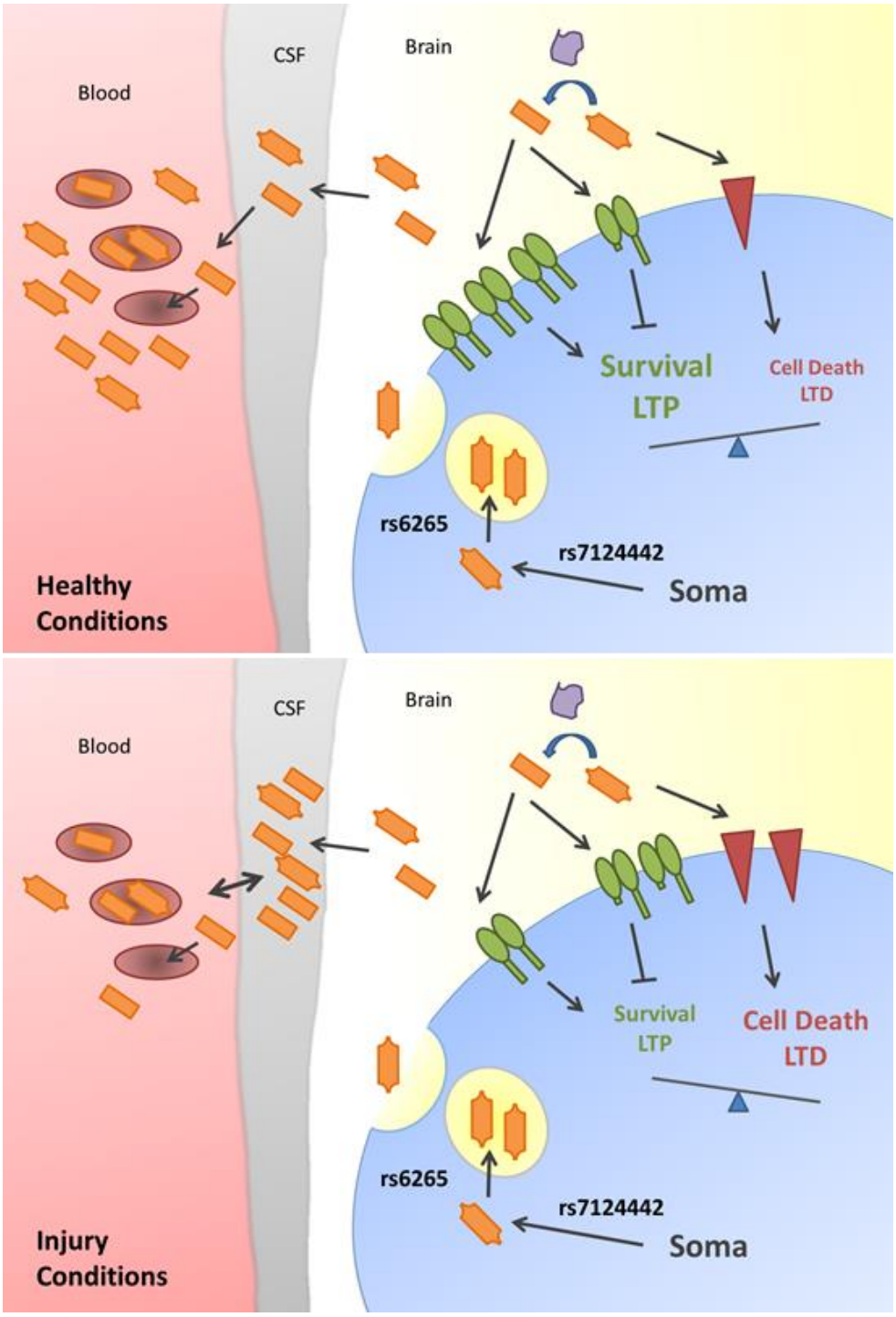


Figure 20. Schematic of relationships affecting serum and CSF BDNF levels following TBI.

Under normal conditions, BDNF serum levels are thought to reflect CSF and brain tissue levels. BDNF moves from the interstitial space to CSF and then into the serum, where it is stored in platelets. After brain injury, permeability of the BBB increases, allowing serum BDNF to leak into the CSF, resulting in a negative correlation between CSF and serum. BDNF rs6265 Val allele results in reduced efficiency of BDNF into synaptic vesicles for release. BDNF rs7124442 C-allele results in reduced trafficking of BDNF to dendrites, reducing spine density and formation. Both rs6265 and rs7124442 may result in reduced BDNF in the intracellular space, thus less signaling on target receptors. While under normal conditions, TrkB receptors are the most ubiquitous receptor, both age and injury (Rostami et al., 2013) upregulate truncated TrkB receptors and pro-apoptotic p75 receptors, altering the balance of pro-survival to pro-apoptotic signaling. In injury, understanding the underlying receptor milieu in tissue immediately following injury, and in association with aging, can support interpretation of BDNF CSF and serum as a biomarker for mortality following brain injury. Serum BDNF levels may be related to secretion of BDNF as understood by BDNF gene risk score (GRS, rs6265 and rs7124442) as well as an understanding of GRS to age-altered balance of receptors. CSF BDNF may be most heavily related to BBB permeability.

In chronic time-points, BDNF levels may be more reflective of a "carryover effect" of neurotrophic support in acute recovery, rather than depressive symptomology, as were not able to show a clear relationship between BDNF levels and current depressive symptoms. While in non-injured populations with depression it is not wholly clear whether decreases in BDNF levels occur prior to the depressive symptomology or are due to the depression. Yet in injury, we have the advantage of tracking BDNF levels prior to PTD development and may better be able to delineate the time-course of BDNF levels that lead to depressive symptomology.

8.4 INTERACTIONS BETWEEN OBSERVED EFFECTS: CONVERGING LINES OF EVIDENCE

Much of the work presented here borrows from the extensive literature on monoaminerigic and neurotrophic signaling in normal conditions or in idiopathic depression. Yet, understanding this literature in the context of TBI is difficult as TBI likely disrupts many signaling pathways important for long term function and recovery. For example, there is a paucity of literature on serotonergic function post-TBI, thus it is unclear how serotonergic signaling capacity may be altered post-TBI, and how that dysfunction contributes to our findings in genetic studies. While better characterized after TBI than the serotonergic system, contemporary understanding of dopaminergic function post-TBI (reviewed (Bales *et al.*, 2009)) is still in a formative state of development. Thus, understanding monoaminergic and neurotrophic signaling in PTD risk is limited by our lack of fundamental understanding of TBI-induced alterations in these pathways.

The work presented here speaks to interacting pathways among serotonin, dopamine, and BDNF signaling. Future work will examine interacting effects as many of these lines of evidence converge on similar mechanisms involving a functional hypomonoaminergic state. As eluded to in **Figure 18** many of the findings we report may converge on functional connectivity of fronto-limbic circuits. We show that acute BDNF levels are associated with depression and cognition at chronic time-points (**Chapter 6**). We hypothesize these findings may be due to neurotrophic signaling during acute recovery that results in fronto-limbic atrophy, and thus, impaired functional connectivity of fronto-limbic circuits. Future studies can examine if acute BDNF levels predict hippocampal volume, for example, and if therapies like exercise, which may improve BDNF signaling, may rescue this effect. As all of our targeted genes have been shown to be relevant to either fronto-limbic atrophy (in gray or white matter) and function (5-HTTLPR (Pezawas *et al.*,

2005), *BDNF* (Kim *et al.*, 2013), *DAT* (Bertolino *et al.*, 2009), *DRD2* (Bartrés-Faz *et al.*, 2002), and *COMT* (Honea *et al.*, 2009)), we also plan to develop a gene risk score, accounting for variation in multiple targeted genes, known to influence fronto-limbic volumes. Additionally, it will be important to understand how the non-uniform atrophy identified in **Chapter 7** may relate to functional alterations. It is possible that monoaminergic or neurotrophic signaling may explain some of the non-uniform atrophy pattern observed.

However, the lack of association between cognition and depression in TBI complicates the hypothesis of fronto-limbic dysfunction in PTD. Further work is needed to address the possibility of more nuanced effects of depression on cognition post-TBI (like emotional regulation, motivation). As we demonstrate strong associations for dopaminergic functioning in cognition, we hypothesized overlapping relationships with depression. A role for dopamine in depression has been proposed in idiopathic depression, but we observed no clear relationship to depression after TBI among the DA genes evaluated thus far. However, future work in development now suggests a role for dopamine in behavioral complications that may moderate depressive symptoms. Similarly, future work will investigate the functional recovery of individuals with depression and *DRD2*-related cognitive deficits to elucidate the effects of the dopaminergic system on PTD-Cognition interactions.

8.5 CONSIDERATION OF OTHER RELATED SIGNALING PATHWAYS IN PTD AND COGNITION POST-TBI

8.5.1 Stress and HPA Axis

Stress is likely an important factor in recovery following TBI. The psychosocial adjustments and enduring cognitive/physical impairments after injury can create a chronically stressful environment for those with TBI. The hypothalamus-pituitary-adrenal (HPA) axis modulates the body's reaction to both physical and emotional stress. Cortisol (in humans) or corticosterone (rodents), or CORT, is a known stress hormone and an end product of the HPA axis that binds to brain glucocorticoid receptors (Joëls, 2008). The stress of major trauma results in increase adrenocorticotropin and CORT (Woolf, 1992). Our published work suggests that serum CORT is elevated for many subjects after clinical TBI and linked to other adrenal/peripheral hormone production (estradiol) that is associated with outcome (Wagner et al., 2011c). CSF increases in CORT early after TBI are even more striking after TBI and associated with poor outcomes (Santarsieri et al., 2013). Elevated cortisol is a known biological characteristic of depression. While there is limited data on cortisol levels and cognitive function in depressed subjects, several lines of evidence suggest cortisol has effects on cognition, including performance on memory testing (Hinkelmann et al., 2009; Newcomer et al., 1994).

Experimental TBI studies show important relationships to stress response following TBI. One study showed that stressed and injured rats showed increased levels of CORT up to 2 months following blast TBI that was related to altered anxiety-like behaviors (Kwon et al., 2011). Forced exercise therapies increase CORT in rodents and result in reduced benefits of exercise following experimental TBI (Griesbach et al., 2012). Importantly, the benefits of exercise in experimental

TBI also seem to be dependent on recovery of the stress response prior to induction of exercise (Griesbach et al., 2013).

Given our work with BDNF and survival, and the likelihood of our survival associations being related to modulation of stress circuitry, examining the role of BDNF in relationship to stress and neuroendocrine function in PTD and cognition will likely provide additional insight. In addition, understanding the stress response in relationship to PTD and cognitive outcomes post-TBI, will greatly impact neurorehabilitation research design and study interpretation.

8.5.2 Inflammation

A growing line of research in the field indicates chronic, maladaptive inflammation may play a role in PTD. Inflammatory processes associated with chronic disease, including cardiovascular disease, multiple sclerosis, and others, have been associated with increased propensity for depressive symptoms and depression (Miller et al., 2009). Causative studies show that immune-stressors (e.g. lipopolysaccharide) can induce depressed mood(Frenois et al., 2007). We have shown acute inflammation can predict chronic depressive-like symptoms in clinical populations TBI (Juengst et al., 2014). Studies in experimental TBI have demonstrated a link between chronic inflammation and depressive symptoms as well (Fenn et al., 2013). Given that neuroinflammation is a hallmark of acute TBI, and there is evidence of long-term inflammation even several years post-injury (Nagamoto-Combs et al., 2007), inflammation is an intriguing line of research in PTD.

Many of the cytokines that might be biomarkers for PTD have relationships with monoaminergic neurotransmission. For example, IL-6 and TNF-alpha activate the HPA axis, and, in turn, can affect levels of tryptophan (precursor for 5-HT). Cytokines also impact neurotrophic support. One study of acute inflammation suggested that TNF-alpha is protective through its

transcriptional facilitation of BDNF via stimulation of nf-kappa-b and CCAAT-enhancer-binding proteins (or C/EBPs) (Saha et al., 2006). However, in chronic stress, pro-inflammatory cytokine expression in the hippocampus decreases mRNA BDNF levels (You et al., 2011). These studies suggest that chronic inflammation could impact depressive symptomology dependent on the timing and duration of inflammation.

8.6 REHABILOMICS: A ROLE FOR MONOAMINERGIC AND NEUROTROPHIC SIGNALING IN SURVIVAL, DEPRESSION AND COGNITION POST-TBI

Within our proposed framework of Rehabilomics (A K Wagner, 2010; Wagner and Zitelli, 2012), we sought to understand how a wide-range of individual factors (like genetics, age, and sex) that interact with injury parameters that could influence response to rehabilitation and alter outcomes following TBI. It is imperative for clinical studies, in addition to experimental paradigms, to rigorously evaluate important factors in rehabilitation like timing, chronicity of treatment, maintenance of treatment effects and influences of acute management on chronic care. Much of the work presented here, supports these important caveats. With an integrative approach, the Rehabilomics-framework combined with rigorous experimental models of neurorehabilitation will likely yield rapid improvements in the current state of neurorehabilitation research and clinical care for individuals dealing with the chronic effects of TBI.

8.6.1 Injury parameters and chronicity in PTD and Cognition post-TBI

TBI is an inherently heterogenous disorder with varying mechanisms of injury, a wide range of injury severities, and a chronic, evolving symptom profile. These are all important considerations in the studies described here. For example, in understanding genetic studies post-TBI, the context of injury severity may interact with findings. In **Chapter 4**, we suggest that some of the discrepancies in the literature regarding *DRD2* may be related to differences in injury severity between study populations. Genetic effects in more mild TBI cohorts may be more reflective of non-injury genetic risk relationships as the associated injury pathology burden may be relatively less. It is also possible these studies examining more mild injuries have greater potential to be reflective of pre-injury functioning or environmental effects. It is also important to use a wide range of clinical studies and experimental models to examine important issues like how acute care can influence response to chronic rehabilitation, focusing on any unintended consequences of acute management of injuries. One example of this effect is evidenced by how acute BDNF levels map to chronic outcomes better than chronic BDNF levels (**Chapter 6**).

8.6.2 Influence on treatment paradigms

The studies presented here demonstrate a number of considerations in development of new treatments for depression and cognition post-TBI. Importantly, cognitive difficulties can impede treatment for depression when utilizing cognitive-behavioral interventions, yet, in **Chapter 2**, we suggest the addition of PTD may not increase cognitive deficits per se on individuals. Future work is needed to evaluate how cognitive functioning, in concert with PTD, may impact efficacy of and participation in cognitive-based therapies. Along this line, our work suggests antidepressant use

may negatively impact cognitive performance. Comprehensive studies are needed to understand how antidepressants affect cognition post-TBI, and whether this potential "side-effect" out-weighs any benefit of depression remittance, thus, aiding clinical decision making.

Similarly, the biomarker and genetic work presented here has not been validated in the context of treatment or rehabilitation. Examining the biomarkers we presented here (i.e. BDNF) for relationships to treatment effects is also important when considering how to improve prognostication or personalize treatments. It is not known if BDNF levels return to healthy control levels following remittance of depression and cognitive rehabilitation, which may suggest BDNF as a required substrate for TBI-related recovery. How our observed genetic effects would interact with depression treatment may provide a greater understanding about the signaling pathways that underlie symptomology. Studying genetic risk relationships could also inform clinical pharmacological interventions. Importantly, work in transgenic animals will likely lead to important pharmacogenetic studies elucidating individual responses to targeted pharmacological interventions post-TBI.

Preclinical testing of rehabilitation focused treatments are far less common compared to early intervention studies, and for the field of translational TBI research to continue to evolve and progress, rehabilitation relevant treatment designs are an important contribution to the field. However, one of the major challenges of translational rehabilitation research is the ability to model complex, higher order behaviors and deficits following TBI. Modeling depression in experimental TBI research is important for understanding treatment effects and mechanisms. Yet, language or cultural barriers, social support, and participation in rehabilitation are important factors in the clinic that cannot be addressed using experimental models.

In the future, it will be important to determine therapeutic windows. For example, are there treatments that can be administered for a limited time period that will have lasting effects, or will chronic treatments require long-term exposure to address ongoing symptom profiles? Similarly, the understanding of how multiple treatments interact with each other to affect recovery, and susceptibility to overlapping complications like depression, apathy, and cognition, will be important to evaluate. It may be that treatment timing and order are necessary to maximize patient benefits. Further, it will be important to assess pharmacological interventions in combination with non-pharmacological interventions in neurorehabilitation.

8.6.3 Conclusions and Summary

As monoaminergic and neurotrophic signaling have been heavily studied in idiopathic depression and cognition, we sought to understand how these interactive signaling pathways may influence survival, depression, and cognition post-TBI. By employing the Rehabilomics framework, we were able to identifying risk for complications in an individual's recovery from TBI that are likely relevant for treatment and early intervention. The studies presented here were both multidisciplinary and multimodal to discern relevant biological mechanisms contributing to PTD and cognitive deficits post-TBI, in order to aid in identifying and treating an "at-risk" and highly complex, population. The results of this work will advance both clinical investigation into the monoaminergic and neurotrophin pathology of TBI as well as basic research into fronto-limbic system vulnerabilities and their involvement in depressive symptomology and cognitive impairments post-TBI.

8.6.4 Limitations and Future Directions

One of the major limitations of the work presented here is the lack of causal studies. The findings suggests highly intriguing correlative evidence for a contribution of monoaminerigic and neurotrophic associations with post-TBI survival, depression, and cognition. While this does not reduce the caliber of the work, these studies will need support from experimental work in rodent models and interventional studies in humans. Mapping portions of our holistic Rehabilomics approach to an experimental rehabilitation paradigm will likely accelerate the pace in which rehabilitation research can translate the work presented here into individualized treatment plans while yielding important insights into rehabilitation-relevant mechanisms.

We suggest future studies should focus on four major lines of research. First, there is an important need to elucidate the mechanisms of our genetic association studies in experimental models of TBI. While we have proposed likely mechanisms for each of our findings, there is a need for evidentiary support from animal models. Secondly, the work in **Chapter 2** suggests a major gap in our understanding of PTD regarding its relationship to cognitive deficits. As this assumption borrowed from extensive MDD literature that demonstrates this relationship, it is imperative to rigorously examine aspects of cognitive dysfunction vs. functional cognition impairment in the context of PTD. Thirdly, we suggest that several of these findings implicate fronto-limbic circuit function in PTD. Critical evaluation of this pathology in clinical studies, using task-based and resting state fMRI studies, are likely to inform the role of fronto-limbic function in PTD development. Lastly, all of these findings suggest new lines scientific inquiry about multiple potential therapeutic targets that have yet to be explored.

APPENDIX A

A.1 VARIATION IN THE BDNF GENE INTERACTS WITH AGE TO PREDICT MORTALITY IN A PROSPECTIVE, LONGITUDINAL COHORT WITH SEVERE

Published in Neurorehabilitation and Neural Repair (2014)

Michelle D. Failla, BS[1,2]; Raj Kumar, MPH[2]; Andrew Peitzman, MD[3]; Yvette P. Conley, PhD[4,5]; Robert E. Ferrell, PhD[5]; Amy K. Wagner, MD[1,2]

A.1.1 Abstract

Background: Mortality predictions following traumatic brain injury (TBI) may be improved by including genetic risk in addition to traditional prognostic variables. One promising target is the gene coding for brain-derived neurotrophic factor (BDNF), a ubiquitous neurotrophin important for neuronal survival and neurogenesis.

Objective: We hypothesized the addition of *BDNF* genetic variation would improve mortality prediction models and that *BDNF* Met-carriers (rs6265) and C-carriers (rs7124442) would have the highest mortality rates post-TBI.

Methods: This study examined *BDNF* functional single nucleotide polymorphisms (SNPs) rs6265r (val66met) and rs7124442 (T>C) in relation to mortality in a prospective, longitudinal cohort with severe TBI. We examined 315 individuals receiving care for a closed head injury within the University of Pittsburgh Medical Center, aged 16–79. Mortality was examined acutely (0-7 days post-injury) and post-acutely (8-365 days post-injury). A gene risk score (GRS) was

developed to examine both *BDNF* loci. Cox proportional hazards models were used to calculate hazard ratios for survivability post-TBI while controlling for covariates.

Results: *BDNF* GRS was significantly associated with acute mortality, regardless of age. Interestingly, subjects in the hypothesized no-risk allele group had the *lowest* survival probability. Post-acutely, *BDNF*-GRS interacted with age such that younger participants in the no-risk group had the *highest* survival probability, while older participants in the hypothesized no-risk group had the *lowest* probability of survival.

Conclusions: These data suggest complex relationships between *BDNF* and TBI mortality that interact with age to influence survival predictions beyond clinical variables alone. Evidence supporting dynamic, temporal balances of pro-survival/pro-apoptotic target receptors may explain injury and age-related gene associations.

A.1.2 Introduction

In the US, ~52,000 deaths are attributed to traumatic brain injury (TBI) yearly ("CDC - Injury - TBI - TBI in the US Report," n.d.). As TBI is a highly heterogeneous disease, it is difficult to predict immediate and long-term outcomes. Understanding factors that predict mortality post-TBI may improve treatment. With the recent focus on personalized medicine, this study utilizes a Rehabilomics (Wagner and Zitelli, 2012) approach, examining genetic factors that capture innate heterogeneity across recovery to improve mortality predictions beyond traditional prognostic factors.

The International Mission on Prognosis and Analysis of randomized Controlled Trials in TBI (IMPACT) has demonstrated high predictability of post-TBI mortality/outcome using a core model of age, injury severity (motor subscale of the Glasgow Coma Scale, GCS (Teasdale and

Jennett, 1974a)), and pupillary reactivity (Maas et al., 2013). Further studies support extending this model by adding neurological findings (evidence of midline shift or presence of subarachnoid hemorrhage (Roozenbeek et al., 2012)). However, these studies have not examined genetic factors, nor have they addressed the possibility of evolving or dynamic predictors of mortality. As influences on mortality likely change across recovery, it is important to examine mortality predictors over time.

Age is a consistent determinant of TBI survival. Older adults comprise a large segment of the population sustaining TBI with comparatively worse outcomes and higher mortality rates despite similar injury parameters (Susman et al., 2002). This phenomenon may be due to adverse age effects on secondary injury cascades. Older age leads to greater susceptibility to glutamate-mediated oxidative stress and damage (Wagner et al., 2004). In experimental models, older animals show decreased neuronal survival post-injury (Onyszchuk et al., 2008).

A ubiquitous neurotrophin in the brain, brain-derived neurotrophic factor (BDNF), may interact with age to influence TBI pathology. BDNF is important for synaptic plasticity, neurogenesis and neuronal survival (Z.-Y. Chen et al., 2004). Yet in areas like the hypothalamus, BDNF can regulate metabolism (Kernie et al., 2000; Pelleymounter et al., 1995). Evidence suggests BDNF may also affect brainstem control of cardiovascular function (Brady et al., 1999; Wang and Zhou, 2002; Wan et al., 2014). Thus, BDNF may affect autonomic regulation and reflect the current energy state (Rothman et al., 2012), suggesting multiple mechanisms through which BDNF signaling could influence TBI mortality and recovery. While BDNF may affect mortality in other populations (Halldén et al., 2013; Krabbe et al., 2009; Ritter et al., 2012), no study has examined BDNF in mortality post-TBI.

BDNF signals through the tyrosine-related kinase-B (TrkB) receptor, full length (TrkB.FL) and truncated (TrkB.T), as well as the $p75^{NTR}$ receptor, activating antagonistic signaling cascades that are dependent on receptor milieu. Studies suggest TrkB.FL/TrkB.T/$p75^{NTR}$ expression ratios may vary across the lifespan (Croll et al., 1998) and following ischemia (Gomes et al., 2012). Similarly, there are dynamic receptor expression changes following experimental TBI (Rostami et al., 2013). Thus, BDNF's role in TBI recovery may be dependent on the relative balance of these target receptors.

The *BDNF* gene has a common, functional, single nucleotide polymorphism (SNP), Val66Met (rs6265) that alters activity-dependent secretion of BDNF *in vitro* (Egan et al., 2003) and shows an age-dependent relationship in cognition (Erickson et al., 2012). Importantly, rs6265 is in linkage disequilibrium (LD) with another *BDNF* SNP, rs7124442. The rs7124442 variant reportedly affects neuronal BDNF mRNA trafficking (Orefice et al., 2013). While rs6265 (Krueger et al., 2011) and rs7124442 (Rostami et al., 2011) have been associated with TBI recovery, no studies have examined how these variants influence other aspects of TBI recovery, specifically mortality. As rs6265 can influence hypothalamus-pituitary-adrenal (HPA) axis reactivity (Alexander et al., 2010; Shalev et al., 2009) and autonomic control of heart rate (A. C. Yang et al., 2010), there may be important contributions for *BDNF* variants in TBI recovery outside of cognitive outcomes.

We examined *BDNF* variation in survivorship post-TBI. We hypothesized that the Met-allele (rs6265), with its decreased activity-dependent BDNF secretion, and the C-allele (rs7124442), with its impaired BDNF mRNA trafficking, would be risk alleles in mortality predictions. We chose to examine a cumulative gene risk score (GRS) incorporating variation at both loci when developing mortality prediction models. In this report, we demonstrate a dynamic,

temporal relationship between *BDNF* GRS and post-TBI survivorship. Our data show a *BDNF* gene risk relationship with survivorship 0-7d post-injury that is contrary to hypothesized relationships. Yet, when evaluating mortality models 8-365d post-injury, we show a *BDNF* age*GRS interaction in survivorship. These findings underscore the importance of understanding age and BDNF signaling relationships following TBI.

Demographics

This prospective cohort study was approved by the University of Pittsburgh Institutional Review Board. Enrollment criteria for this study included age, ≥16 and <75 years, and an admission GCS score ≤8 indicating severe TBI. Exclusion criteria included documented prolonged hypoxia prior to admission or a history of seizures. Subjects were consecutively recruited and consent obtained from a next of kin. To minimize genetic stratification effects,(Freedman et al., 2004) associations are reported in Caucasians only (n=284, reported findings). Important for generalizability of findings, reported analyses in Caucasians-only were similar to results in the total population (n=315, data not shown).

The analyzed cohort (284 participants) was aged 16–74 yrs. (mean: 35.96±15.46; median=33) with closed head injury receiving care within the University of Pittsburgh Medical Center (UPMC). GCS scores (Teasdale and Jennett, 1974a) ranged from 3-15 (mean GCS: 6.20±2.54; median=6) when using the best GCS obtained within 24 hours post-injury. Demographic information (age, race, sex, and mechanism of injury) was collected through clinical chart review and subject/caregiver interviews. Age was treated as a continuous and categorical variable, split at the 75% quartile (Q3) of our population (above and below, Q3=45 yrs). By 365d post-injury, 25.8% of subjects had died and distribution of Glasgow Outcome Scale (GOS)(Wilson et al., 1998) scores for survivors was as follows: 2, n=13; 3, n=66; 4, n=49; 5, n=22.

Table 1. Complications categories.

Category	Complications Listed in Trauma Registry
Pulmonary	Acute Respiratory Distress Syndrome (ARDS) Acute Respiratory Failure Aspiration/ Pneumonia Atelectasis Pleural Effusion Pneumonia Pulmonary Embolus Bronchial Mainstem Intubation Acute Sinusitis Empyema
Infection	CNS Infection Sepsis/Septicemia
Cardiovascular	Acute Arterial Occlusion Cardiopulmonary Arrest (not cause of death) Major Arrhythmia Myocardial Infarction
Musculoskeletal	Extremity Compartment Syndrome
Hematological	Coagulopathy Post-Operative Hemorrhage
Renal	Acute Renal Failure Renal Failure Urinary Tract Infection
Wounds	Wound Infection Decubitis Ulcer Wound Dehiscence
Gastrointestinal	C Difficile Colitis Esophageal Intubation GI Bleed Bowel Obstruction Pancreatitis Small Bowel Obstruction
Neurological	CNS Infection Diabetes Insipidus Neuro-sequelae Progression of Neurologic Insult Seizures

The University of Pittsburgh Trauma Registry provided abstracted information from the acute care medical record regarding post-injury complications. These complications were categorized as: pulmonary, infection, cardiovascular, musculoskeletal, hematological, renal, wound, gastrointestinal, and neurological complications (**Table 1)**.

Neurological injury assessments were abstracted from admission head CT reports and were categorized by the following injury subtypes: contusion, subdural hematoma, subarachnoid hemorrhage (SAH), intraventricular hemorrhage (IVH), intracranial hemorrhage (ICH), epidural hematoma (EDH), and diffuse axonal injury (DAI). A neurological burden score (NBS) was calculated by summing injury types that significantly impacted survival for a given mortality group. NBS was only utilized in the acute mortality analysis and consisted of SDH, EDH, and contusion. For the post-acute mortality group, only the ICH category survived correction for age and GCS in Cox model predictions of survivorship.

Seizure information was abstracted from available medical records and coded as time to first post-traumatic seizure (PTS), up to 365d post-injury. Time to first seizure was divided into two groups, consistent with mortality cohorts and standard PTS nomenclature: *acute* (0-7d) and *post-acute* PTS (8-365d) (Temkin et al., 1990). Notation in medical records referring to convulsions, seizures, status epilepticus, or seizure disorder was documented as a PTS episode.

Mortality

Time until death was recorded in days post-injury, up to 1 year post-injury, using the Social Security Death Index.("Social Security Death Index," n.d.) Mortality was evaluated over two time-epochs, 0-7d post-injury (*acute*) and 8d-365d post-injury (*post-acute*). For 0-7d, survivorship was right censored at 7d post-injury. For 8-365d, subjects were only included if they survived greater than 7d, and survivorship was right censored at 365d. For logistic regression and receiver operating curve (ROC) analysis, mortality was examined as a binary outcome at 7d post-injury (acute) and 365d post-injury (post-acute, excluding subjects who died before 7d). Acutely, 11.62% of subjects died (time until death: median=3d, min=0d, max=7d, Q1=2d, Q3=6d). An additional

14.16% died post-acutely (time until death: median=19d, min=8d, max=301d, Q1=11.5d, Q3=31d).

Genotyping and SNP Selection

DNA was isolated from blood using a simple salting out procedure(S.A. Miller et al., 1988) or from cerebrospinal fluid using the Qiamp protocol from Qiagen. *BDNF* rs6269 and rs7124442 were genotyped by TaqMan allele discrimination assay using Assay on Demand reagents (Applied Biosystems). This assay utilized fluorescent labeled probes to detect allele(s) present for DNA sample. All allele frequencies were in Hardy-Weinberg equilibrium.

Selected SNPs (rs6265, rs7124442) have been reported as functional. Both SNPs have a minor allele frequency >20%. Each SNP represents a different haplotype block of *BDNF* covering variation corresponding to isoform a. A cumulative *BDNF* GRS was developed using rs6265 Met (Val/Met or Met/Met) and rs7124442 C (T/C or C/C) carrier status as hypothesized risk alleles based on the literature (Egan et al., 2003; Orefice et al., 2013). Thus, a GRS of 0 was the hypothesized no risk group (Val/Val, T/T); a GRS of 1 included carriers for 1 risk allele (Val/Val, C-carriers or Met-carriers, T/T); and a GRS of 2 included carriers of both risk alleles (Met-carriers, C-carriers).

Statistical Analysis

Analysis was conducted using Statistical Analysis Software (version 9.2; SAS Institute) and the Statistical Package for Social Sciences (version 21.0; SPSS). Descriptive analysis included mean$\pm$standard deviation (STD) for continuous variables. Frequencies were calculated for categorical variables. Genetic analysis utilized categorizations based on allele carrier status, and the *BDNF* GRS was used to examine cumulative genetic risk associations with mortality. Demographic and clinical information was compared with mortality status and genotype using

Student's t-tests and ANOVA (Mann-Whitney or Kruskal-Wallis where appropriate) to compare means, and Chi-Square or Fisher's Exact test to compare frequencies.

Demographic, clinical, and genotype information was examined for survivorship associations using either a Kaplan-Meier or Cox proportional hazards model (Cox, 1972). The Log-rank test was used to determine significant differences between two survival curves (significant if $p<0.05$). To control for covariates, we used multivariate Cox proportional hazards regression. Demographic and clinical variables remained in the final Cox models if they survived correction for age and GCS ($p\leq0.2$). The proportionality of hazards assumption was tested and confirmed for relevant variables.

Mortality status was examined using multivariate receiver operating curve (ROC) analysis to quantify model prediction capacity and to relate to published studies (Roozenbeek et al., 2012). Mortality was assessed at 7d and 365d post-injury. Using area under the curve (AUC), ROCs estimated incremental increases in model sensitivity and specificity gained when including GRS and/or GRS interactions, compared to base models of relevant clinical variables. Base models were compared to final models using a chi-square test for significant differences in AUC ($p<0.05$ considered significant).

A.1.3 Results

Genetic Associations with Demographics

Table 2. Demographic variables by genotypes.

	Rs6265	***Rs7124442***

	Val/Val (n=170)	Val/Met, Met/Met (n=114)	p value	C/C, C/T (n=126)	T/T (n=158)	p value
Age	35.55±15.48	36.57±15.46	0.962	33.38±14.02	38.01±16.25	***0.011***
Gender (% Male)	134 (78.8%)	96 (84.2%)	0.283	102 (80.9%)	128 (81.0%)	0.989
Mechanism of Injury			0.779			0.776
Automobile/ Motorcycle	128 (75.3%)	85 (75.2%)		97 (77.0%)	116 (73.9%)	
Fall/Jump	25 (14.7%)	19 (16.8%)		19 (15.1%)	25 (15.9%)	
Other	17 (10.0%)	9 (8.0%)		10 (7.9%)	16 (10.2%)	
GCS	6	6	0.678	6	6	0.368
Length of Hospital Stay (days)	19.72 ± 11.38	21.02 ± 12.32	0.517	19.32 ± 11.69	20.99 ± 11.80	0.306
ISS	35.17 ± 10.01	34.69 ± 8.91	0.625	35.04 ± 9.26	34.92 ± 9.83	0.925
Seizures	25 (14.8%)	15 (13.8%)	0.697	14 (11.2%)	26 (16.5%)	0.208
Complication Type (% Present)						
Pulmonary	108 (63.5%)	63 (55.3%)	**0.175**	75 (59.5%)	96 (60.8%)	0.833
Infection	32 (15.3%)	29 (25.4%)	0.471	31 (24.6%)	34 (21.5%)	0.538
Cardio-vascular	6 (3.5%)	7 (6.1%)	0.387	4 (3.2%)	9 (5.7%)	0.312
MSK	3 (1.80%)	0 (0%)	0.227	2 (1.6%)	1 (0.6%)	0.434
Heme	27 (15.9%)	12 (10.5%)	0.222	13 (10.3%)	26 (16.5%)	**0.135**
Renal	23 (13.5%)	16 (14.0%)	0.999	19 (15.1%)	20 (12.7%)	0.556
Wounds	8 (4.7%)	11 (9.6%)	**0.145**	10 (7.9%)	9 (5.7%)	0.453
GI	18 (10.6%)	9 (7.9%)	0.538	14 (11.1%)	13 (8.2%)	0.411
Neuro	29 (17.9%)	22 (19.3%)	0.639	22 (17.5%)	29 (18.4%)	0.845
Neurological Injury Type (% Present)						
SDH	107 (62.9%)	73 (64.0%)	0.900	82 (65.1%)	98 (62.0%)	0.596
DAI	51 (30.0%)	33 (28.9%)	0.895	45 (35.7%)	39 (24.7%)	***0.043***
EDH	22 (12.9%)	23 (20.2%)	**0.135**	21 (16.7%)	24 (15.2%)	0.735
Contusion	76 (44.7%)	60 (52.6%)	0.226	62 (49.2%)	74 (46.8%)	0.691
IVH	44 (25.9%)	36 (31.6%)	0.346	35 (27.8%)	45 (28.5%)	0.896
ICH	67 (39.4%)	37 (32.5%)	0.259	44 (34.9%)	60 (38.0%)	0.596
SAH	116 (68.2%)	74 (64.9%)	0.608	87 (69.0%)	103 (65.2%)	0.493

Demographic distributions were examined for both *BDNF* loci (**Table 2**). The mean age for T/T homozygotes (rs7124442) was higher versus C-carriers (38.0±16.3 versus 33.4±14.0,

p=0.011). DAI was less common among T/T homozygotes versus C-carriers (24.7% vs. 35.7%, p=0.043). However, subjects with DAI were significantly younger compared to subjects without DAI (28.7±11.9 vs. 39.0±15.8, p=0.038). There were no demographic variable differences by rs6265 Met-carrier status.

Mortality Associations with Demographics

Demographic associations with acute/post-acute mortality and survivorship were examined **(Table 3)**. Survivors had lower mean age (all group, p<0.001) and higher GCS scores (all group, p=0.002) compared to non-survivors. In Cox proportional hazards regression, age and GCS predicted survival probability for both 0-7d and 8-365d models (p<0.03, all comparisons). Pulmonary (adjusted for age, GCS: p<0.001, HR=0.156) and cardiac (adjusted for age, GCS: p=0.196, HR=2.065) complications survived correction for age and GCS (p<0.2) and were added to the overall models for acute survival. Pulmonary complications were less frequent in subjects who died acutely (**Table 3**), but subjects with pulmonary complications were significantly younger than those without pulmonary complications (34.1±15.1 versus 38.8±15.6, p=0.009). Cardiac complications were more frequent in subjects who died acutely compared to survivors (12.1% vs. 2.8%, all group, p=0.026). For the 8-365d model, a wound complication (adjusted for age, GCS: p=0.020, HR=2.857) was the only complication to significantly predict survival probability.

While there were no differences in frequency of radiological findings between acute and post-acute mortality groups, there were significant associations with survivorship. For the 0-7d model, the NBS (including SAH, epidural hematoma, and contusion; range of 0-3), tended to predict survivorship (corrected for age, GCS: p=0.046, HR=1.379). SAH and contusions were more frequent in subjects who died (0-7d or 8-365d) versus survivors (**Table 3**). For the 8-365d

model, those with ICH tended to have higher survival frequencies (age and GCS corrected, p=0.079, HR=0.541).

Table 3: Demographic information across mortality groups (acute, post-acute, and survivor).(Kruskal-Wallis, Fischer's Exact, significance at p<0.05)

Variable	Acute Mortality (0-7d, n=33)	Post-Acute Mortality (8-365d, n=40)	Survivors (at 365d, n=211)	All Groups (*p* value)
Age, years (mean±STD)	42.69±17.30	47.5±16.39	32.72±13.54	***<0.001***
Gender (n, % Male)	23 (69.7%)	31 (77.5%)	176 (83.4%)	0.141
Mechanism of Injury (n, %)				***0.004***
Automobile/Motorcycle	17 (51.5%)	27 (67.5%)	169 (80.5%)	
Fall/Jump	13 (39.4%)	9 (22.5%)	22 (10.5%)	
Other	3 (9.1%)	4 (10.0%)	19 (9.0%)	
GCS, median	5	6	6	***0.002***
ISS, mean ± STD	35.61±10.86	36.58 ± 9.89	34.57±9.29	0.589
Length of Hospital Stay, days, mean±STD	3.82±2.42	18.62±8.63	23.24±10.92	***<0.001***
Seizures	1 (3.0%)	4 (10.0%)	35 (16.5%)	**0.081**
Complication Type (% Present)				
Pulmonary	8 (24.2%)	28 (70.0%)	135 (64.0%)	***<0.001***
Infection	4 (12.1%)	10 (25.0%)	51 (24.2%)	0.293
Cardiovascular	4 (12.1%)	3 (7.5%)	6 (2.8%)	***0.026***
Heme	4 (12.1%)	4 (10.0%)	31 (14.7%)	0.844
Renal	3 (9.1%)	6 (15.0%)	30 (14.2%)	0.746
Wounds	0 (0%)	6 (15.0%)	13 (6.2%)	***0.037***
GI	0 (0%)	3 (7.5%)	24 (11.4%)	**0.093**
Neuro	6 (18.2%)	10 (25.0%)	35 (16.6%)	0.440
Neurological Injury Type (% Present)				
SDH	23 (69.7%)	28 (70.0%)	129 (61.1%)	0.254
DAI	5 (15.2%)	7 (17.5%)	72 (34.1%)	**0.074**
EDH	2 (6.1%)	6 (15.0%)	37 (17.5%)	0.291
Contusion	21 (63.6%)	24 (60.0%)	91 (43.1%)	***0.004***
IVH	7 (21.2%)	14 (35.0%)	59 (28.0%)	0.338
ICH	12 (36.4%)	14 (35.0%)	78 (37.5%)	0.947
SAH	29 (87.9%)	30 (75.0%)	131 (62.1%)	***0.005***

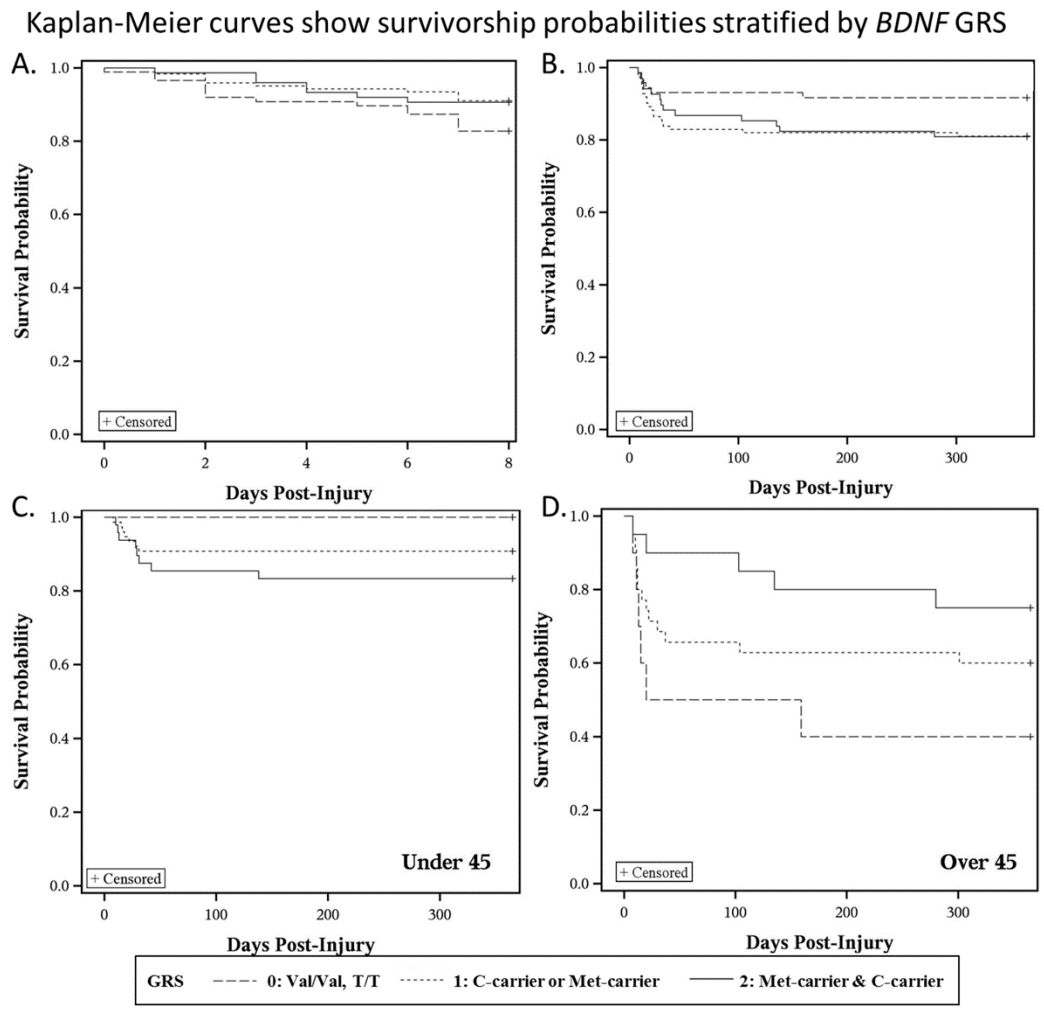


Figure 1. Kaplan-Meier curves show survivorship probabilities stratified by *BDNF* GRS.

Kaplan-Meier curves stratified by *BDNF* GRS at (**A**) 0-7d (GRS, p=0.144) and **(B)** 8-365d (GRS, p=0.134). GRS was significant in the Cox model for acute mortality, correcting for covariates. The post-acute (8-365d) is further examined in age cohorts split at age 45, **(C)** <45 (GRS, p=0.006) and **(D)** ≥45 (GRS, p=0.106). The GRS*age interaction was significant in the Cox model for post-acute mortality, correcting for covariates (Gene risk score, GRS: GRS=0, Val/Val, T/T; GRS=1, Val/Val, C-carriers or Met-carriers, T/T; GRS=2, Met-carriers, C-carriers).

Genetic Associations with Mortality

Kaplan-Meier curves reflecting mortality 0-7d post-injury tended to have different survivorship probabilities based on *BDNF* GRS (p=0.144, **Figure 1A**). Those with a GRS=0 hypothesized risk alleles had the *lowest* probability of survival. Multivariate Cox regression predicting survivorship 0-7d post-injury showed *BDNF* GRS became significant after adjusting for age, GCS, NBS, pulmonary complications, and cardiac complications (**Table 4A**). Those with a GRS of 1 or 2 had a higher probability of survival compared to those with a GRS of 0 (GRS=1, *p*=0.0107, HR=0.351, 95% CI: 0.157-0.784; GRS=2, *p*=0.0286, HR=0.349, 95% CI: 0.136-0.895). For 8-365d post-injury, there was a trend for different survivorship probabilities based on *BDNF* GRS (*p*=0.134, **Figure 1B**), where those with a GRS=0 had the *highest* survival probability. In this case, multivariate Cox regression demonstrated that this trend for *BDNF* GRS did not survive covariate adjustment (**Table 4B**).

Next, age*GRS interactions with survival probability were examined. There was no significant age*GRS interaction in 0-7d survivorship probability (data not shown). However, **Table 4C** shows a significant age*GRS interaction for 8-365d survivorship (Age*GRS interaction, *p*=0.0003, HR=0.933, 95% CI: 0.898-0.968). To increase the interpretability of this interaction, we tested the relationship of GRS with survivorship prediction in 8-365d post-injury across different age cut-points. The population was stratified, below and above Q3 (age=45), and GRS was examined in Kaplan-Meier curves for the two age strata. Among participants <45 years, GRS associations with survivorship demonstrated that those with a GRS=0 had the highest probability of survival (p=0.006), while participants >45 with a GRS=0 had the lowest survivorship probability (p=0.106) **(Figure 1C-D)**.

Table 4: Cox Model of BDNF Gene Risk Score (GRS

Variable	Parameter Estimate	Standard Error	Chi-Square	*p* value	Hazard Ratio	95% Hazard Ratio CI[#]
A. ***BDNF GRS predicting time until death, 0-7d post-injury (n=279, with 33 events)***						
Age	0.02419	0.01118	4.6814	*0.0305*	1.024	(1.002 - 1.047)
GCS*	-0.35982	0.08939	16.2041	*<0.0001*	0.698	(0.586-0.831)
NBS¥	0.47135	0.23476	4.0312	*0.0447*	1.602	(1.011-2.538)
Pulmonary Complication	-2.16656	0.43392	24.9300	*<0.0001*	0.115	(0.049-0.268)
Cardiac Complication	1.69773	0.59712	8.0839	*0.0045*	5.462	(1.695-17.603)
GRS (1 risk SNP)**	-1.04771	0.41069	6.5081	*0.0107*	0.351	(0.157-0.784)
GRS (2 risk SNP)**	-1.05395	0.48134	4.7944	*0.0286*	0.349	(0.136-0.895)
B. ***BDNF GRS predicting time until death, 8-365d post-injury (n=246, with 39 events)***						
Age	0.07138	0.01113	41.1539	*<0.0001*	1.074	(1.051-1.098)
GCS*	-0.29592	0.07565	15.3018	*<0.0001*	0.744	(0.641-0.863)
Intracranial Hemorrhage	-0.65714	0.35339	3.4578	0.0630	0.518	(0.259-1.036)
Wound Complication	1.08121	0.45695	5.5986	*0.0180*	2.948	(1.204-7.220)
GRS (1 risk SNP)**	0.35669	0.48177	0.5482	0.4591	1.429	(0.556-3.673)
GRS (2 risk SNP)**	0.36536	0.51130	0.5106	0.4749	1.441	(0.529-3.925)
C. ***BDNF GRS x Age interaction predicting time until death, 8-365d post-injury (n=246, with 39 events)***						
Age	0.13921	0.02388	33.9730	*<0.0001*	1.149	(1.149-1.204)
GCS*	-0.27425	0.07230	14.3896	*0.0001*	0.760	(0.660-0.876)
Intracranial Hemorrhage	-0.53117	0.35590	2.2274	0.1356	0.588	(0.293-1.181)
Wound Complication	1.22736	0.46444	6.9838	*0.0082*	3.412	(1.373-8.479)
GRS (1 risk SNP)**	2.90541	1.00668	8.3298	*0.0039*	18.273	(2.540-131.428)
GRS (2 risk SNP)**	5.46647	1.68218	10.5601	*0.0012*	236.62	(8.754-6396.365)
GRS x Age Interaction	-0.05889	0.01791	10.8113	*0.0010*	0.943	(0.910-0.976)

*** GCS (Glasgow Coma Scale, Best in 24 hours)**
¥ Neurological Burden Score
****GRS (0 risk SNP) was the reference category**
 # CI: Confidence Interval

We further validated these models in multivariate ROC examining post-TBI mortality status. Using a similar censorship strategy and covariate selection as with our Cox models, we

examined mortality status for acute (0-7d) and post-acute (8-365d) mortality. The 0-7d base model had an AUC=0.8412. The addition of *BDNF* GRS and a GRS*age interaction did not significantly improve this model. The 8-365d base model (excluding subjects who died in the acute phase) had an AUC=0.836. The addition of the GRS*age interaction increased the AUC above the base model (from 0.836 to 0.876, p=0.021, **Figure 2**).

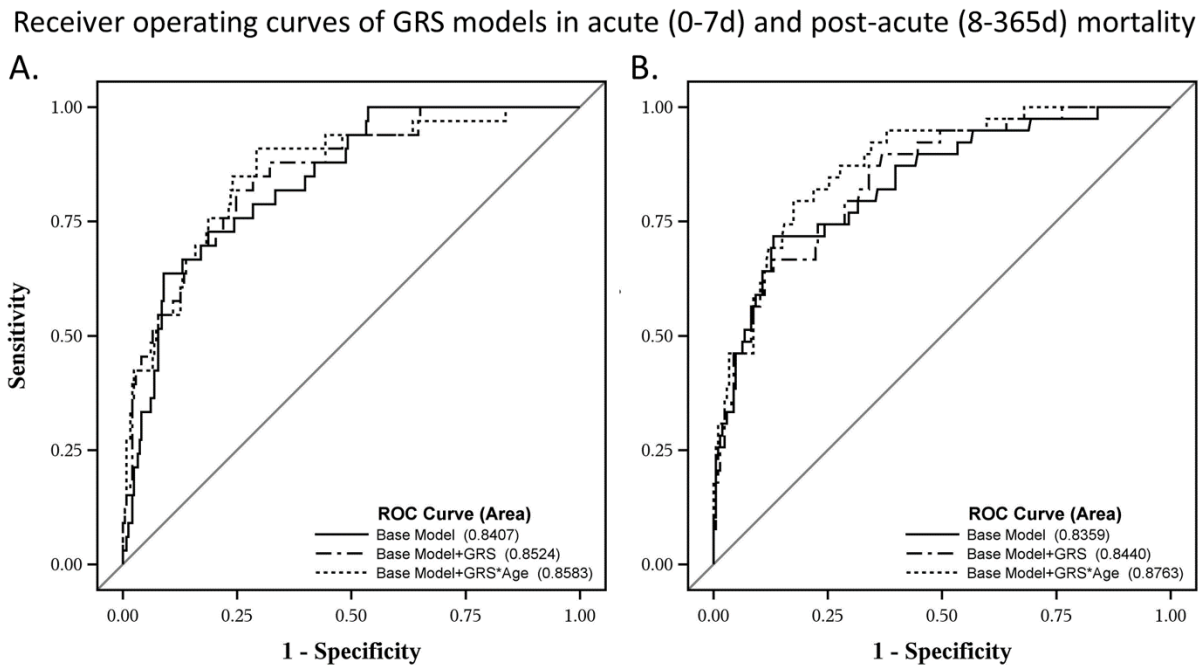


Figure 2. Receiver operating curves of GRS models in acute (0-7d) and post-acute (8-365d) mortality.

(A) Mortality at 7d. The base model of age, GCS, pulmonary complications, cardiac complications, and neurological burden score did not differ from the base model+GRS (AUC=0.8412, 0.8532; p=0.501) or base model+GRS*age (AUC=0.8412, 0.8571; p=0.664). **(B)** Mortality at 365d (excluding subjects who died <7d). The AUC of the base model of age, GCS, wound complications and ICH plus the GRS*age interaction (base model+GRS*age, AUC=0.876) was increased from the base model (AUC=0.836, p=0.021) and the base model+GRS (AUC=0.844, p=0.248). (AUC, area under the curve; GCS, Glasgow Coma Score; Gene risk score, GRS: GRS=0, Val/Val, T/T; GRS=1, Val/Val, C-carriers or Met-carriers, T/T; GRS=2, Met-carriers, C-carriers).

A.1.4 Discussion

This study demonstrates important gene and gene*age interactions with *BDNF* in post-TBI mortality. First, we identified a dynamic temporal relationship between *BDNF* and survival. Second, we showed the hypothesized *BDNF* gene risk relationship to mortality is not supported acutely (0-7d post-injury), as the hypothesized no risk group had the lowest survival probability. Third, we demonstrated *BDNF* genetics interacts with age to inform survivorship predictions 8-365d post-injury. Here, the hypothesized risk relationship was supported in younger individuals, while older individuals maintained a similar risk pattern to that observed acutely. We speculate dynamic relationships between BDNF and mortality risk post-TBI may be attributable to age and injury effects on BDNF target receptors. Other actions of BDNF in autonomic function and other body systems may also impact its role in TBI-related mortality.

These unique relationships between *BDNF* and TBI mortality may be related to specific alterations in target receptor milieu with regard to TBI and age. BDNF, first synthesized as proBDNF, is processed either in the soma, extracellular space (after release), or dendrites (after endocytosis) (Barker, 2009). If not cleaved, proBDNF targets the pro-apoptotic $p75^{NTR}$ receptor, (Barrett, 2000) while mature BDNF initiates pro-survival signaling through the full length TrkB receptor (TrkB.FL). Following experimental TBI, tissue plasminogen activator (tPA), an enzyme that cleaves proBDNF, shows increased activity ipsilateral to the injury (Sashindranath et al., 2011) and mice lacking tPA show reduced edema and cortical lesion volume (Mori et al., 2001). Additionally, there are two truncated isoforms of TrkB, TrkB.T1 and TrkB.T2, which lack intracellular tyrosine signaling, but are implicated in other pathways (Gomes et al., 2012; Vidaurre et al., 2012). In experimental TBI, there are transient increases in hippocampal TrkB.FL; this study also showed regionally-specific $p75^{NTR}$ increases up to 8 weeks post-TBI (Rostami et al., 2013).

Although controversial, the ratio of TrkB.FL/Trk.T expressed has been suggested to influence cell survival during excitotoxic injury (Gomes et al., 2012),(Vidaurre et al., 2012). Other studies report pre-incubation with BDNF prior to excitotoxic conditions/insults may be neuroprotective (Almeida et al., 2005; Lindvall et al., 1994), but this effect is likely receptor-dependent. As studies demonstrate transient increases in hippocampal BDNF transcription immediately following experimental TBI (Hicks et al., 1997; Rostami et al., 2013), followed by chronically decreased levels,[31] understanding temporal receptor expression changes may be critical in TBI.

Under the assumption that mature BDNF-TrkB.FL signaling is the primary action in adults,(Matsumoto et al., 2008) our risk allele assignment was based on the hypothesis that lower BDNF signaling would result in reduced pro-survival signaling and negatively impact survival. Thus, it would be reasonable to suggest lower BDNF signaling prior to/during an injury would exacerbate neuronal death post-TBI via reduced pro-survival signaling. However, the genetic variant hypothesized to increase BDNF activity-dependent secretion (Val/Val, rs6265) was associated with increased acute (0-7d) mortality risk. Consistent with animal literature (Rostami et al., 2013), this relationship may be the result of an injury-specific balance of BDNF's target receptors, with relative increases in TrkB.T or p75NTR compared to uninjured adults.

We show an important age*gene interaction with the *BDNF* GRS in post-acute mortality prediction. One explanation for age-specific risk profiles is differential expression patterns of target receptors across aging (Croll et al., 1998; Tapia-Arancibia et al., 2008), Romanczyk *et al* reports dynamic prefrontal cortex TrkB expression across the lifespan, with a peak in young to middle adulthood that decreases with age (Romanczyk et al., 2002). Webster *et al* reported similar TrkB expression patterns in the hippocampus and temporal lobe (Webster et al., 2006). Compared to young adult rats, aged rats have reduced TrkB.FL, but not TrkB.T,(Silhol et al., 2005) altering

receptor ratios. Post-TBI, an age-specific shift in the balance of BDNF receptor ratios, from pro-survival to pro-apoptotic, could diminish recovery. Thus, older individuals with *BDNF* genotypes associated with higher baseline BDNF signaling may have a disadvantage.

This study focuses on two variants, rs6265 and rs7124442. While the rs6265 Met-allele impairs secretion and intracellular processing of mature BDNF in hippocampal neurons,(Egan et al., 2003) there is currently no evidence that it alters proBDNF/BDNF ratios. The rs7124442 C-allele reduces BDNF mRNA trafficking from the soma to dendrites in hippocampal cultures.[20] Given that studies suggest BDNF mRNA translated in dendrites are more likely to be secreted in the proBDNF form (An et al., 2008), it is possible rs7124442 alters proBDNF/BDNF ratios. While these variants are hypothesized to affect BDNF signaling, it is unclear if this remains true with age or injury. We suggest these variants are indicative of variability in neurotrophic support post-injury, and thus, may interact with receptor expression to produce TBI-specific dynamic risk profiles.

Previous studies with the *BDNF* gene suggest it interacts with age and environment to affect cognitive function (Kaplan et al., 2010). One study showed Met-carriers, despite theoretically lower activity-dependent BDNF secretion, have greater cognitive recovery following penetrating TBI decades after injury (Krueger et al., 2011). These data are part of a growing literature demonstrating TBI-specific risk genetic relationships in TBI outcomes (Failla et al., 2013; Graham et al., 2013; Wagner et al., 2012). However, in contrast to our findings, *BDNF* variation has also been examined in stroke, where the Met-allele was linked to poor recovery, regardless of age (Siironen et al., 2007), though it is unclear if age interactions were explored in this relatively older population compared to our cohort. However, a preclinical study examining Val66Met in a rodent stroke model showed the Met-allele enhanced motor recovery chronically,

further supporting the concept of injury-specific associations for this variant.(Qin et al., 2014) Yet these studies focused on cognitive/plasticity effects of BDNF. It will be important to understand regional and temporal roles for BDNF in recovery or rehabilitation-based interventions compared to mortality risk.

In addition, BDNF can regulate energy metabolism and autonomic function. Evidence suggests that BDNF may be involved in brainstem regulation of cardiovascular function.(Brady et al., 1999; Wang and Zhou, 2002; Wan et al., 2014) Similarly, BDNF modulates the sympathetic/parasympathetic balance in cardiovascular function (Yang et al., 2002). Interestingly, rs6265 is associated with differences in heart rate variability(A. C. Yang et al., 2010) and acute stress heart rate reactivity in healthy populations (Alexander et al., 2010). In fact, one study showed that local BDNF administration following surgical sympathectomy induced hippocampal vascular changes and edema (Kasselman et al., 2006). This study suggests BDNF effects during a state of compromised autonomic function (e.g. immediately following TBI (Goldstein et al., 1998)) could impact TBI pathology, particularly at early time-points when mortality rates are highest. There is a dearth of research about BDNF function outside of cognition or plasticity post-TBI, limiting speculation about how BDNF and CNS-peripheral modulation of autonomic function post-TBI might occur. It is also not clear how age may interact with BDNF in autonomic regulation.

Our data also suggest temporally specific prognostic factors for mortality across recovery. Many TBI survival studies use a cross-sectional approach to mortality. Our results suggest there are different factors contributing to mortality predictions over time that are not captured within the current literature. Acutely, a higher NBS significantly reduced survivorship probability, consistent with published studies showing the addition of neuroradiological findings improves mortality

prediction (Roozenbeek et al., 2012). While pulmonary complications occur less frequently in survivors, subjects with pulmonary complications also were significantly younger, consistent with published studies in TBI (Rincon et al., 2012). However, pulmonary complication effects were independent of age. One consideration for this finding is the possibility that there is a delayed onset for pulmonary complications, such as acute respiratory distress syndrome (ARDS), that may be secondary to TBI-specific ICP management, and thus, may not be a large negative factor within acute mortality models (Contant et al., 2001). Also, cardiac complications were a negative predictor of survival acutely. GCS was a significant factor in mortality predictions, consistent with previous studies on the relationship of injury severity to mortality post-TBI (Roozenbeek et al., 2012).

Subjects in the post-acute survival model had a median time until death of 19d, suggesting that in this early post-acute time frame there are unique factors in mortality prediction. Early post-traumatic seizures were not related to mortality predictions in acute cohort. Yet, acute seizure occurrence negatively impacted survivorship post-acutely, consistent with epidemiological studies linking seizures to higher mortality risk after TBI (Harrison-Felix et al., 2006), suggesting the pathology associated with early seizures is also relevant to post-acute mortality. Subjects with wound complications had reduced survival probability, likely reflecting other important health/recovery factors, such as mobility, as contributing post-acute mortality risk (Zhan C and Miller MR, 2003). Wounds may also be a surrogate measure for infections or sepsis that could impact mortality post-TBI. Subjects with ICH had a higher probability of survival post-acutely compared to those without ICH. As these subjects survived acutely, they were likely monitored closely for surgical intervention, standard care that may have increased survival probability post-acutely.

This study also shows that, in our post-acute model, our GRS significantly added prognostic capacity to the base mortality model. While different cohorts, assessed over different time-frames, our AUC displays better mortality discrimination ability compared to previously published prediction models that include age, GCS, and pupil dilation only (AUC=0.787 (Roozenbeek et al., 2012)). Further, the use of Cox models strengthens our survivorship predictions. These data suggest that, while clinical variables (eg. GCS) inform outcome, genetic factors can influence mortality predictions beyond what standard clinical variables are able to accomplish, particularly for the post-acute period when most primary neurological injury effects on mortality outcomes have already occurred. While novel and promising prognostic models, these findings need validation in independent studies with larger populations.

There are some limitations in interpretation and generalizability to consider. While BDNF is primarily expressed in the brain,[33] BDNF is also synthesized and secreted from vascular endothelial cells and may have a peripheral action (see review, Caporali *et al* (Caporali and Emanueli, 2009)). Also, there is a substantial peripheral store of BDNF in platelets,[51] yet it is unclear if platelet release is altered in TBI. One study suggests plasma BDNF levels predict mortality in ICU patients without direct brain injury, but the mechanism of this association is unclear(Ritter et al., 2012). Plasma BDNF levels were also related to all-cause mortality in a cohort of older women (Krabbe et al., 2009). With altered metabolic homeostasis immediately following TBI, there may be a vascular action of BDNF that could influence mortality.

There are some additional considerations in this study as it uses a candidate gene approach. The study findings are specific to a racially homogenous population, though models incorporating our small population with other racial backgrounds were stable with similar results as reported (data not shown). Future studies should evaluate *BDNF* risk in populations with diverse racial

backgrounds to increase generalizability to the TBI population. The variants studied also had relatively low frequency for some genotypes, warranting a carrier approach, similar to published Val66Met studies (Krueger et al., 2011; Shalev et al., 2009). Similarly, these variants only cover the most well-studied isoform of BDNF, yet emerging research indicates that there are multiple isoforms for BDNF (Pruunsild et al., 2007). Future research may require additional genotyping to assess the relevance of these isoforms on TBI pathology and outcome prognostication.

Future studies may examine how these findings relate to other genetic variants such as apolipoprotein E(*APOE*) that have shown associations to TBI mortality.(Teasdale et al., 2005) It was also difficult to examine genetic relationships to injury type given the severity of injury in this cohort, as many subjects showed multiple injury sub-types. As we do not have specific cause of deaths for this study, future studies may examine specific cause of death relationships to genetic risk.

Importantly, this study implicates BDNF signaling in TBI mortality prediction, supporting the need for validation studies and a better understanding of BDNF signaling post-TBI across body systems. This work supports the need for examination of specific regional TrkB.FL/TrkB.T/$p75^{NTR}$ receptor ratios in experimental TBI models and post-mortem tissue. As serum BDNF is decreased acutely in clinical TBI (Kalish and Phillips, 2010), future studies may investigate BDNF as a biomarker, with age/gene variation as possible BDNF modifiers post-TBI. Future studies focusing on the dynamic roles of BDNF in mortality compared to rehabilitation and recovery following TBI are needed and may yield different associations reflective of unique pathology and/or recovery mechanisms.

A.2 COMT VAL158MET GENOTYPE AND SEX INTERACT TO AFFECT COGNITIVE DYSFUNCTION AFTER TBI

John M. Myrga[1], **Michelle D. Failla, BS[1,2]**, Joseph H. Ricker, PhD[3], C., Yvette P. Conley, PhD[4,5], Patricia M. Arenth, PhD[1], Amy K. Wagner, MD[1,2,6]

A.2.1 Introduction

With over 1.7 million traumatic brain injuries (TBI) each year, 17-23% of which are moderate-severe, it is important to understand how these injuries impact patient outcomes("CDC - TBI in the US Report - Traumatic Brain Injury - Injury Center," n.d.). A major area of concern for individuals with TBI is cognitive dysfunction(Arciniegas, 2002). Those with moderate-severe TBI often exhibit cognitive dysfunction lasting 6 months or longer, which can significantly impact functional recovery, supervision needs, and caregiver burden of individuals post-TBI(Levin et al., 1979). Another important issue when attempting to discern prognosis for recovery and cognitive dysfunction is the variability observed in individuals who have similar injury complexes and clinical care factors. Variation in personal biology as it relates to overall recovery, biosusceptibility to complications, and individual prognosis is a relatively contemporary concept in TBI research, and this variability can be examined through a Rehabilomics framework to determine the role of biomarkers, as surrogate markers of personal biology, in categorizing these diverse outcomes. A growing body of work suggests that analyzing how genetic heterogeneity affects biological systems of interest is an important application of the Rehabilomics model and may elucidate genetic contributions to cognitive dysfunction following TBI(A. K. Wagner, 2010),(Wagner and Zitelli, 2013).

One important neurotransmitter system that modulates cognition is the dopamine (DA) system. DA systems, particularly those involving the prefrontal cortex (PFC), influences executive function, memory consolidation, verbal language skills, and attention. PFC DA release is regulated primarily by the mesocortical pathway, which contains DA neurons that project from the ventral tegmental area (VTA) to the dorsolateral pre-frontal cortex (PFC) (Seamans and Robbins, 2010). Recent studies in healthy and diseased populations have suggested an important role for DA in cognitive outcomes (Seamans and Robbins, 2010). The "inverted U" hypothesis further states that too much or too little DA can negatively impact cognition via D1 receptors, particularly in the context of ADHD and other pathological conditions (Cools and D'Esposito, 2011b). Mounting evidence within animal models of TBI suggests that functional hypodopaminergia can occur after TBI (James W. Bales et al., 2009) and we hypothesize that DA dysfunction post-injury may be further affected by genetic variation. Currently published work implicates genetic variation within the DA transporter (DAT) as influencing both acute (Shimada et al., 2014b), (Hong Qu Yan et al., 2002), (Amy K. Wagner et al., 2007b) and chronic DA relevant pathology (Amy K. Wagner et al., 2014). In addition, genetic variation within the ANKK1 Taq1a polymorphism has been implicated as affecting both striatal DA physiology and cognition during the first year after moderate to severe TBI(Failla et al., n.d.)

In TBI-specific studies, Bales et al summarizes clinical and experimental data showing efficacy with DA treatments in addressing cognitive dysfunction and in further supporting a DA hypothesis for providing clinical treatments that address cognitive dysfunction (James W. Bales et al., 2009). This knowledge has translated to clinical care, with multiple DA agonists considered as potentially effective agents in treating cognitive dysfunction after TBI (Neurobehavioral Guidelines Working Group et al., 2006). However, it is unknown which patients would most

benefit from such treatments; additional knowledge about DA system genetic heterogeneity and its effects on endogenous DA function may further refine our understanding of which individuals might benefit the most from specific DA therapies.

In addition to controlled DA release from VTA projection neurons, the brain has other mechanisms that modulate DA levels. Most brain regions use DAT as the primary method of DA removal from the synapse (Ciliax et al., 1995). In the PFC, DAT expression is lower than structures like the striatum (Sesack et al., 1998) so DA clearance is primarily mediated by the enzyme catechol-o-methyltransferase (COMT) encoded by the *COMT* gene(Jingshan Chen et al., 2004). COMT inactivates catecholamines through the addition of a methyl group. Within *COMT*, there exists a well-characterized functional single-nucleotide polymorphism, which causes a substitution from valine to methionine at position 158. Studies have shown that Val158Met (rs4680) substitution leads to instability of the enzyme and up to a 40% decrease in enzyme activity, resulting in higher levels of DA available at PFC synapses for those carrying either one or two Met alleles (Männistö and Kaakkola, 1999). In addition to the importance of COMT to PFC DA levels, studies have suggested COMT may play a role in altering tonic DA levels that impact subcortical regions of the brain such as the hippocampus and striatum (Robert M Bilder et al., 2004).

In healthy populations, the Val158Met variation can modulate behavioral and cognitive functioning (Malhotra et al., 2002). Val-carriers have increased enzyme activity, leading to lower DA levels, and often perform worse on memory tasks (Egan et al., 2001). However, Val-carriers have greater levels of cognitive flexibility and less anxiety (Bellander et al., 2014). The Met allele, with greater synaptic DA levels, has been associated with greater baseline cognitive scores on memory but lower levels of cognitive flexibility (Mattay et al., 2003). Few studies have studied the relationship between Val158Met and cognition in a TBI population. Lipsky et al showed that

Met/Met homozygotes performed better than Val/Val homozygotes on perseverative responses in a 113 participant, predominantly male military population (Lipsky et al., 2005b). However, Willmott and colleagues did not find significant differences between genotype status and various cognitive outcomes measures in a 223 participant study that included women and men evaluated at an early time point following TBI (Willmott et al., 2014b).

Since studies have indicated a sex based dimorphism within the DA system (Munro et al., 2006), (Becker, 1999), recent work has considered the possibility of a Val158Met interaction with sex affecting outcomes. Significant sex and Val158Met genotype interactions have been demonstrated when investigating sensation seeking personality traits(Lang et al., 2007a) and risk-taking propensity in youth (Amstadter et al., 2012). Various studies have also shown the potential of a DAergic gene – sex interaction in cognition in both healthy and diseased populations (Harrison and Tunbridge, 2007), (Soeiro-De-Souza et al., 2013), (Amy K. Wagner et al., 2007a). Jacobs and Esposito have further reported that Val158Met status has an important role in understanding the observed impact of estradiol on cognition(Jacobs and D'Esposito, 2011). We investigated a potential gene x sex interaction in a TBI specific population to further elucidate the role of the Val158Met mutation on multiple domains of cognitive performance as well as functional cognitive impairment.

A.2.2 Methods

Participants

106 participants were recruited from both inpatient and outpatient clinics located at the University of Pittsburgh Medical Center (UPMC) as part of a larger genetic TBI study approved by the University of Pittsburgh Institutional Review Board. Enrollment criteria for this study

included a non-penetrating TBI, admission Glasgow Coma Scale (GCS)≤8 indicating severe TBI, a CT scan with evidence of intracranial injury, and age ≥16 and <75 years. Subjects with evidence of extended hypoxia prior to admission were excluded. Other studies have suggested racial differences in rs4680 allelic frequencies(Cross et al., 2010). Therefore, this study was restricted to Caucasians only (n=98). Of note, findings in Caucasian-only analyses were similar to results in the entire population (n=106).

For these 98 participants, demographic information was obtained from medical record review, and/or participant/caregiver interview. The best GCS within 24 hrs post-injury was used for analysis, as this injury severity index has greater accuracy when considering cognitive outcomes (Udekwu et al., 2004b)(Cifu et al., 1997b). At both 6 and 12-month post-injury, trained staff members administered a battery of neuropsychological tests. 94 participants had sufficient neuropsychological data recorded at the 6-month time point, and 64 participants at the 12-month time point had sufficient neuropsychological data to be included in this analysis.

Sample Collection and Genotyping

All subjects were genotyped for the Val158Met, a missense mutation in the COMT gene (rs4680). DNA was obtained from whole blood, using a simple salting out procedure(S. A. Miller et al., 1988). Genotyping was completed using TaqMan allele discrimination technology and available 5′ exonuclease Assay-on-Demand TaqMan assays (Applied Biosystems).

Cognitive Outcome Measures

A brief overview of the cognitive composite score methods are outlined here but for a more thorough explanation, refer to Failla et al(Failla et al., n.d.) for more detailed description about creating the composite score. The cognitive composite score for this population was formulated using eight neuropsychological tests organized into four domain-specific groupings. The *verbal*

fluency domain includes the Delis-Kaplan Executive Function Systems (DKEFS) Verbal Fluency section(Delis et al., 2001) and the Controlled Oral Word Association (COWA)(John Borkowski et al., 1967), both designed to target verbal fluency. The *attention domain* includes Trail Making Test A(Ralph Reitan and Wolfson, 1985) and the Digit span subsection from the Wechsler Adult Intelligence Scale-R(G. J. Larrabee and Curtiss, 1995) designed to focus on attention deficits. The memory domain includes The California Verbal Learning Test-Long Delay Free Recall section(Delis, 2000) and the Rey-Osterrieth Complex Figure Test(P Osterrieth, 1944), and the executive function domain used the Trail Making Test B(Ralph Reitan and Wolfson, 1985) and Stroop Interference Score(J. Stroop, 1935).

Raw scores for these tests were converted into T-scores using provider recommended normative data; correcting for age, race, sex, and education level when indicated. To obtain a domain score, participants needed to complete at least one of the two tests for each domain. If the results from both tests were present, the individual T-scores were averaged to create the reported T-score. The overall composite score consisted of an equally weighted average of the four domains specified above. In order to be included into our analysis, individuals were required to have valid cognitive composite scores.

Functional cognition was evaluated by the Cognitive Function subscale of the Functional Independence Measures (FIM-Cog) at 6 and 12 months after TBI. The FIM-Cog is numerically scored from 1-7 with a greater number indicating fewer functional cognitive impairments across five questions involving - expression, comprehension, social interaction, problem solving, and memory.

Depression

Self-reported depressive symptoms were obtained from participants at 6 and 12 months post-injury using the Patient Health Questionnaire-9 (PHQ9), a measurement tool that is derived from the DSM-IV criteria for Major Depressive Disorder (MDD). This instrument requires participants to rate their depressive symptoms over a two-week period on a scale from 0-3, with 3 indicating the more severe or frequent appearance of symptoms. Categorization as depressed required the self-selection of a minimum of five symptoms, with at least one of which was cardinal symptom (i.e anhedonia, or depressive mood). This metric has been successfully used previously to identify and assess depressive symptoms in TBI populations(Cook et al., 2011b)·(Fann et al., 2009b).

Statistical Analysis

Statistical analyses were completed using SPSS (Version 22). Mean, median, standard deviation, and standard error of the mean were calculated when appropriate for GCS, age, and education levels. Categorical variables, such as MDD and sex, are presented as frequencies. Comparison of individual neuropsychological tests and demographic variables were grouped by variable and compared using Mann-Whitney, ANOVA, Kruskall-Wallis, T-Tests, or Chi-Square tests as appropriate.

Since a "dose dependent" effect has been observed in other studies, analyses were first completed comparing the Val/Val, Val/Met, and Met/Met genotypes. If warranted, carrier analysis was then performed grouping carriers with non-significant differences in post-hoc analysis and comparing them to the remaining variant of interest. To control for confounding variables and injury specific events, a multivariate model was created for each overall cognitive composite score using covariates that were either associated with COMT Met158Val genotype or cognitive composite scores or both. A backwards linear regression was conducted where any

variables with a $p>0.2$ were removed from the final model. Associated variables with a $p\leq0.05$ were considered statistically significant.

A.2.3 Results

The average age of the population was 34.37±13.80 with a range of 17-71. The best GCS within 24hr averaged 7.93±3.08 for the population, with a range of 3-15. Demographic data by genotype is shown in **Table 1**. There were no significant differences in age, sex, GCS, education, or depression status between carrier groups.

Table 1. Demographics of Caucasian only populations with composite scores at 6 & 12 month time points.

	6 Month n=94					***12 Month n=64***				
	Met/Met	n	Val Carriers	n	p value	Met/Met	n	Val Carriers	n	p value
Age	36.03 ± 15.270	29	33.42 ± 13.435	65	0.589	32.86 ± 13.752	21	33.72 ± 14.158	43	0.753
Sex	3 (10.34%)	29	13 (20.0%)	65	0.253	3 (14.28%)	21	14 (32.55%)	43	0.225
GCS	7	29	8	65	0.428	7	21	7	43	0.212
Education	12.48 ± 1.765	29	12.95 ± 1.988	65	0.593	12.95 ± 1.717	21	12.74 ± 1.787	43	0.659
Depressed	10 (37.04%)	27	23 (37.09%)	62	1.00	6 (28.6%)	21	11 (26.83%)	41	1.00

Figure 1. Genotypic breakdown of sex x genotype interaction (6 month given only as a reference). Some evidence for the possibility of a "dose dependent" effect for the Met allele. Using post-hoc analysis no difference was seen between the Val/Met and Val/Val groups. Data presented as overall composite average with SEM.

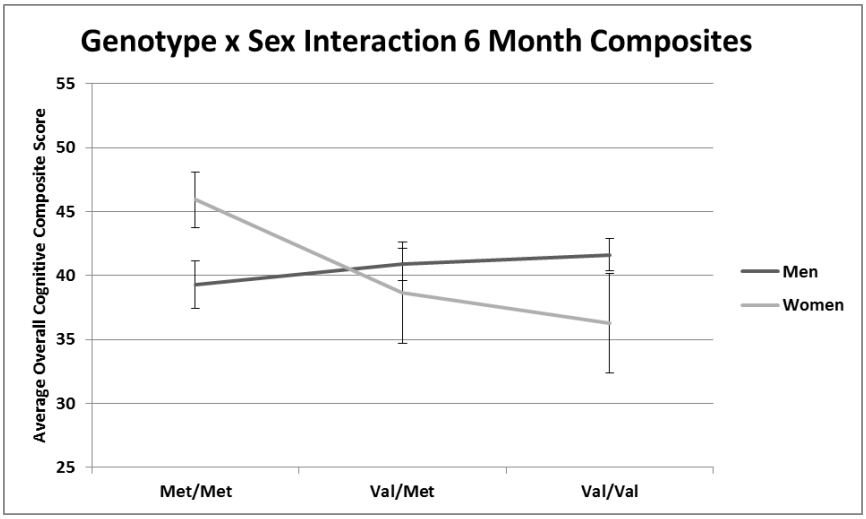


Figure 1 shows the overall composite scores by each genotype as a function of sex. There was no significant difference in cognitive performance between women who were Val-homozygotes and women Val-carriers, while significant differences were observed at both 6 and 12 months when comparing cognitive performance for Met-homozygotes to their Val/Val and Val/Met counterparts. Therefore, in further analysis, Met-homozygotes were compared to Val-carriers as consistent with the literature (Willmott et al., 2014b). PTD status (data not shown), sex, and carrier status alone had no significant impact on any individual neuropsychological test, cognitive composite domain score, or the overall composite score at 6 or 12-month time-points as shown in **Table 2**. There were no differences in overall FIM-Cog score by sex or genotype. At 6 months, PTD negatively impacted overall FIM-Cog (p=0.011).

Table 2. Bivariate analysis of gender and carrier impact on cognitive outcomes alone. A) 6 Month and B) 12 Months. Presented scores are T-scores with standard deviation.

A.

6 Month	Sex Analysis		Sex Sig	Carrier Analysis		Carrier Sig
	Female	Male		Val-Carrier	Met/Met	
Attention	35.7 ±7.80	38.2 ± 10.35	0.352	38.04 ± 9.65	37.08 ± 10.74	0.668
Trails A	31.11 ± 12.71	36.44 ± 13.23	0.134	35.43 ± 13.44	35.57 ± 12.95	0.963
Digit Span	42.30 ± 7.9622	41.87 ± 6.84	0.841	42.29 ± 6.83	41.06 ± 7.59	0.528
Memory	37.76 ± 14.46	39.21± 10.97	0.701	38.46 ± 11.48	40.05 ± 11.99	0.542
CVLT: LDFR	38.00 ± 20.12	35.00 ± 14.69	0.591	35.42 ± 15.92	35.71 ± 15.39	0.936
Rey Complex Figure	38.88 ± 12.33	42.92 ± 11.01	0.184	41.23 ± 10.77	44.34 ± 12.33	0.219
Language Fluency	38.21 ± 15.03	38.43 ± 10.20	0.954	39.05 ± 10.92	36.91 ± 11.65	0.394
COWA: Animals	31.69 ± 16.39	34.32 ± 12.09	0.510	34.00 ± 12.51	33.42 ± 14.28	0.870
DKEFS: Verbal Fluency	45.71 ± 9.98	40.73 ± 10.37	0.104	42.57 ± 9.64	39.46 ± 11.86	0.211
Executive Function	44.74 ± 7.82	46.16 ± 9.68	0.572	45.94 ± 8.53	45.83 ± 11.14	0.958
Trails Making Test B	36.47 ± 16.41	40.23 ± 13.45	0.322	39.83 ± 12.61	38.85 ± 17.07	0.762
Stroop Task Interference	53.38 ± 8.26	53.58 ± 8.10	0.928	53.67 ± 7.08	53.22 ± 10.26	0.822
Overall	39.10 ± 9.85	40.50 ± 7.65	0.520	40.37 ± 7.60	39.96 ± 9.13	0.824
FIM Cog	29.94 4.56	31.16 ± 3.79	0.308	31.084 ± 4.05	30.52 ± 3.64	0.386

B.

12 Month	Sex Analysis		SEX Sig	Carrier Analysis		Carrier Sig
	Female	Males		Val Carrier	Met/Met	
Attention	38.19 ± 11.96	39.74 ± 6.47	0.626	38.49 ± 7.70	41.12 ± 8.82	0.226
Trails A	36.47 ± 20.41	37.66 ± 11.61	0.832	36.17 ± 13.02	40.06 ± 16.57	0.336
Digit Span	40.67 ± 8.56	42.04 ± 7.04	0.536	41.30 ± 7.48	42.38 ± 7.37	0.609
Memory	40.31 ± 13.73	41.31 ± 11.15	0.771	41.02 ± 11.08	41.14 ± 13.29	0.970
CVLT: LDFR	38.71 ± 19.45	38.48 ± 16.06	0.964	39.66 ± 16.02	36.11 ± 18.39	0.449
Rey Complex Figure	41.13 ± 11.30	44.48 ± 10.12	0.272	42.19 ± 10.12	46.60 ± 10.75	0.121
Language Fluency	40.00 ± 11.41	38.02 ± 10.03	0.511	38.46 ± 10.14	38.62 ± 10.97	0.956
COWA: Animals	33.64 ± 12.31	34.15 ± 12.83	0.896	33.15 ± 11.85	35.67 ± 14.07	0.466
DKEFS: Verbal Fluency	47.00 ± 9.69	41.07 ± 10.36	*0.063*	43.20 ± 10.26	41.05 ± 10.92	0.466
Executive Function	44.88 ± 12.44	47.48 ± 8.56	0.353	46.21 ± 9.47	48.09 ± 10.06	0.466
Trails Making Test B	36.60 ± 20.76	40.89 ± 11.12	0.455	38.80 ± 13.74	42.00 ± 14.91	0.418
Stroop Task Interference	51.75 ± 7.51	54.28 ± 7.62	0.255	53.07 ± 8.28	54.8 ± 5.98	0.408
Overall	40.84 ± 10.19	41.64 ± 6.75	0.723	41.05 ± 7.36	42.24 ± 8.39	0.562
FIM Cog	30.125 ± 4.09	31.68 ± 3.31	0.127	31.38 ± 3.66	31.00 ± 3.25	0.583

We then examined sex*gene interactions with cognitive composites. As shown in **Figure 2** there was a significant gene*sex interaction in the Language Fluency domain (p=0.046) at 6

months. There were similar trends in terms of memory domain scores (p=0.075) and the overall composite (p=0.067). Met homozygote women performed significantly better than Met homozygote men at 6 months in the language fluency domain (p=0.001). At 12 months, a significant sex*carrier interaction was observed in the attention domain (p=0.003), memory domain (p=0.021), and the overall composite score (p=0.008). Met Homozygote women performed better than their male counterparts in the attention (p=0.029) and overall (p=0.025) subscales while there was a trend towards significance observed with the language fluency (p=0.081) and memory (p=0.091) domains. **Figure 3** shows the average cognitive composite T-scores for the sex*carrier interaction, with women who were Met/Met homozygotes performing ~8 points better on their t-score than their Val-Carriers counterparts and also better than both the Met/Met and Val-Carrier men at 6 months post-injury. At 12 months, women Met-Homozygotes performed ~12 points higher on their cognitive composite t-score, and these women performed even better than Val-Carrier men (p=0.045), Met-Homozygote men (p=0.025), and Val-Carrier women (p=0.008). As reported in **Figure 4,** genetic comparisons with functional cognition followed the same pattern with Met-Homozygote women performing ~2-4 points higher than Val-carrier women as well as both Val-Carrier and Met-Homozygote men. Post-hoc analysis showed a significant difference between Val-Carrier men and Val-Carrier women at both 6 (p=0.046) and 12 (p=0.027) months. There was also a trend towards significance observed between Met-Homozygote women and Val carrier women at 6 (p=0.099) and 12 (p=0.053) months.

Figure 2. Domain and overall average T-scores scores by sex*gene groups at A) 6 months and B) 12 months. Error bars presented as SEM. A star above signifies a significant sex*gene interaction in the associated domain. An ampersand signifies a significant difference between Women Met Homozygotes and Men Met Homozygotes.

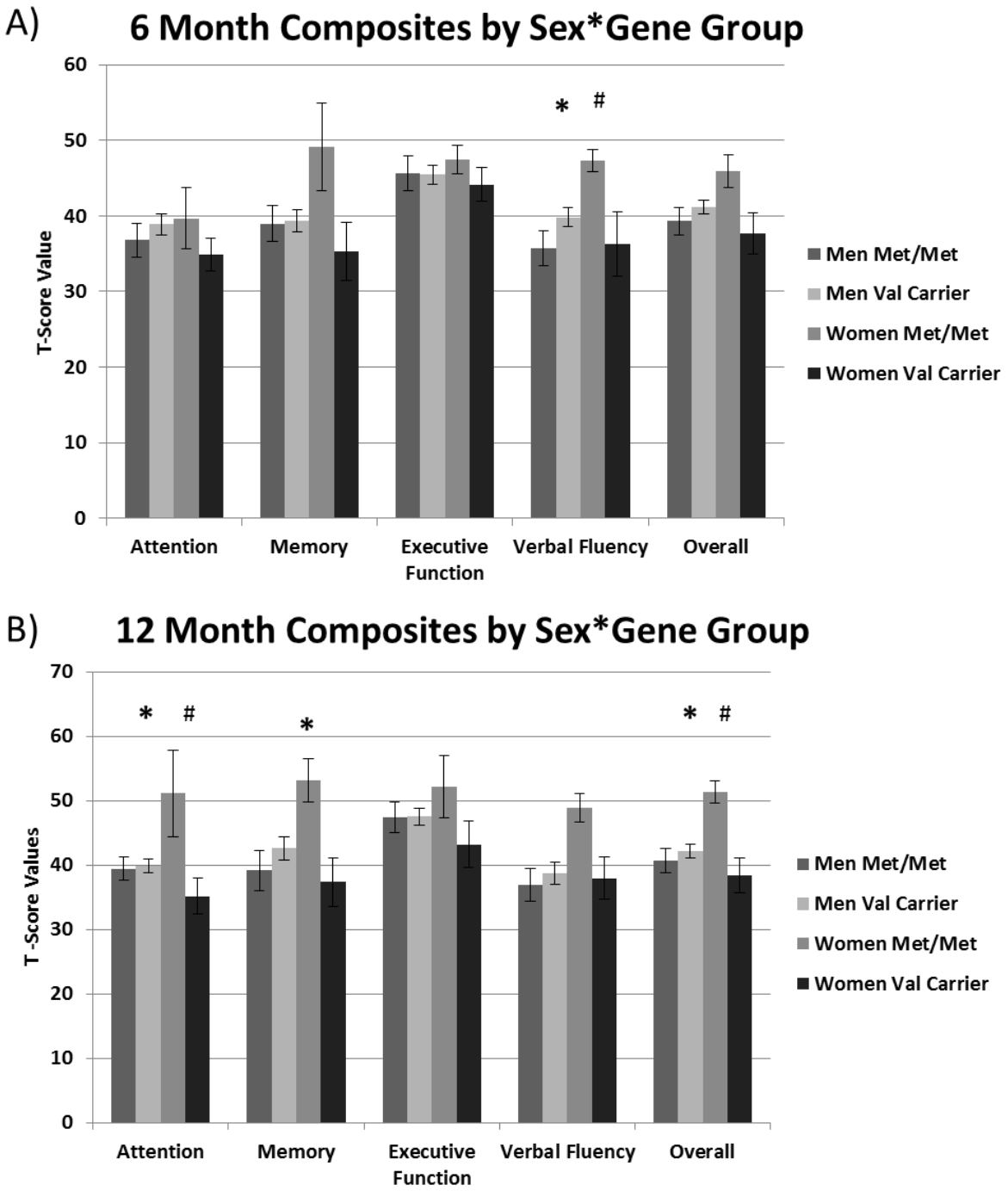


Figure 3. Overall cognitive composite T-scores A) 6 months B) 12 months. Error bars presented as SEM. A star indicates the group is significantly different from all three other groups.

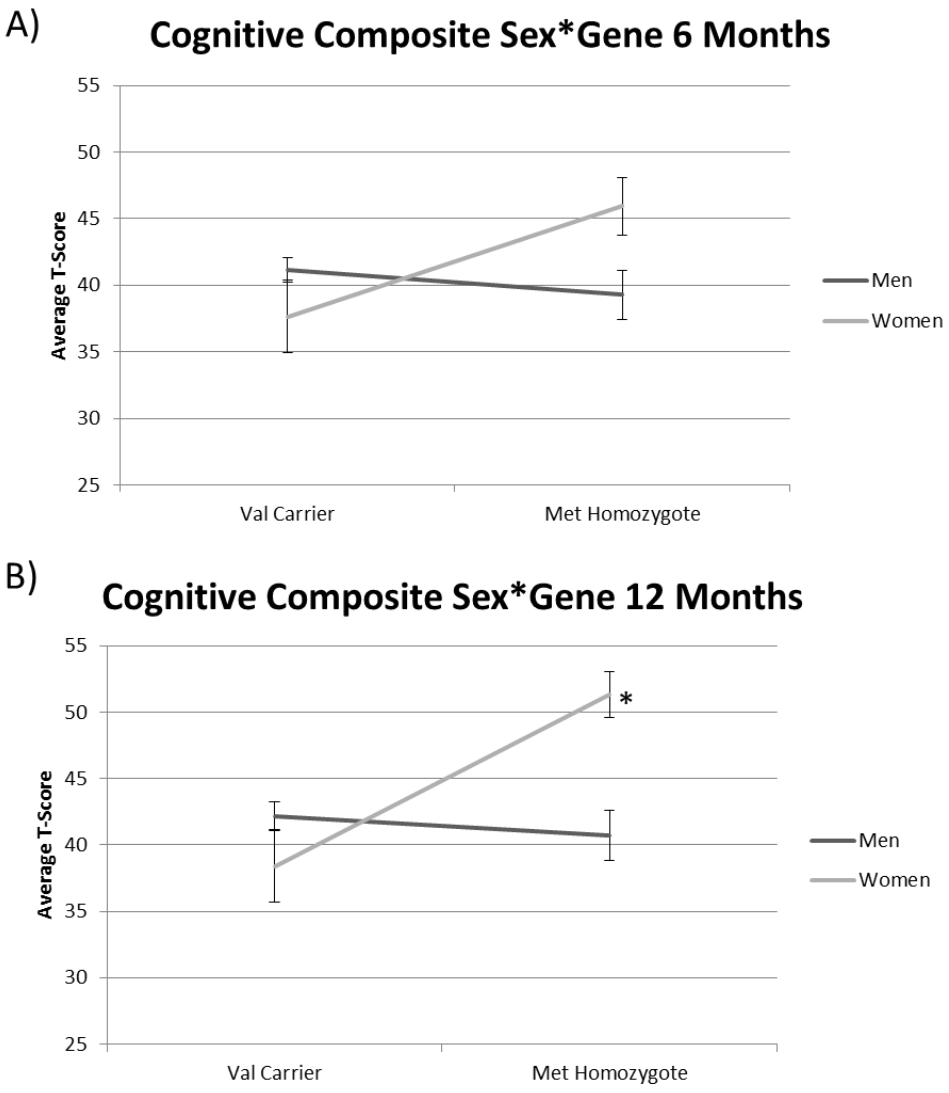
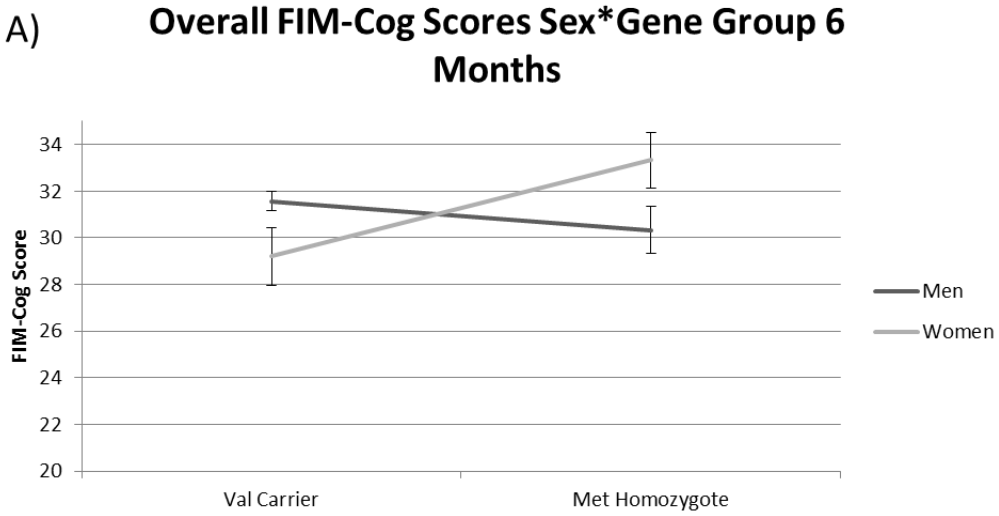
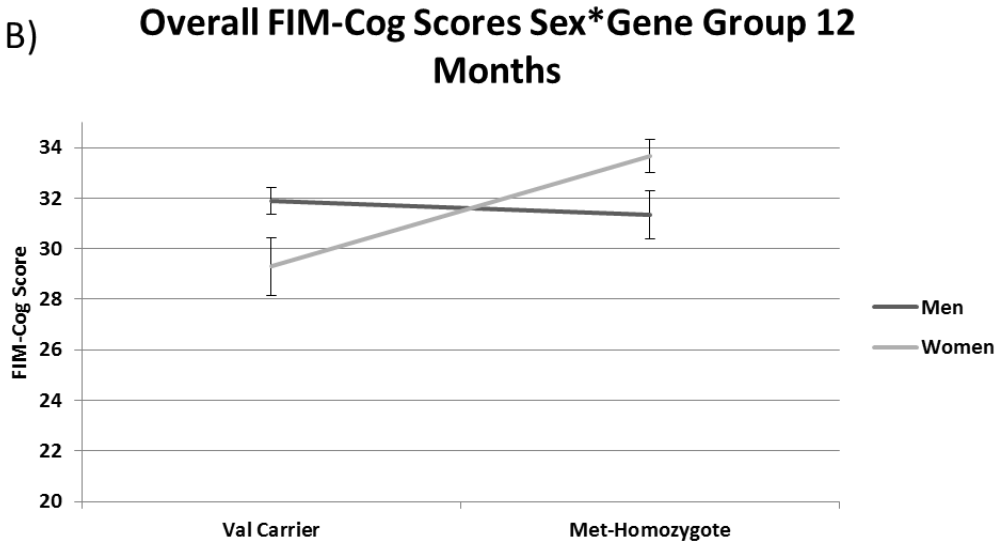


Figure 4. FIM-Cog scores by sex*gene grouping A) 6 months and B) 12 months after TBI. Data presented as average of the group with SEM.

A)



B)



To adjust for potential demographic, clinical, and injury specific factors, a backwards step-wise multivariate linear regression model was used to predict overall cognitive composite scores

at 6 and 12 months. The sex*gene interaction was significant at 6 months (p=0.024) and at 12 months (p=0.002) even after adjusting for age, education, and GCS as shown in **Table 3.** Met-Homozygote women performed significantly better than their Val-carrier counterparts as well as men who were Met-homozygotes. The addition of the sex*gene interaction term to the cognitive composite model resulted in a significant increase in incremental variance as measured by change in R-square at both 6 ($F=5.24$, $p<0.05$) and 12 ($F=10.09$, $p<0.01$) months. Using the same model, functional cognition as measured by FIM-Cog was also had a significant sex*gene interaction at 6 months (p=0.038) and tended to be significant at 12 months (p=0.058). The addition of the sex*gene interaction term to the FIM-Cog model resulted in a significant in incremental variance as measured by change in R-square at 6 ($F=4.45$, $p<0.05$) but was not significant at 12 months ($F=3.735$, $p>0.05$). While depression was added to the FIM-Cog model, it did not meet the significance threshold and was removed from the model at both 6 and 12 months.

Table 3. Parameters used to model functional cognition and overall composite scores at both 6 and 12 months.

Variable	Parameter Estimate	Standard Error	t value	p value	95% Confidence Interval
A. 6 months – Cognitive Composite, R Squared = .258 (Adjusted R Squared = .207)					
Age	-0.099	0.056	-1.763	0.081	(-0.211- 0.013)
GCS	0.924	0.266	3.48	0.001	(0.396 – 1.452)
Education	1.272	0.393	3.237	0.002	(0.491 – 2.053)
Sex	9.184	4.540	2.023	0.046	(.160– 18.208)
Rs4680	0.667	1.755	0.380	0.705	(-2.822 – 4.156)
Sex*Rs4680	-11.400	4.980	-2.289	***0.024***	(-21.297 – -1.502)
B. 12 months – Cognitive Composite, R Squared = .374 (Adjusted R Squared = .308)					
Age	-0.066	0.062	-1.073	0.288	(0.191- 1.443)
GCS	0.817	0.313	2.613	0.011	(0.396 – 1.452)
Education	1.685	0.479	3.520	0.001	(0.726 – 2.643)
Sex	11.219	4.566	2.709	0.113	(2.927– 19.511)
Rs4680	1.316	1.937	0.679	0.013	(-2.563 – 5.195)
Sex*Rs4680	-14.506	4.980	-3.177	***0.002***	(-21.297 – -1.502)
C. 6 months – Functional Cognition, R Squared = .176 (Adjusted R Squared = .120)					
Age	-.056	0.029	-1.915	0.059	(-0.113 - 0.002)
GCS	0.326	0.137	2.377	0.020	(0.053 - 0.598)
Education	0.404	0.203	1.990	0.050	(0.000 - 0.807)
Sex	3.509	2.343	1.497	0.138	(-1.149 – 8.166)
Rs4680	0.754	0.906	0.832	0.408	(-1.047 – 2.555)
Sex*Rs4680	-5.423	2.570	-2.110	***0.038***	(-10.532 - -0.315)
D. 12 months – Functional Cognition, R Squared = .325 (Adjusted R Squared = .254)					
Age	-0.096	0.030	-3.232	0.002	(-0.155 - -0.036)
GCS	0.476	0.150	3.168	0.002	(0.175 - 0.777)
Education	0.302	0.230	1.310	0.195	(-0.159 - -0.763)
Sex	2.689	1.992	1.350	0.182	(-1.299 – 6.678)
Rs4680	0.163	0.932	0.175	0.862	(-1.703 – 2.029)
Sex*Rs4680	-4.244	2.196	-1.933	***0.058***	(-8.642 - 0.154)

A.2.4 Discussion

Following a TBI, cognitive recovery is often difficult to predict, and current methodologies provide little insight into reasons for observed heterogeneity. This study investigated the functional polymorphism Val158Met in *COMT* with cognitive outcomes, aiming to identify genetic influences on cognition post-TBI. The current study is the first to establish a gene*sex interaction with *COMT* on cognitive outcomes in a severe TBI population. We show that women who were Met-homozygotes performed significantly better than women Val-carriers with both functional and cognitive performance measures. We did not observe this relationship among men. Furthermore, the addition of the sex*gene interaction term to our model resulted in significant improvements in its predictive power. Although this work requires validation in an independent cohort, the work suggests a possible role for considering sex based genetics in a clinical setting when considering pharmacological and cognitive therapies to use for individuals following TBI.

In the literature, there is evidence of a sex*Val158Met interaction on cognitive and behavioral measures, but often with inconsistent results. Some studies show significant sex based relationships between *COMT* and behavioral traits like sensation seeking (Lang et al., 2007a), risk taking (Amstadter et al., 2012), and general emotionality (C. Chen et al., 2011). When considering gene*sex interactions, the literature is quite variable without a consistent relationship between Val158Met status and outcomes (Soeiro-De-Souza et al., 2013),(O'Hara et al., 2006),(Barnett et al., 2007),(Harrison and Tunbridge, 2007). Lipsky et al showed that the Met-Homozygote genotype was associated with higher outcomes on the Wisconsin Card Sorting Test (WCST) following TBI (Lipsky et al., 2005b), while Willmott and colleagues reported no difference in measures of executive function based on Val158Met status following TBI (Willmott et al., 2014c).

However, Lipsky et al studied a primarily male population, and Willmott's neuropsychological data was taken at an acute time point and did not examine a gene*sex interaction.

COMT in an important regulator of DA, which is critical for attention, memory, and planning, and DA deficiencies have often been associated with cognitive deficits in schizophrenia and bipolar disorder (Harrison and Tunbridge, 2007)·(Egan et al., 2001)·(Seamans and Yang, 2004). One way to interpret these results is using the "inverted U" curve of the DA hypothesis observed in other conditions (Mattay et al., 2003), which suggests there is an optimal level of DA; levels above or below this optimal level lead to suboptimal outcomes via D1 receptor stimulation (Vijayraghavan et al., 2007). This point was further corroborated in control populations by pharmacological studies using methylphenidate and bromocriptine where excessive dosages led to negative cognitive outcomes and where increasing DA did not have a linear relationship with cognitive improvements (Cools and D'Esposito, 2011b). In this framework, understanding control mechanisms for basal DA levels is critical to determining their role in cognitive outcomes (Cools and D'Esposito, 2011b).

Prefrontal COMT activity and DA levels are highly dependent on Val158Met status (Tunbridge et al., 2006). Studies have shown that the Met allele leads to lower enzyme activity and higher levels of prefrontal DA (Tunbridge et al., 2006). This genetic state results in higher prefrontal DA levels which may be why healthy Met-carriers often perform better on working memory tasks (Tunbridge et al., 2006). Greater synaptic DA levels can increase D1 receptor activation associated with efficient memory and attentional processes (Sawaguchi and Goldman-Rakic, 1991). Val/Val individuals have greater enzyme activity and lower PFC DA levels(Egan et al., 2001). One hypothesis put forth by Bilder and Volkva suggests that since COMT is still expressed in subcortical regions, COMT may play a role in altering tonic and phasic DA levels

subcortically in addition to DA regulation under control by the PFC (Robert M. Bilder et al., 2004). Therefore, COMT may still influence subcortical DA levels, even though it is COMT effects on PFC DA transmission that are better characterized. The literature reported here may help explain why there were significant associations with *COMT* status with memory and attention, domains that are not solely PFC focused. In addition, studies have suggested a "dose-dependent" effect for the Met allele, with the heterozygote having an intermediate outcome(Egan et al., 2001). While not statistically significant, our data does suggest this possibility in women as shown in **Figure 1.**

Estradiol can enhance DA activity by increasing DA synthesis and altering tonic firing rates of DA neurons (Becker, 1999), (Xiao and Becker, 1994). Kritzer and Creutz have even shown that mesocortical pathways in female rats consist of 50% DA neurons where male rats only have 30% (Kritzer and Creutz, 2008). A post-mortem study has shown that independent of Val158Met status, men have a 17% greater activity in PFC COMT than women(Jingshan Chen et al., 2004). Estradiol also specifically regulates COMT activity and expression (ref). Further, studies have reported two estrogen response elements (EREs) on the *COMT* promoter region, and physiological estradiol levels inhibit *COMT* expression in rat livers (Xie et al., 1999). However, for similar levels of transcription, men still have higher COMT activity than women (Harrison and Tunbridge, 2007). Recently, Jacobs and D'Esposito have shown baseline DA levels are controlled by Val158Met interactions with estradiol levels that vary during the female menstrual cycle to impact PFC DA levels and functioning (Jacobs and D'Esposito, 2011). Met/Met women perform better under low estradiol conditions, with Val/Val women having opposite relationships. Although the precise mechanism is unclear, an estradiol related component to DAergic control over cognition is present. Those with TBI can also exhibit hypopituitarism (Tanriverdi et al., 2006) and altered gonadotropin levels as far as 12 months post injury (Bondanelli et al., 2005), for which outcomes

are impaired in men with persistent hypogonadism. This work suggests a role for an interaction between TBI and systemic estradiol levels suggesting the importance of this hormone when considering sex and Val158Met status in context of DA dysfunction.

TBI causes a functional hypodopaminergic state (James W. Bales et al., 2009), leaving many individuals with TBI with low basal DA levels. In the case of the inverted U hypothesis, TBI likely shifts individuals toward the low DA left portion of the curve. Since women have lower COMT enzyme activity, even prior to TBI, they would be expected to have a rightward shift on the curve compared to men. All Met/Met carriers would exhibit a rightward shift relative to Val-carriers. Since we hypothesize that a TBI induced hypodopaminergic state shifts DA levels to the left of the U-shaped curve, reasonable rightward DA increases at baseline would be beneficial to cognition. We observed this as our Met/Met women had the best cognitive outcomes. However, this framework would also suggest that male Val-carriers would have the worst cognitive outcomes, which we did not observe. This finding suggests that these there may be more factors that interact to impact baseline DA levels other just the observed Val158Met*sex interaction when considering the inverted U framework for interpreting DA system pathophysiology.

While COMT is typically associated with the PFC, the mesolimbic DAergic pathway also innervates the hippocampus. Since we included a memory component in the cognitive score, it is important to consider the role of COMT and DA in the hippocampus. Studies using tolcapone to block COMT activity in rats have shown increases in DA levels in the hippocampus and better performance on hippocampal dependent cognitive activities (Laatikainen et al., 2012). There are also numerous areas of the literature suggesting that Val158Met status impacts functional connectivity, which shows synchronous activation of biologically relevant connections between brain regions. Liu and colleagues have shown that Val homozygotes have decreased connectivity

between cingulate cortices and PFC regions compared to Val/Met heterozygotes, providing complementary imaging evidence that Val-carrier participants with lower levels of connectivity had lower cognitive outcomes that Met homozygotes. Similarly, evidence from Bertolino and colleagues shows that Val158Met status impacts coupling between the PFC and the hippocampal formation during memory processing. In this study, the Val allele led to reduced hippocampal activation at encoding and retrieval, uncoupled from the PFC (Bertolino et al., 2006). Thus, while memory is not PFC centered, Val-alleles may have reduced ability to modulate hippocampal function. FIM-Cog score analysis helps to illustrate that these genetic associations are not limited to neuropsychiatric tests, but also play a role in functional cognitive impairments that can limit daily activity.

The literature also provides evidence of other potential Val158Met interactions, of which the age and Val158Met interaction received the most focus (Dumontheil et al., 2011)·(Frias et al., 2005). However, we were unable to find such an association with cognitive outcomes in our study, perhaps due to the fact the standardized scores account for age in their calculations. One limitation of this study is our relatively small sample size for a genetics study, so our outcomes may not be large enough to delineate more subtle differences that might be present in the population. Also, since estrogen may play an important role in Val158Met transcription and activity, estrogen levels at time of cognitive testing would be useful. We also limited our analysis to Caucasians only since allelic distributions were significantly different by race, limiting our generalizability. Future studies in a larger and more diverse population that focus on additional factors that impact baseline DA levels are warranted. While this study looked at only cognitive outcomes, future studies should also look at more in depth behavioral measures also important in TBI populations since they have also been shown to be relevant to Val158Met status. Combined knowledge of how multiple DA

genetic factors impact cognition following TBI are important to determine which patients may benefit the most from DA targeted therapies post-TBI.

BIBLIOGRAPHY

Aalto, S., Brück, A., Laine, M., Någren, K., Rinne, J.O., 2005. Frontal and Temporal Dopamine Release during Working Memory and Attention Tasks in Healthy Humans: a Positron Emission Tomography Study Using the High-Affinity Dopamine D2 Receptor Ligand [11C]FLB 457. J. Neurosci. 25, 2471–2477. doi:10.1523/JNEUROSCI.2097-04.2005

Åberg, E., Fandiño-Losada, A., Sjöholm, L.K., Forsell, Y., Lavebratt, C., 2011. The functional Val158Met polymorphism in catechol-O-methyltransferase (COMT) is associated with depression and motivation in men from a Swedish population-based study. J. Affect. Disord. 129, 158–166. doi:10.1016/j.jad.2010.08.009

Alexander, N., Osinsky, R., Schmitz, A., Mueller, E., Kuepper, Y., Hennig, J., 2010. The BDNF Val66Met polymorphism affects HPA-axis reactivity to acute stress. Psychoneuroendocrinology 35, 949–953. doi:10.1016/j.psyneuen.2009.12.008

Almeida, R.D., Manadas, B.J., Melo, C.V., Gomes, J.R., Mendes, C.S., Grãos, M.M., Carvalho, R.F., Carvalho, A.P., Duarte, C.B., 2005. Neuroprotection by BDNF against glutamate-induced apoptotic cell death is mediated by ERK and PI3-kinase pathways. Cell Death Differ. 12, 1329–1343. doi:10.1038/sj.cdd.4401662

Alonso, M., Vianna, M.R.M., Depino, A.M., Mello e Souza, T., Pereira, P., Szapiro, G., Viola, H., Pitossi, F., Izquierdo, I., Medina, J.H., 2002. BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation. Hippocampus 12, 551–560. doi:10.1002/hipo.10035

Amstadter, A.B., MacPherson, L., Wang, F., Banducci, A.N., Reynolds, E.K., Potenza, M.N., Gelernter, J., Lejuez, C.W., 2012. The relationship between risk-taking propensity and the COMT Val158Met polymorphism among early adolescents as a function of sex. J. Psychiatr. Res. 46, 940–945. doi:10.1016/j.jpsychires.2012.04.010

Andersson, S., Bergedalen, A.-M., 2002. Cognitive correlates of apathy in traumatic brain injury. Cogn. Behav. Neurol. 15, 184–191.

An, J.J., Gharami, K., Liao, G.-Y., Woo, N.H., Lau, A.G., Vanevski, F., Torre, E.R., Jones, K.R., Feng, Y., Lu, B., Xu, B., 2008. Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell 134, 175–187. doi:10.1016/j.cell.2008.05.045

Arciniegas, D.B., 2002. Cognitive impairment following traumatic brain injury. Curr. Treat. Options Neurol. 4, 43+.

Arciniegas, D.B., Topkoff, J.L., Rojas, D.C., Sheeder, J., Teale, P., Young, D.A., Sandberg, E., Reite, M.L., Adler, L.E., 2001. Reduced Hippocampal Volume in Association With P50 Nonsuppression Following Traumatic Brain Injury. J. Neuropsychiatry Clin. Neurosci. 13, 213–221. doi:10.1176/appi.neuropsych.13.2.213

Arenth, P.M., Russell, K.C., Scanlon, J.M., Kessler, L.J., Ricker, J.H., 2012. Encoding and recognition after traumatic brain injury: Neuropsychological and functional magnetic resonance imaging findings. J. Clin. Exp. Neuropsychol. 34, 333–344. doi:10.1080/13803395.2011.633896

Ashman, T.A., Cantor, J.B., Gordon, W.A., Spielman, L., Flanagan, S., Ginsberg, A., Engmann, C., Egan, M., Ambrose, F., Greenwald, B., 2009. A randomized controlled trial of sertraline

for the treatment of depression in persons with traumatic brain injury. Arch. Phys. Med. Rehabil. 90, 733–740. doi:10.1016/j.apmr.2008.11.005

Bales, J.W., Wagner, A.K., Kline, A.E., Dixon, C.E., 2009. Persistent cognitive dysfunction after traumatic brain injury: A dopamine hypothesis. Neurosci. Biobehav. Rev. 33, 981–1003. doi:10.1016/j.neubiorev.2009.03.011

Bales, J.W., Wagner, A.K., Kline, A.E., Dixon, C.E., 2009. Persistent cognitive dysfunction after traumatic brain injury: A dopamine hypothesis. Neurosci. Biobehav. Rev. 33, 981–1003. doi:10.1016/j.neubiorev.2009.03.011

Baños, J.H., Novack, T.A., Brunner, R., Renfroe, S., Lin, H.-Y., Meythaler, J., 2010. Impact of early administration of sertraline on cognitive and behavioral recovery in the first year after moderate to severe traumatic brain injury. J. Head Trauma Rehabil. 25, 357–361. doi:10.1097/HTR.0b013e3181d6c715

Barker, P.A., 2009. Whither proBDNF? Nat. Neurosci. 12, 105–106. doi:10.1038/nn0209-105

Barnett, J.H., Heron, J., Ring, S.M., Golding, J., Goldman, D., Xu, K., Jones, P.B., 2007. Gender-specific effects of the catechol-O-methyltransferase Val108/158Met polymorphism on cognitive function in children. Am. J. Psychiatry 164, 142–149. doi:10.1176/appi.ajp.164.1.142

Barrett, G.L., 2000. The p75 neurotrophin receptor and neuronal apoptosis. Prog. Neurobiol. 61, 205–229.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.

Bartrés-Faz, D., Junqué, C., Serra-Grabulosa, J.M., López-Alomar, A., Moya, A., Bargalló, N., Mercader, J.M., Moral, P., Clemente, I.C., 2002. Dopamine DRD2 Taq I polymorphism associates with caudate nucleus volume and cognitive performance in memory impaired subjects. Neuroreport 13, 1121–1125.

Başkaya, M.K., Rao, A.M., Doğan, A., Donaldson, D., Dempsey, R.J., 1997. The biphasic opening of the blood-brain barrier in the cortex and hippocampus after traumatic brain injury in rats. Neurosci. Lett. 226, 33–36.

Bath, K.G., Lee, F.S., 2006. Variant BDNF (Val66Met) impact on brain structure and function. Cogn. Affect. Behav. Neurosci. 6, 79–85.

Baune, B.T., Hohoff, C., Berger, K., Neumann, A., Mortensen, S., Roehrs, T., Deckert, J., Arolt, V., Domschke, K., 2007. Association of the COMT val158met variant with antidepressant treatment response in major depression. Neuropsychopharmacology 33, 924–932.

Bayley, M.T., Tate, R., Douglas, J.M., Turkstra, L.S., Ponsford, J., Stergiou-Kita, M., Kua, A., Bragge, P., 2014. INCOG Guidelines for Cognitive Rehabilitation Following Traumatic Brain Injury: Methods and Overview. J. Head Trauma Rehabil. 29, 290–306. doi:10.1097/HTR.0000000000000070

Bayly, P.V., Cohen, T.S., Leister, E.P., Ajo, D., Leuthardt, E.C., Genin, G.M., 2005. Deformation of the Human Brain Induced by Mild Acceleration. J. Neurotrauma 22, 845–856. doi:10.1089/neu.2005.22.845

Becker, J.B., 1999. Gender Differences in Dopaminergic Function in Striatum and Nucleus Accumbens. Pharmacol. Biochem. Behav. 64, 803–812. doi:10.1016/S0091-3057(99)00168-9

Bekinschtein, P., Cammarota, M., Izquierdo, I., Medina, J.H., 2008a. Reviews: BDNF and Memory Formation and Storage. The Neuroscientist 14, 147 –156. doi:10.1177/1073858407305850

Bekinschtein, P., Cammarota, M., Katche, C., Slipczuk, L., Rossato, J.I., Goldin, A., Izquierdo, I., Medina, J.H., 2008b. BDNF is essential to promote persistence of long-term memory storage. Proc. Natl. Acad. Sci. 105, 2711–2716. doi:10.1073/pnas.0711863105

Bellander, M., Bäckman, L., Liu, T., Schjeide, B.-M.M., Bertram, L., Schmiedek, F., Lindenberger, U., Lövdén, M., 2014. Lower baseline performance but greater plasticity of working memory for carriers of the val allele of the comt val158met polymorphism. Neuropsychology. doi:10.1037/neu0000088

Bendlin, B.B., Ries, M.L., Lazar, M., Alexander, A.L., Dempsey, R.J., Rowley, H.A., Sherman, J.E., Johnson, S.C., 2008. Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. NeuroImage 42, 503–514. doi:10.1016/j.neuroimage.2008.04.254

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300.

Berridge, K.C., Kringelbach, M.L., 2008. Affective neuroscience of pleasure: reward in humans and animals. Psychopharmacology (Berl.) 199, 457–480. doi:10.1007/s00213-008-1099-6

Bertolino, A., Fazio, L., Di Giorgio, A., Blasi, G., Romano, R., Taurisano, P., Caforio, G., Sinibaldi, L., Ursini, G., Popolizio, T., Tirotta, E., Papp, A., Dallapiccola, B., Borrelli, E., Sadee, W., 2009. Genetically determined interaction between the dopamine transporter and the D2 receptor on prefronto-striatal activity and volume in humans. J. Neurosci. Off. J. Soc. Neurosci. 29, 1224–1234. doi:10.1523/JNEUROSCI.4858-08.2009

Bertolino, A., Rubino, V., Sambataro, F., Blasi, G., Latorre, V., Fazio, L., Caforio, G., Petruzzella, V., Kolachana, B., Hariri, A., Meyer-Lindenberg, A., Nardini, M., Weinberger, D.R., Scarabino, T., 2006. Prefrontal-hippocampal coupling during memory processing is modulated by COMT val158met genotype. Biol. Psychiatry 60, 1250–1258. doi:10.1016/j.biopsych.2006.03.078

Bhang, S., Ahn, J.-H., Choi, S.-W., 2010. Brain-derived neurotrophic factor and serotonin transporter gene-linked promoter region genes alter serum levels of brain-derived neurotrophic factor in humans. J. Affect. Disord. doi:10.1016/j.jad.2010.07.008

Bilder, R.M., Volavka, J., Lachman, H.M., Grace, A.A., 2004. The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 29, 1943–1961. doi:10.1038/sj.npp.1300542

Bilder, R.M., Volavka, J., Lachman, H.M., Grace, A.A., 2004. The Catechol-O-Methyltransferase Polymorphism: Relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. Neuropsychopharmacology 29, 1943–1961. doi:10.1038/sj.npp.1300542

Bleiberg, J., Garmoe, W., Cederquist, J., Reeves, D., 1993. Effect of dexedrine on performance consistency following brain injury: A double-blind placebo crossover case study. Neuropsychiatry. Neuropsychol. Behav. Neurol.

Bombardier, C.H., Fann, J.R., Temkin, N.R., Esselman, P.C., Barber, J., Dikmen, S.S., 2010a. Rates of Major Depressive Disorder and Clinical Outcomes Following Traumatic Brain Injury. JAMA 303, 1938–1945. doi:10.1001/jama.2010.599

Bombardier, C.H., Fann, J.R., Temkin, N.R., Esselman, P.C., Barber, J., Dikmen, S.S., 2010b. Rates of Major Depressive Disorder and Clinical Outcomes Following Traumatic Brain Injury. JAMA 303, 1938–1945. doi:10.1001/jama.2010.599

Bondanelli, M., Ambrosio, M.R., Zatelli, M.C., Marinis, L.D., Uberti, E.C. degli, 2005. Hypopituitarism after traumatic brain injury. Eur. J. Endocrinol. 152, 679–691. doi:10.1530/eje.1.01895

Borkowski, J., Benton, A., Spreen, O., 1967. Word fluency and brain damage. Neuropsychologia 5, 135–140.

Borkowski, J., Benton, A., Spreen, O., 1967. Word Fluency and Brain Damage. Neuropsychologia 135–140.

Bornstein, R.A., Miller, H.B., van Schoor, J.T., 1989. Neuropsychological deficit and emotional disturbance in head-injured patients. J. Neurosurg. 70, 509–513. doi:10.3171/jns.1989.70.4.0509

Brady, R., Zaidi, S.I., Mayer, C., Katz, D.M., 1999. BDNF is a target-derived survival factor for arterial baroreceptor and chemoafferent primary sensory neurons. J. Neurosci. Off. J. Soc. Neurosci. 19, 2131–2142.

Brandeis, R., Brandys, Y., Yehuda, S., 1989. The use of the Morris water maze in the study of memory and learning. Int. J. Neurosci. 48, 29–69.

Braver, T.S., Cohen, J.D., 2000. On the control of control: The role of dopamine in regulating prefrontal function and working memory. Control Cogn. Process. Atten. Perform. XVIII 713–737.

Brooks, J.C., Strauss, D.J., Shavelle, R.M., Paculdo, D.R., Hammond, F.M., Harrison-Felix, C.L., 2013. Long-Term Disability and Survival in Traumatic Brain Injury: Results From the National Institute on Disability and Rehabilitation Research Model Systems. Arch. Phys. Med. Rehabil. 94, 2203–2209. doi:10.1016/j.apmr.2013.07.005

Brooks, J., Fos, L.A., Greve, K.W., Hammond, J.S., 1999. Assessment of executive function in patients with mild traumatic brain injury. J. Trauma 46, 159–163.

Brunoni, A.R., Lopes, M., Fregni, F., 2008. A Systematic Review and Meta-Analysis of Clinical Studies on Major Depression and BDNF Levels: Implications for the Role of Neuroplasticity in Depression. Int. J. Neuropsychopharmacol. 11, 1169–1180. doi:10.1017/S1461145708009309

Buckley, M.J., 2005. The role of the perirhinal cortex and hippocampus in learning, memory, and perception. Q. J. Exp. Psychol. B 58, 246–268. doi:10.1080/02724990444000186

Burt, D.B., Zembar, M.J., Niederehe, G., 1995. Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. Psychol. Bull. 117, 285–305.

Bushnik, T., Englander, J., Wright, J., 2008. Patterns of fatigue and its correlates over the first 2 years after traumatic brain injury. J. Head Trauma Rehabil. 23, 25–32. doi:10.1097/01.HTR.0000308718.88214.bb

Busto, R., Dietrich, W.D., Globus, M.Y.-T., Alonso, O., Ginsberg, M.D., 1997. Extracellular Release of Serotonin following Fluid-Percussion Brain Injury in Rats. J. Neurotrauma 14, 35–42. doi:10.1089/neu.1997.14.35

Bylsma, L.M., Morris, B.H., Rottenberg, J., 2008. A meta-analysis of emotional reactivity in major depressive disorder. Clin. Psychol. Rev. 28, 676–691.

Canli, T., Lesch, K.-P., 2007. Long story short: the serotonin transporter in emotion regulation and social cognition. Nat Neurosci 10, 1103–1109. doi:10.1038/nn1964

Caporali, A., Emanueli, C., 2009. Cardiovascular actions of neurotrophins. Physiol. Rev. 89, 279–308. doi:10.1152/physrev.00007.2008

Carrera, E., Tononi, G., 2014. Diaschisis: past, present, future. Brain J. Neurol. 137, 2408–2422. doi:10.1093/brain/awu101

CDC - Injury - TBI - TBI in the US Report [WWW Document], n.d. URL http://www.cdc.gov/traumaticbraininjury/tbi_ed.html (accessed 11.22.10a).

CDC - Injury - TBI - TBI in the US Report [WWW Document], n.d. URL http://www.cdc.gov/traumaticbraininjury/tbi_ed.html (accessed 11.22.10b).

CDC - TBI in the US Report - Traumatic Brain Injury - Injury Center [WWW Document], n.d. URL http://www.cdc.gov/traumaticbraininjury/tbi_ed.html (accessed 6.26.13).

Cernak, I., Chang, T., Ahmed, F.A., Cruz, M.I., Vink, R., Stoica, B., Faden, A.I., 2010. Pathophysiological response to experimental diffuse brain trauma differs as a function of developmental age. Dev. Neurosci. 32, 442–453. doi:10.1159/000320085

Chamelian, L., Feinstein, A., 2006. The effect of major depression on subjective and objective cognitive deficits in mild to moderate traumatic brain injury. J. Neuropsychiatry Clin. Neurosci. 18, 33–38. doi:10.1176/appi.neuropsych.18.1.33

Chan, F., Lanctôt, K.L., Feinstein, A., Herrmann, N., Strauss, J., Sicard, T., Kennedy, J.L., McCullagh, S., Rapoport, M.J., 2008. The serotonin transporter polymorphisms and major depression following traumatic brain injury. Brain Inj. BI 22, 471–479. doi:10.1080/02699050802084886

Chen, C., Chen, C., Moyzis, R., Dong, Q., He, Q., Zhu, B., Li, J., Li, H., Li, J., Lessard, J., 2011. Sex modulates the associations between the COMT gene and personality traits. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 36, 1593–1598. doi:10.1038/npp.2011.39

Chen, H., Poon, A., Yeung, C., Helms, C., Pons, J., Bowcock, A.M., Kwok, P.-Y., Liao, W., 2011. A genetic risk score combining ten psoriasis risk loci improves disease prediction. PloS One 6, e19454. doi:10.1371/journal.pone.0019454

Chen, J.-K., Johnston, K.M., Petrides, M., Ptito, A., 2008a. Neural Substrates of Symptoms of Depression Following Concussion in Male Athletes With Persisting Postconcussion Symptoms. Arch Gen Psychiatry 65, 81–89. doi:10.1001/archgenpsychiatry.2007.8

Chen, J.-K., Johnston, K.M., Petrides, M., Ptito, A., 2008b. Neural Substrates of Symptoms of Depression Following Concussion in Male Athletes With Persisting Postconcussion Symptoms. Arch Gen Psychiatry 65, 81–89. doi:10.1001/archgenpsychiatry.2007.8

Chen, J., Lipska, B.K., Halim, N., Ma, Q.D., Matsumoto, M., Melhem, S., Kolachana, B.S., Hyde, T.M., Herman, M.M., Apud, J., Egan, M.F., Kleinman, J.E., Weinberger, D.R., 2004. Functional Analysis of Genetic Variation in Catechol-O-Methyltransferase (COMT): Effects on mRNA, Protein, and Enzyme Activity in Postmortem Human Brain. Am. J. Hum. Genet. 75, 807–821. doi:10.1086/425589

Chen, J., Lipska, B.K., Halim, N., Ma, Q.D., Matsumoto, M., Melhem, S., Kolachana, B.S., Hyde, T.M., Herman, M.M., Apud, J., others, 2004. Functional Analysis of Genetic Variation in Catechol-O-Methyltransferase (< i> COMT</i>): Effects on mRNA, Protein, and Enzyme Activity in Postmortem Human Brain. Am. J. Hum. Genet. 75, 807–821.

Chen, X., Li, Y., Kline, A.E., Dixon, C.E., Zafonte, R.D., Wagner, A.K., 2005. Gender and environmental effects on regional brain-derived neurotrophic factor expression after experimental traumatic brain injury. Neuroscience 135, 11–17. doi:10.1016/j.neuroscience.2005.05.041

Chen, Z.-Y., Patel, P.D., Sant, G., Meng, C.-X., Teng, K.K., Hempstead, B.L., Lee, F.S., 2004. Variant Brain-Derived Neurotrophic Factor (BDNF) (Met66) Alters the Intracellular Trafficking and Activity-Dependent Secretion of Wild-Type BDNF in Neurosecretory

Cells and Cortical Neurons. J Neurosci 24, 4401–4411. doi:10.1523/JNEUROSCI.0348-04.2004

Chiaretti, A., Piastra, M., Polidori, G., Di Rocco, C., Caresta, E., Antonelli, A., Amendola, T., Aloe, L., 2003. Correlation between neurotrophic factor expression and outcome of children with severe traumatic brain injury. Intensive Care Med. 29, 1329–1338. doi:10.1007/s00134-003-1852-6

Cicerone, K.D., Langenbahn, D.M., Braden, C., Malec, J.F., Kalmar, K., Fraas, M., Felicetti, T., Laatsch, L., Harley, J.P., Bergquist, T., Azulay, J., Cantor, J., Ashman, T., 2011. Evidence-based cognitive rehabilitation: updated review of the literature from 2003 through 2008. Arch. Phys. Med. Rehabil. 92, 519–530. doi:10.1016/j.apmr.2010.11.015

Cifu, D.X., Keyser-Marcus, L., Lopez, E., Wehman, P., Kreutzer, J.S., Englander, J., High, W., 1997a. Acute predictors of successful return to work 1 year after traumatic brain injury: a multicenter analysis. Arch. Phys. Med. Rehabil. 78, 125–131.

Cifu, D.X., Keyser-Marcus, L., Lopez, E., Wehman, P., Kreutzer, J.S., Englander, J., High, W., 1997b. Acute predictors of successful return to work 1 year after traumatic brain injury: a multicenter analysis. Arch. Phys. Med. Rehabil. 78, 125–131.

Ciliax, B.J., Heilman, C., Demchyshyn, L.L., Pristupa, Z.B., Ince, E., Hersch, S.M., Niznik, H.B., Levey, A.I., 1995. The dopamine transporter: immunochemical characterization and localization in brain. J. Neurosci. 15, 1714–1723.

Clarke, H.F., Walker, S.C., Dalley, J.W., Robbins, T.W., Roberts, A.C., 2007. Cognitive Inflexibility after Prefrontal Serotonin Depletion Is Behaviorally and Neurochemically Specific. Cereb. Cortex 17, 18–27. doi:10.1093/cercor/bhj120

Comings, D.E., MacMurray, J.P., 2000. Molecular heterosis: a review. Mol. Genet. Metab. 71, 19–31. doi:10.1006/mgme.2000.3015

Contant, C.F., Valadka, A.B., Gopinath, S.P., Hannay, H.J., Robertson, C.S., 2001. Adult respiratory distress syndrome: a complication of induced hypertension after severe head injury. J. Neurosurg. 95, 560–568. doi:10.3171/jns.2001.95.4.0560

Conti, A.C., Cryan, J.F., Dalvi, A., Lucki, I., Blendy, J.A., 2002. cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. J. Neurosci. Off. J. Soc. Neurosci. 22, 3262–3268. doi:20026293

Cook, K.F., Bombardier, C.H., Bamer, A.M., Choi, S.W., Kroenke, K., Fann, J.R., 2011a. Do Somatic and Cognitive Symptoms of Traumatic Brain Injury Confound Depression Screening? Arch. Phys. Med. Rehabil. 92, 818–823. doi:16/j.apmr.2010.12.008

Cook, K.F., Bombardier, C.H., Bamer, A.M., Choi, S.W., Kroenke, K., Fann, J.R., 2011b. Do somatic and cognitive symptoms of traumatic brain injury confound depression screening? Arch. Phys. Med. Rehabil. 92, 818–823. doi:10.1016/j.apmr.2010.12.008

Cools, R., D'Esposito, M., 2011a. Inverted-U-shaped dopamine actions on human working memory and cognitive control. Biol. Psychiatry 69, e113–125. doi:10.1016/j.biopsych.2011.03.028

Cools, R., D'Esposito, M., 2011b. Inverted-U–Shaped Dopamine Actions on Human Working Memory and Cognitive Control. Biol. Psychiatry 69, e113–e125. doi:10.1016/j.biopsych.2011.03.028

Cooper, B.R., Hester, T.J., Maxwell, R.A., 1980. Behavioral and biochemical effects of the antidepressant bupropion (Wellbutrin): evidence for selective blockade of dopamine uptake in vivo. J. Pharmacol. Exp. Ther. 215, 127.

Cope, E.C., Morris, D.R., Scrimgeour, A.G., VanLandingham, J.W., Levenson, C.W., 2011. Zinc supplementation provides behavioral resiliency in a rat model of traumatic brain injury. Physiol. Behav. 104, 942–947. doi:10.1016/j.physbeh.2011.06.007

Cox, D.R., 1972. Regression models and life-tables. J. R. Stat. Soc. Ser. B Methodol. 187–220.

Croll, S.D., Ip, N.Y., Lindsay, R.M., Wiegand, S.J., 1998. Expression of BDNF and trkB as a function of age and cognitive performance. Brain Res. 812, 200–208.

Cross, D.S., Ivacic, L.C., Stefanski, E.L., McCarty, C.A., 2010. Population based allele frequencies of disease associated polymorphisms in the Personalized Medicine Research Project. BMC Genet. 11, 51. doi:10.1186/1471-2156-11-51

Damberg, M., 2005. Transcription factor AP-2 and monoaminergic functions in the central nervous system. J. Neural Transm. 112, 1281–1296. doi:10.1007/s00702-005-0325-1

Damsa, C., Bumb, A., Bianchi-Demicheli, F., Vidailhet, P., Sterck, R., Andreoli, A., Beyenburg, S., others, 2004. " Dopamine-dependent" side effects of selective serotonin reuptake inhibitors: a clinical review. J. Clin. Psychiatry 65, 1064.

Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9, 46–56. doi:10.1038/nrn2297

Darrah, S.D., Miller, M.A., Ren, D., Hoh, N.Z., Scanlon, J.M., Conley, Y.P., Wagner, A.K., 2012. Genetic variability in glutamic acid decarboxylase genes: Associations with post-traumatic seizures after severe TBI. Epilepsy Res. doi:10.1016/j.eplepsyres.2012.07.006

Dawood, T., Anderson, J., Barton, D., Lambert, E., Esler, M., Hotchkin, E., Haikerwal, D., Kaye, D., Lambert, G., 2007. Reduced overflow of BDNF from the brain is linked with suicide risk in depressive illness. Mol. Psychiatry 12, 981–983. doi:10.1038/sj.mp.4002059

De Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 463–475. doi:10.1038/nrn1683

Delis, D., 2000. The California Verbal Learning Test. The Psychological Corporation, San Antonio.

Delis, D.C., et al., 2000. The California Verbal Learning Test. The Psychological Corporation, San Antonio.

Delis, D., Kaplan, E., Kramer, J.H., 2001. D-KEFS: examiners manual. The Psychological Corporation, San Antonio.

Depue, B.E., Olson-Madden, J.H., Smolker, H.R., Rajamani, M., Brenner, L.A., Banich, M.T., 2014. Reduced Amygdala Volume Is Associated with Deficits in Inhibitory Control: A Voxel- and Surface-Based Morphometric Analysis of Comorbid PTSD/Mild TBI. BioMed Res. Int. 2014. doi:10.1155/2014/691505

Dias, V.V., Brissos, S., Frey, B.N., Andreazza, A.C., Cardoso, C., Kapczinski, F., 2009. Cognitive function and serum levels of brain-derived neurotrophic factor in patients with bipolar disorder. Bipolar Disord. 11, 663–671. doi:10.1111/j.1399-5618.2009.00733.x

Disner, S.G., Beevers, C.G., Haigh, E.A.P., Beck, A.T., 2011. Neural mechanisms of the cognitive model of depression. Nat. Rev. Neurosci. 12, 467–477. doi:10.1038/nrn3027

Dixon, C., Kochanek, P.M., Yan, H.Q., Schiding, J.K., Griffith, R.G., Baum, E., Marion, D.W., DeKosky, S.T., 1999. One-year study of spatial memory performance, brain morphology, and cholinergic markers after moderate controlled cortical impact in rats. J. Neurotrauma 16, 109–122.

Dodds, T.A., Martin, D.P., Stolov, W.C., Deyo, R.A., 1993. A validation of the functional independence measurement and its performance among rehabilitation inpatients. Arch. Phys. Med. Rehabil. 74, 531–536.

Donnemiller, E., Brenneis, C., Wissel, J., Scherfler, C., Poewe, W., Riccabona, G., Wenning, G.K., 2000. Impaired dopaminergic neurotransmission in patients with traumatic brain injury: a SPET study using 123 I-\ss-CIT and 123 I-IBZM. Eur. J. Nucl. Med. Mol. Imaging 27, 1410–1414.

Drueke, B., Schlaegel, S.M.A., Seifert, A., Moeller, O., Gründer, G., Gauggel, S., Boecker, M., 2013. The role of 5-HT in response inhibition and re-engagement. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. 23, 830–841. doi:10.1016/j.euroneuro.2013.05.005

D'Sa, C., Duman, R.S., 2002. Antidepressants and neuroplasticity. Bipolar Disord. 4, 183–194. doi:10.1034/j.1399-5618.2002.01203.x

Duan, J., Wainwright, M.S., Comeron, J.M., Saitou, N., Sanders, A.R., Gelernter, J., Gejman, P.V., 2003. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. Hum. Mol. Genet. 12, 205–216.

Dumontheil, I., Roggeman, C., Ziermans, T., Peyrard-Janvid, M., Matsson, H., Kere, J., Klingberg, T., 2011. Influence of the COMT Genotype on Working Memory and Brain Activity Changes During Development. Biol. Psychiatry 70, 222–229. doi:10.1016/j.biopsych.2011.02.027

Dunlop, B.W., Nemeroff, C.B., 2007. The Role of Dopamine in the Pathophysiology of Depression. Arch Gen Psychiatry 64, 327–337. doi:10.1001/archpsyc.64.3.327

Edenberg, H., Reynolds, J., 1998. Improved method for detecting the long and short promoter alleles of the serotonin transporter gene HTT (SLC6A4). Psychiatr. Genet. 8, 193.

Egan, M.F., Goldberg, T.E., Kolachana, B.S., Callicott, J.H., Mazzanti, C.M., Straub, R.E., Goldman, D., Weinberger, D.R., 2001. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc. Natl. Acad. Sci. 98, 6917–6922. doi:10.1073/pnas.111134598

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257–269.

Eker, C., Kitis, O., Taneli, F., Eker, O.D., Ozan, E., Yucel, K., Coburn, K., Gonul, A.S., 2010. Correlation of serum BDNF levels with hippocampal volumes in first episode, medication-free depressed patients. Eur. Arch. Psychiatry Clin. Neurosci. 260, 527–533. doi:10.1007/s00406-010-0110-5

Elovic, E.P., Lansang, R., Li, Y., Ricker, J.H., 2003. The use of atypical antipsychotics in traumatic brain injury. J. Head Trauma Rehabil. 18, 177–195.

Erickson, K.I., Miller, D.L., Roecklein, K.A., 2012. The aging hippocampus: interactions between exercise, depression, and BDNF. Neurosci. Rev. J. Bringing Neurobiol. Neurol. Psychiatry 18, 82–97. doi:10.1177/1073858410397054

Erickson, K.I., Prakash, R.S., Voss, M.W., Chaddock, L., Heo, S., McLaren, M., Pence, B.D., Martin, S.A., Vieira, V.J., Woods, J.A., McAuley, E., Kramer, A.F., 2010. Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. J. Neurosci. Off. J. Soc. Neurosci. 30, 5368–5375. doi:10.1523/JNEUROSCI.6251-09.2010

Erritzoe, D., Holst, K., Frokjaer, V.G., Licht, C.L., Kalbitzer, J., Nielsen, F.Å., Svarer, C., Madsen, J., Knudsen, G.M., 2010. A Nonlinear Relationship between Cerebral Serotonin Transporter and 5-HT2A Receptor Binding: An In Vivo Molecular Imaging Study in Humans. J. Neurosci. 30, 3391–3397. doi:10.1523/JNEUROSCI.2852-09.2010

Etkin, A., Egner, T., Kalisch, R., 2011. Emotional processing in anterior cingulate and medial prefrontal cortex. Trends Cogn. Sci. 15, 85–93. doi:10.1016/j.tics.2010.11.004

Failla, M.D., Burkhardt, J.N., Miller, M.A., Scanlon, J.M., Conley, Y.P., Ferrell, R.E., Wagner, A.K., 2013. Variants of SLC6A4 in depression risk following severe TBI. Brain Inj. BI 27, 696–706. doi:10.3109/02699052.2013.775481

Failla, M.D., John Myrga M., Ricker, J.H., Dixon, C.E., Conley, Y.P., Wagner, A.K., n.d. Post-TBI Cognitive Performance is moderated by variation within ANKK1 and DRD2 genes. J. Head Trauma Rehabil.

Failla, M.D., Kumar, R.G., Peitzman, A.B., Conley, Y.P., Ferrell, R.E., Wagner, A.K., 2014. Variation in the BDNF Gene Interacts With Age to Predict Mortality in a Prospective, Longitudinal Cohort with Severe TBI. Neurorehabil. Neural Repair. doi:10.1177/1545968314542617

Fales, C.L., Barch, D.M., Rundle, M.M., Mintun, M.A., Snyder, A.Z., Cohen, J.D., Mathews, J., Sheline, Y.I., 2008. Altered emotional interference processing in affective and cognitive-control brain circuitry in major depression. Biol. Psychiatry 63, 377–384. doi:10.1016/j.biopsych.2007.06.012

Fann, J.R., Bombardier, C.H., Dikmen, S., Esselman, P., Warms, C.A., Pelzer, E., Rau, H., Temkin, N., 2005. Validity of the Patient Health Questionnaire-9 in assessing depression following traumatic brain injury. J. Head Trauma Rehabil. 20, 501–511.

Fann, J.R., Hart, T., Schomer, K.G., 2009a. Treatment for Depression after Traumatic Brain Injury: A Systematic Review. J. Neurotrauma 26, 2383–2402. doi:10.1089/neu.2009.1091

Fann, J.R., Hart, T., Schomer, K.G., 2009b. Treatment for Depression after Traumatic Brain Injury: A Systematic Review. J. Neurotrauma 26, 2383–2402. doi:10.1089/neu.2009.1091

Fann, J.R., Uomoto, J.M., Katon, W.J., 2001. Cognitive Improvement With Treatment of Depression Following Mild Traumatic Brain Injury. Psychosomatics 42, 48–54. doi:10.1176/appi.psy.42.1.48

Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A., Sklar, P., 2005. Molecular genetics of attention-deficit/hyperactivity disorder. Biol. Psychiatry 57, 1313–1323. doi:10.1016/j.biopsych.2004.11.024

Faul, M., Xu, L., Wald, M.M., Coronado, V.G., 2010. Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths 2002–2006. Atlanta GA Cent. Dis. Control Prev. Natl. Cent. Inj. Prev. Control.

Fenn, A.M., Gensel, J.C., Huang, Y., Popovich, P.G., Lifshitz, J., Godbout, J.P., 2013. Immune Activation Promotes Depression 1 Month After Diffuse Brain Injury: A Role for Primed Microglia. Biol. Psychiatry. doi:10.1016/j.biopsych.2013.10.014

Fischl, B., 2012. FreeSurfer. NeuroImage. doi:10.1016/j.neuroimage.2012.01.021

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355.

Fischl, B., van der Kouwe, A., Destrieux, C., Halgren, E., Ségonne, F., Salat, D.H., Busa, E., Seidman, L.J., Goldstein, J., Kennedy, D., Caviness, V., Makris, N., Rosen, B., Dale, A.M.,

2004. Automatically parcellating the human cerebral cortex. Cereb. Cortex N. Y. N 1991 14, 11–22.

Fleming, J., Tooth, L., Hassell, M., Chan, W., 1999. Prediction of community integration and vocational outcome 2-5 years after traumatic brain injury rehabilitation in Australia. Brain Inj. BI 13, 417–431.

Freedman, M.L., Reich, D., Penney, K.L., McDonald, G.J., Mignault, A.A., Patterson, N., Gabriel, S.B., Topol, E.J., Smoller, J.W., Pato, C.N., Pato, M.T., Petryshen, T.L., Kolonel, L.N., Lander, E.S., Sklar, P., Henderson, B., Hirschhorn, J.N., Altshuler, D., 2004. Assessing the impact of population stratification on genetic association studies. Nat. Genet. 36, 388–393. doi:10.1038/ng1333

Frenette, A.J., Kanji, S., Rees, L., Williamson, D.R., Perreault, M.M., Turgeon, A.F., Bernard, F., Fergusson, D.A., 2012. Efficacy and safety of dopamine agonists in traumatic brain injury: a systematic review of randomized controlled trials. J. Neurotrauma 29, 1–18. doi:10.1089/neu.2011.1812

Frenois, F., Moreau, M., O'Connor, J., Lawson, M., Micon, C., Lestage, J., Kelley, K.W., Dantzer, R., Castanon, N., 2007. Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. Psychoneuroendocrinology 32, 516–531. doi:10.1016/j.psyneuen.2007.03.005

Frias, C.M. de, Annerbrink, K., Westberg, L., Eriksson, E., Adolfsson, R., Nilsson, L.-G., 2005. Catechol O-Methyltransferase Val158Met Polymorphism is Associated with Cognitive Performance in Nondemented Adults. J. Cogn. Neurosci. 17, 1018–1025. doi:10.1162/0898929054475136

Frodl, T., Koutsouleris, N., Bottlender, R., Born, C., Jager, M., Morgenthaler, M., Scheuerecker, J., Zill, P., Baghai, T., Schule, C., Rupprecht, R., Bondy, B., Reiser, M., Moller, H.-J., Meisenzahl, E.M., 2008. Reduced gray matter brain volumes are associated with variants of the serotonin transporter gene in major depression. Mol Psychiatry 13, 1093–1101.

Frodl, T., Meisenzahl, E.M., Zill, P., Baghai, T., Rujescu, D., Leinsinger, G., Bottlender, R., Schule, C., Zwanzger, P., Engel, R.R., Rupprecht, R., Bondy, B., Reiser, M., Moller, H.-J., 2004. Reduced Hippocampal Volumes Associated With the Long Variant of the Serotonin Transporter Polymorphism in Major Depression. Arch Gen Psychiatry 61, 177–183. doi:10.1001/archpsyc.61.2.177

Fujimura, H., Altar, C.A., Chen, R., Nakamura, T., Nakahashi, T., Kambayashi, J., Sun, B., Tandon, N.N., 2002. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thromb. Haemost. 87, 728–734.

Fujiwara, E., Schwartz, M.L., Gao, F., Black, S.E., Levine, B., 2008. Ventral frontal cortex functions and quantified MRI in traumatic brain injury. Neuropsychologia 46, 461–474. doi:10.1016/j.neuropsychologia.2007.08.027

Gale, S.D., Baxter, L., Roundy, N., Johnson, S.C., 2005. Traumatic brain injury and grey matter concentration: a preliminary voxel based morphometry study. J. Neurol. Neurosurg. Psychiatry 76, 984–988. doi:10.1136/jnnp.2004.036210

Gao, W.-M., Chadha, M.S., Kline, A.E., Clark, R.S.B., Kochanek, P.M., Dixon, C.E., Jenkins, L.W., 2006. Immunohistochemical analysis of histone H3 acetylation and methylation--evidence for altered epigenetic signaling following traumatic brain injury in immature rats. Brain Res. 1070, 31–34. doi:10.1016/j.brainres.2005.11.038

García, M.A., Vázquez, J., Giménez, C., Valdivieso, F., Zafra, F., 1996. Transcription Factor AP-2 Regulates Human Apolipoprotein E Gene Expression in Astrocytoma Cells. J. Neurosci. 16, 7550–7556.

Gass, P., Hellweg, R., 2010. Peripheral brain-derived neurotrophic factor (BDNF) as a biomarker for affective disorders? Int. J. Neuropsychopharmacol. Off. Sci. J. Coll. Int. Neuropsychopharmacol. CINP 13, 1–4. doi:10.1017/S1461145709991039

Gelernter, J., Cubells, J.F., Kidd, J.R., Pakstis, A.J., Kidd, K.K., 1999. Population studies of polymorphisms of the serotonin transporter protein gene. Am. J. Med. Genet. 88, 61–66. doi:10.1002/(SICI)1096-8628(19990205)88:1<61::AID-AJMG11>3.0.CO;2-K

Gfeller, J.D., Chibnall, J.T., Duckro, P.N., 1994. Postconcussion symptoms and cognitive functioning in posttraumatic headache patients. Headache 34, 503–507.

Goldman, D., Brown, G.L., Albaugh, B., Robin, R., Goodson, S., Trunzo, M., Akhtar, L., Lucas-Derse, S., Long, J., Linnoila, M., 1993. DRD2 dopamine receptor genotype, linkage disequilibrium, and alcoholism in American Indians and other populations. Alcohol. Clin. Exp. Res. 17, 199–204.

Goldstein, B., Toweill, D., Lai, S., Sonnenthal, K., Kimberly, B., 1998. Uncoupling of the autonomic and cardiovascular systems in acute brain injury. Am. J. Physiol. 275, R1287–1292.

Gomes, J.R., Costa, J.T., Melo, C.V., Felizzi, F., Monteiro, P., Pinto, M.J., Inácio, A.R., Wieloch, T., Almeida, R.D., Grãos, M., Duarte, C.B., 2012. Excitotoxicity downregulates TrkB.FL signaling and upregulates the neuroprotective truncated TrkB receptors in cultured hippocampal and striatal neurons. J. Neurosci. Off. J. Soc. Neurosci. 32, 4610–4622. doi:10.1523/JNEUROSCI.0374-12.2012

Gotlib, I.H., Joormann, J., 2010. Cognition and depression: current status and future directions. Annu. Rev. Clin. Psychol. 6, 285–312. doi:10.1146/annurev.clinpsy.121208.131305

Gould, K.R., Ponsford, J.L., Johnston, L., Sch�nberger, M., 2011. The Nature, Frequency and Course of Psychiatric Disorders in the First Year After Traumatic Brain Injury: A Prospective Study. Psychol. Med. FirstView, 1–11. doi:10.1017/S003329171100033X

Goyal, A., Failla, M.D., Niyonkuru, C., Amin, K., Fabio, A., Berger, R.P., Wagner, A.K., 2013. S100b as a prognostic biomarker in outcome prediction for patients with severe traumatic brain injury. J. Neurotrauma 30, 946–957. doi:10.1089/neu.2012.2579

Grade, C., Redford, B., Chrostowski, J., Toussaint, L., Blackwell, B., 1998. Methylphenidate in early poststroke recovery: a double-blind, placebo-controlled study. Arch. Phys. Med. Rehabil. 79, 1047–1050.

Graham, D.P., Helmer, D.A., Harding, M.J., Kosten, T.R., Petersen, N.J., Nielsen, D.A., 2013. Serotonin transporter genotype and mild traumatic brain injury independently influence resilience and perception of limitations in Veterans. J. Psychiatr. Res. 47, 835–842. doi:10.1016/j.jpsychires.2013.02.006

Graybiel, A.M., 2005. The basal ganglia: learning new tricks and loving it. Curr. Opin. Neurobiol. 15, 638–644. doi:10.1016/j.conb.2005.10.006

Green, R.E., Colella, B., Christensen, B., Johns, K., Frasca, D., Bayley, M., Monette, G., 2008. Examining moderators of cognitive recovery trajectories after moderate to severe traumatic brain injury. Arch. Phys. Med. Rehabil. 89, S16–24. doi:10.1016/j.apmr.2008.09.551

Greicius, M.D., Supekar, K., Menon, V., Dougherty, R.F., 2009. Resting-state functional connectivity reflects structural connectivity in the default mode network. Cereb. Cortex 19, 72–78.

Griesbach, G.S., Hovda, D.A., Gomez-Pinilla, F., 2009. Exercise-induced improvement in cognitive performance after traumatic brain injury in rats is dependent on BDNF activation. Brain Res. 1288, 105–115. doi:10.1016/j.brainres.2009.06.045

Griesbach, G.S., Tio, D.L., Nair, S., Hovda, D., 2013. Recovery of Stress Response Coincides with Responsiveness to Voluntary Exercise after Traumatic Brain Injury. J. Neurotrauma. doi:10.1089/neu.2013.3151

Griesbach, G.S., Tio, D.L., Vincelli, J., McArthur, D.L., Taylor, A.N., 2012. Differential Effects of Voluntary and Forced Exercise on Stress Responses after Traumatic Brain Injury. J. Neurotrauma 29, 1426–1433. doi:10.1089/neu.2011.2229

Griffin, É.W., Mullally, S., Foley, C., Warmington, S.A., O'Mara, S.M., Kelly, A.M., 2011. Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males. Physiol. Behav. 104, 934–941. doi:10.1016/j.physbeh.2011.06.005

Guillin, O., Diaz, J., Carroll, P., Griffon, N., Schwartz, J.-C., Sokoloff, P., 2001. BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. Nature 411, 86–89. doi:10.1038/35075076

Gunstad, J., Benitez, A., Smith, J., Glickman, E., Spitznagel, M.B., Alexander, T., Juvancic-Heltzel, J., Murray, L., 2008. Serum brain-derived neurotrophic factor is associated with cognitive function in healthy older adults. J. Geriatr. Psychiatry Neurol. 21, 166–170. doi:10.1177/0891988708316860

Halldén, S., Sjögren, M., Hedblad, B., Engström, G., Narkiewicz, K., Hoffmann, M., Wahlstrand, B., Hedner, T., Melander, O., 2013. Smoking and obesity associated BDNF gene variance predicts total and cardiovascular mortality in smokers. Heart Br. Card. Soc. 99, 949–953. doi:10.1136/heartjnl-2013-303634

Hamani, C., Mayberg, H., Stone, S., Laxton, A., Haber, S., Lozano, A.M., 2011. The subcallosal cingulate gyrus in the context of major depression. Biol. Psychiatry 69, 301–308.

Hamilton, J.P., Siemer, M., Gotlib, I.H., 2008. Amygdala volume in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. Mol. Psychiatry 13, 993–1000. doi:10.1038/mp.2008.57

Hammond, F.M., Grattan, K.D., Sasser, H., Corrigan, J.D., Rosenthal, M., Bushnik, T., Shull, W., 2004. Five years after traumatic brain injury: A study of individual outcomes and predictors of change in function. NeuroRehabilitation 19, 25–35.

Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F., Weinberger, D.R., 2002. Serotonin Transporter Genetic Variation and the Response of the Human Amygdala. Science 297, 400 –403. doi:10.1126/science.1071829

Harrison-Felix, C., Whiteneck, G., Devivo, M.J., Hammond, F.M., Jha, A., 2006. Causes of death following 1 year postinjury among individuals with traumatic brain injury. J. Head Trauma Rehabil. 21, 22–33.

Harrison, P.J., Tunbridge, E.M., 2007. Catechol-O-Methyltransferase (COMT): A Gene Contributing to Sex Differences in Brain Function, and to Sexual Dimorphism in the Predisposition to Psychiatric Disorders. Neuropsychopharmacology 33, 3037–3045. doi:10.1038/sj.npp.1301543

Hashimoto, K., 2010. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. Psychiatry Clin. Neurosci. 64, 341–357. doi:10.1111/j.1440-1819.2010.02113.x

Heaton, R.K., 1981. Wisconsin Card Sorting Test Manual. Psychological Assesment Resources, Odessa, FL.

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., Lesch, K.P., 2002. Allelic Variation of Human Serotonin Transporter Gene Expression. J. Neurochem. 66, 2621–2624. doi:10.1046/j.1471-4159.1996.66062621.x

Hernández, T.D., 2006. Post-traumatic neural depression and neurobehavioral recovery after brain injury. J. Neurotrauma 23, 1211–1221. doi:10.1089/neu.2006.23.1211

Hertel, P.T., 1998. Relation between rumination and impaired memory in dysphoric moods. J. Abnorm. Psychol. 107, 166–172.

Hertel, P.T., Rude, S.S., 1991. Depressive deficits in memory: focusing attention improves subsequent recall. J. Exp. Psychol. Gen. 120, 301–309.

Hibbard, M.R., Ashman, T.A., Spielman, L.A., Chun, D., Charatz, H.J., Melvin, S., 2004. Relationship between depression and psychosocial functioning after traumatic brain injury. Arch. Phys. Med. Rehabil. 85, S43–53.

Hicks, R.R., Numan, S., Dhillon, H.S., Prasad, M.R., Seroogy, K.B., 1997. Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma. Brain Res. Mol. Brain Res. 48, 401–406.

Hinkelmann, K., Moritz, S., Botzenhardt, J., Riedesel, K., Wiedemann, K., Kellner, M., Otte, C., 2009. Cognitive impairment in major depression: association with salivary cortisol. Biol. Psychiatry 66, 879–885. doi:10.1016/j.biopsych.2009.06.023

Hoffman, J.M., Bell, K.R., Powell, J.M., Behr, J., Dunn, E.C., Dikmen, S., Bombardier, C.H., 2010. A randomized controlled trial of exercise to improve mood after traumatic brain injury. PM R 2, 911–919. doi:10.1016/j.pmrj.2010.06.008

Hohenadel, M.G., Thearle, M.S., Grice, B.A., Huang, H., Dai, M.-H., Tao, Y.-X., Hunter, L.A., Palaguachi, G.I., Mou, Z., Kim, R.C., Tsang, M.M., Haack, K., Voruganti, V.S., Cole, S.A., Butte, N.F., Comuzzie, A.G., Muller, Y.L., Baier, L.J., Krakoff, J., Knowler, W.C., Yanovski, J.A., Han, J.C., 2014. Brain-derived neurotrophic factor in human subjects with function-altering melanocortin-4 receptor variants. Int. J. Obes. 38, 1068–1074. doi:10.1038/ijo.2013.221

Hoh, N.Z., Wagner, A.K., Alexander, S.A., Clark, R.B., Beers, S.R., Okonkwo, D.O., Ren, D., Conley, Y.P., 2010. BCL2 genotypes: functional and neurobehavioral outcomes after severe traumatic brain injury. J. Neurotrauma 27, 1413–1427. doi:10.1089/neu.2009.1256

Homberg, J.R., Lesch, K.-P., 2011. Looking on the bright side of serotonin transporter gene variation. Biol. Psychiatry 69, 513–519. doi:10.1016/j.biopsych.2010.09.024

Honea, R., Verchinski, B.A., Pezawas, L., Kolachana, B.S., Callicott, J.H., Mattay, V.S., Weinberger, D.R., Meyer-Lindenberg, A., 2009. Impact of interacting functional variants in COMT on regional gray matter volume in human brain. NeuroImage 45, 44–51. doi:10.1016/j.neuroimage.2008.10.064

Hoofien, D., Gilboa, A., Vakil, E., Donovick, P.J., 2001. Traumatic brain injury (TBI) 10-20 years later: a comprehensive outcome study of psychiatric symptomatology, cognitive abilities and psychosocial functioning. Brain Inj. BI 15, 189–209. doi:10.1080/026990501300005659

Hoofien, D., Vakil, E., Gilboa, A., Donovick, P.J., Barak, O., 2002. Comparison of the predictive power of socio-economic variables, severity of injury and age on long-term outcome of traumatic brain injury: sample-specific variables versus factors as predictors. Brain Inj. BI 16, 9–27. doi:10.1080/02699050110088227

Ho, P.-S., Ho, K.K.-J., Huang, W.-S., Yen, C.-H., Shih, M.-C., Shen, L.-H., Ma, K.-H., Huang, S.-Y., n.d. Association study of serotonin transporter availability and SLC6A4 gene

polymorphisms in patients with major depression. Psychiatry Res. Neuroimaging. doi:10.1016/j.pscychresns.2012.04.005
Horch, H.W., Katz, L.C., 2002. BDNF release from single cells elicits local dendritic growth in nearby neurons. Nat Neurosci 5, 1177–1184. doi:10.1038/nn927
Horsfield, S.A., Rosse, R.B., Tomasino, V., Schwartz, B.L., Mastropaolo, J., Deutsch, S.I., 2002. Fluoxetine's effects on cognitive performance in patients with traumatic brain injury. Int. J. Psychiatry Med. 32, 337–344.
Hranilovic, D., Stefulj, J., Schwab, S., Borrmann-Hassenbach, M., Albus, M., Jernej, B., Wildenauer, D., 2004. Serotonin transporter promoter and intron 2 polymorphisms: relationship between allelic variants and gene expression. Biol. Psychiatry 55, 1090–1094. doi:10.1016/j.biopsych.2004.01.029
Hudak, A.M., Hynan, L.S., Harper, C.R., Diaz-Arrastia, R., 2012. Association of depressive symptoms with functional outcome after traumatic brain injury. J. Head Trauma Rehabil. 27, 87–98. doi:10.1097/HTR.0b013e3182114efd
Hudak, A., Warner, M., Marquez de la Plata, C., Moore, C., Harper, C., Diaz-Arrastia, R., 2011. Brain morphometry changes and depressive symptoms after traumatic brain injury. Psychiatry Res. Neuroimaging 191, 160–165.
Hyder, A.A., Wunderlich, C.A., Puvanachandra, P., Gururaj, G., Kobusingye, O.C., 2007. The impact of traumatic brain injuries: A global perspective. NeuroRehabilitation 22, 341–353.
Ikonomidou, C., Turski, L., 2002. Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? Lancet Neurol. 1, 383–386.
Irimia, A., Goh, S.-Y.M., Torgerson, C.M., Vespa, P.M., Van Horn, J.D., 2014. STRUCTURAL AND CONNECTOMIC NEUROIMAGING FOR THE PERSONALIZED STUDY OF LONGITUDINAL ALTERATIONS IN CORTICAL SHAPE, THICKNESS AND CONNECTIVITY AFTER TRAUMATIC BRAIN INJURY. J. Neurosurg. Sci. 58, 129–144.
Jacobs, E., D'Esposito, M., 2011. Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. J. Neurosci. Off. J. Soc. Neurosci. 31, 5286–5293. doi:10.1523/JNEUROSCI.6394-10.2011
Jacobson, L., Sapolsky, R., 1991. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr. Rev. 12, 118–134.
Jennett, B., Bond, M., 1975. Assessment of outcome after severe brain damage. Lancet 1, 480–484.
Jennifer Fleming, Leigh Tooth, Mary, 1999. Prediction of community integration and vocational outcome 2-5 years after traumatic brain injury rehabilitation in Australia. Brain Inj. 13, 417–431. doi:10.1080/026990599121476
Jiang, L., Liu, C., Tong, J., Mao, R., Chen, D., Wang, H., Huang, J., Li, L., 2014. Fluoxetine Pretreatment Promotes Neuronal Survival and Maturation after Auditory Fear Conditioning in the Rat Amygdala. PLoS ONE 9, e89147. doi:10.1371/journal.pone.0089147
Joëls, M., 2008. Functional actions of corticosteroids in the hippocampus. Eur. J. Pharmacol. 583, 312–321. doi:10.1016/j.ejphar.2007.11.064
Joormann, J., Dkane, M., Gotlib, I.H., 2006. Adaptive and maladaptive components of rumination? Diagnostic specificity and relation to depressive biases. Behav. Ther. 37, 269–280.
Jorge, R.E., Arciniegas, D.B., 2014. Mood Disorders After TBI. Psychiatr. Clin. North Am., Neuropsychiatry of Traumatic Brain Injury 37, 13–29. doi:10.1016/j.psc.2013.11.005

Jorge, R.E., Robinson, R.G., Arndt, S.V., Starkstein, S.E., Forrester, A.W., Geisler, F., 1993. Depression following traumatic brain injury: a 1 year longitudinal study. J. Affect. Disord. 27, 233–243. doi:16/0165-0327(93)90047-N

Jorge, R.E., Robinson, R.G., Moser, D., Tateno, A., Crespo-Facorro, B., Arndt, S., 2004. Major depression following traumatic brain injury. Arch. Gen. Psychiatry 61, 42–50. doi:10.1001/archpsyc.61.1.42

Jorge, R.E., Robinson, R.G., Starkstein, S.E., Arndt, S.V., 1994. Influence of major depression on 1-year outcome in patients with traumatic brain injury. J. Neurosurg. 81, 726–733. doi:10.3171/jns.1994.81.5.0726

Juengst, S.B., Kumar, R.G., Failla, M.D., Goyal, A., Wagner, A.K., 2014. Acute Inflammatory Biomarker Profiles Predict Depression Risk Following Moderate to Severe Traumatic Brain Injury. J. Head Trauma Rehabil. doi:10.1097/HTR.0000000000000031

Juengst, S., Skidmore, E., Arenth, P.M., Niyonkuru, C., Raina, K.D., 2013. Unique contribution of fatigue to disability in community-dwelling adults with traumatic brain injury. Arch. Phys. Med. Rehabil. 94, 74–79. doi:10.1016/j.apmr.2012.07.025

Kalish, H., Phillips, T.M., 2010. Analysis of neurotrophins in human serum by immunoaffinity capillary electrophoresis (ICE) following traumatic head injury. J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci. 878, 194–200. doi:10.1016/j.jchromb.2009.10.022

Kaplan, G.B., Vasterling, J.J., Vedak, P.C., 2010. Brain-derived neurotrophic factor in traumatic brain injury, post-traumatic stress disorder, and their comorbid conditions: role in pathogenesis and treatment. Behav. Pharmacol. 21, 427–437. doi:10.1097/FBP.0b013e32833d8bc9

Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J.-M., Bertschy, G., 2005. Low Brain-Derived Neurotrophic Factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biol. Psychiatry 57, 1068–1072. doi:10.1016/j.biopsych.2005.01.008

Karege, F., Perret, G., Bondolfi, G., Schwald, M., Bertschy, G., Aubry, J.-M., 2002. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res. 109, 143–148.

Karg, K., Burmeister, M., Shedden, K., Sen, S., 2011. The Serotonin Transporter Promoter Variant (5-HTTLPR), Stress, and Depression Meta-analysis Revisited: Evidence of Genetic Moderation. Arch Gen Psychiatry 68, 444–454. doi:10.1001/archgenpsychiatry.2010.189

Kasselman, L.J., Sideris, A., Bruno, C., Perez, W.R., Cai, N., Nicoletti, J.N., Wiegand, S.J., Croll, S.D., 2006. BDNF: A missing link between sympathetic dysfunction and inflammatory disease? J. Neuroimmunol. 175, 118–127. doi:10.1016/j.jneuroim.2006.03.008

Katz, D.I., Alexander, M.P., 1994. Traumatic brain injury. Predicting course of recovery and outcome for patients admitted to rehabilitation. Arch. Neurol. 51, 661–670.

Katz, D.I., Alexander, M.P., Klein, R.B., 1998. Recovery of arm function in patients with paresis after traumatic brain injury. Arch. Phys. Med. Rehabil. 79, 488–493.

Kenna, G.A., Roder-Hanna, N., Leggio, L., Zywiak, W.H., Clifford, J., Edwards, S., Kenna, J.A., Shoaff, J., Swift, R.M., 2012. Association of the 5-HTT gene-linked promoter region (5-HTTLPR) polymorphism with psychiatric disorders: review of psychopathology and pharmacotherapy. Pharmacogenomics Pers. Med. 5, 19–35. doi:10.2147/PGPM.S23462

Kennedy, K.M., Rodrigue, K.M., Land, S.J., Raz, N., 2009. BDNF Val66Met polymorphism influences age differences in microstructure of the Corpus Callosum. Front. Hum. Neurosci. 3, 19. doi:10.3389/neuro.09.019.2009

Kernie, S.G., Liebl, D.J., Parada, L.F., 2000. BDNF regulates eating behavior and locomotor activity in mice. EMBO J. 19, 1290–1300. doi:10.1093/emboj/19.6.1290

Kessler, R.C., Chiu, W.T., Demler, O., Walters, E.E., 2005. Prevalence, Severity, and Comorbidity of 12-Month DSM-IV Disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62, 617–627. doi:10.1001/archpsyc.62.6.617

Kim, J., Avants, B., Patel, S., Whyte, J., Coslett, B.H., Pluta, J., Detre, J.A., Gee, J.C., 2008. Structural consequences of diffuse traumatic brain injury: a large deformation tensor-based morphometry study. NeuroImage 39, 1014–1026. doi:10.1016/j.neuroimage.2007.10.005

Kim, J., Whyte, J., Patel, S., Europa, E., Wang, J., Coslett, H.B., Detre, J.A., 2011. Methylphenidate modulates sustained attention and cortical activation in survivors of traumatic brain injury: a perfusion fMRI study. Psychopharmacology (Berl.). doi:10.1007/s00213-011-2622-8

Kim, S.N., Kang, D.-H., Yun, J.-Y., Lee, T.Y., Jung, W.H., Jang, J.H., Kwon, J.S., 2013. Impact of the BDNF Val66Met Polymorphism on Regional Brain Gray Matter Volumes: Relevance to the Stress Response. Psychiatry Investig. 10, 173–179. doi:10.4306/pi.2013.10.2.173

Kline, A.E., Yu, J., Massucci, J.L., Zafonte, R.D., Dixon, C.E., 2002. Protective effects of the 5-HT1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin against traumatic brain injury-induced cognitive deficits and neuropathology in adult male rats. Neurosci. Lett. 333, 179–182. doi:10.1016/S0304-3940(02)01101-1

Kline, A.., Yu, J., HorvÃ¡th, E., Marion, D.., Dixon, C.., 2001. The selective 5-HT1A receptor agonist repinotan HCl attenuates histopathology and spatial learning deficits following traumatic brain injury in rats. Neuroscience 106, 547–555. doi:10.1016/S0306-4522(01)00300-1

Kobiella, A., Reimold, M., Ulshofer, D.E., Ikonomidou, V.N., Vollmert, C., Vollstadt-Klein, S., Rietschel, M., Reischl, G., Heinz, A., Smolka, M.N., 2011. How the serotonin transporter 5-HTTLPR polymorphism influences amygdala function: the roles of in vivo serotonin transporter expression and amygdala structure. Transl Psychiatry 1, e37.

Koolschijn, P.C.M.P., van Haren, N.E.M., Lensvelt-Mulders, G.J.L.M., Hulshoff Pol, H.E., Kahn, R.S., 2009. Brain volume abnormalities in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. Hum. Brain Mapp. 30, 3719–3735. doi:10.1002/hbm.20801

Koponen, S., Taiminen, T., Portin, R., Himanen, L., Isoniemi, H., Heinonen, H., Hinkka, S., Tenovuo, O., 2002. Axis I and II psychiatric disorders after traumatic brain injury: a 30-year follow-up study. Am. J. Psychiatry 159, 1315–1321.

Kouneiher, F., Charron, S., Koechlin, E., 2009. Motivation and cognitive control in the human prefrontal cortex. Nat. Neurosci. 12, 939–945. doi:10.1038/nn.2321

Kovalchuk, Y., Hanse, E., Kafitz, K.W., Konnerth, A., 2002. Postsynaptic Induction of BDNF-Mediated Long-Term Potentiation. Science 295, 1729–1734. doi:10.1126/science.1067766

Krabbe, K.S., Mortensen, E.L., Avlund, K., Pedersen, A.N., Pedersen, B.K., Jørgensen, T., Bruunsgaard, H., 2009. Brain-derived neurotrophic factor predicts mortality risk in older women. J. Am. Geriatr. Soc. 57, 1447–1452. doi:10.1111/j.1532-5415.2009.02345.x

Kraus, M.F., Susmaras, T., Caughlin, B.P., Walker, C.J., Sweeney, J.A., Little, D.M., 2007. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain 130, 2508 –2519. doi:10.1093/brain/awm216

Krishnan, V., Nestler, E.J., 2008. The molecular neurobiology of depression. Nature 455, 894–902. doi:10.1038/nature07455

Kritzer, M.F., Creutz, L.M., 2008. Region and sex differences in constituent dopamine neurons and immunoreactivity for intracellular estrogen and androgen receptors in mesocortical projections in rats. J. Neurosci. Off. J. Soc. Neurosci. 28, 9525–9535. doi:10.1523/JNEUROSCI.2637-08.2008

Krueger, F., Pardini, M., Huey, E.D., Raymont, V., Solomon, J., Lipsky, R.H., Hodgkinson, C.A., Goldman, D., Grafman, J., 2011. The role of the Met66 brain-derived neurotrophic factor allele in the recovery of executive functioning after combat-related traumatic brain injury. J. Neurosci. Off. J. Soc. Neurosci. 31, 598–606. doi:10.1523/JNEUROSCI.1399-10.2011

Kumar, A., Stoica, B.A., Sabirzhanov, B., Burns, M.P., Faden, A.I., Loane, D.J., 2013. Traumatic brain injury in aged animals increases lesion size and chronically alters microglial/macrophage classical and alternative activation states. Neurobiol. Aging 34, 1397–1411. doi:10.1016/j.neurobiolaging.2012.11.013

Kumar, R.G., Boles, J.A., Wagner, A.K., 2014. Chronic Inflammation After Severe Traumatic Brain Injury: Characterization and Associations With Outcome at 6 and 12 Months Postinjury. J. Head Trauma Rehabil. doi:10.1097/HTR.0000000000000067

Kwon, S.-K.C., Kovesdi, E., Gyorgy, A.B., Wingo, D., Kamnaksh, A., Walker, J., Long, J.B., Agoston, D.V., 2011. Stress and Traumatic Brain Injury: A Behavioral, Proteomics, and Histological Study. Front. Neurol. 2. doi:10.3389/fneur.2011.00012

Laakso, A., Pohjalainen, T., Bergman, J., Kajander, J., Haaparanta, M., Solin, O., Syvälahti, E., Hietala, J., 2005. The A1 allele of the human D2 dopamine receptor gene is associated with increased activity of striatal L-amino acid decarboxylase in healthy subjects. Pharmacogenet. Genomics 15, 387–391.

Laasonen-Balk, T., Kuikka, J., Viinamäki, H., Husso-Saastamoinen, M., Lehtonen, J., Tiihonen, J., 1999. Striatal dopamine transporter density in major depression. Psychopharmacology (Berl.) 144, 282–285.

Laatikainen, L.M., Sharp, T., Bannerman, D.M., Harrison, P.J., Tunbridge, E.M., 2012. Modulation of hippocampal dopamine metabolism and hippocampal-dependent cognitive function by catechol-O-methyltransferase inhibition. J. Psychopharmacol. Oxf. Engl. 26, 1561–1568. doi:10.1177/0269881112454228

Laine, T.P., Ahonen, A., Räsänen, P., Pohjalainen, T., Tiihonen, J., Hietala, J., 2001. The A1 allele of the D2 dopamine receptor gene is associated with high dopamine transporter density in detoxified alcoholics. Alcohol Alcohol. Oxf. Oxfs. 36, 262–265.

Lamar, M., Craig, M., Daly, E.M., Cutter, W.J., Tang, C., Brammer, M., Rubia, K., Murphy, D.G.M., 2012. Acute tryptophan depletion promotes an anterior-to-posterior fMRI activation shift during task switching in older adults. Hum. Brain Mapp. doi:10.1002/hbm.22187

Lanctôt, K.L., Rapoport, M.J., Chan, F., Rajaram, R.D., Strauss, J., Sicard, T., McCullagh, S., Feinstein, A., Kiss, A., Kennedy, J.L., Bassett, A.S., Herrmann, N., 2010. Genetic predictors of response to treatment with citalopram in depression secondary to traumatic brain injury. Brain Inj. BI 24, 959–969. doi:10.3109/02699051003789229

Lang, U.E., Bajbouj, M., Sander, T., Gallinat, J., 2007a. Gender-Dependent Association of the Functional Catechol-O-Methyltransferase Val158Met Genotype with Sensation Seeking Personality Trait. Neuropsychopharmacology 32, 1950–1955. doi:10.1038/sj.npp.1301335

Lang, U.E., Hellweg, R., Sander, T., Gallinat, J., 2009. The Met allele of the BDNF Val66Met polymorphism is associated with increased BDNF serum concentrations. Mol Psychiatry 14, 120–122.

Lang, U.E., Hellweg, R., Seifert, F., Schubert, F., Gallinat, J., 2007b. Correlation Between Serum Brain-Derived Neurotrophic Factor Level and An In Vivo Marker of Cortical Integrity. Biol. Psychiatry 62, 530–535. doi:10.1016/j.biopsych.2007.01.002

Larrabee, G.J., Curtiss, G., 1995. Construct validity of various verbal and visual memory tests. J. Clin. Exp. Neuropsychol. 17, 536–547. doi:10.1080/01688639508405144

Larrabee, G.J., Curtiss, G., 1995. Construct validity of various verbal and visual memory tests. J. Clin. Exp. Neuropsychol. 17, 536–547. doi:10.1080/01688639508405144

Larson, E.B., Zollman, F.S., 2010. The effect of sleep medications on cognitive recovery from traumatic brain injury. J. Head Trauma Rehabil. 25, 61–67. doi:10.1097/HTR.0b013e3181c1d1e1

Lazarus, L.W., Winemiller, D.R., Lingam, V.R., Neyman, I., Hartman, C., Abassian, M., Kartan, U., Groves, L., Fawcett, J., 1992. Efficacy and side effects of methylphenidate for poststroke depression. J. Clin. Psychiatry 53, 447–449.

Lee, F.J., Pei, L., Moszczynska, A., Vukusic, B., Fletcher, P.J., Liu, F., 2007. Dopamine transporter cell surface localization facilitated by a direct interaction with the dopamine D2 receptor. EMBO J. 26, 2127–2136. doi:10.1038/sj.emboj.7601656

Lee, H., Kim, S.-W., Kim, J.-M., Shin, I.-S., Yang, S.-J., Yoon, J.-S., 2005. Comparing effects of methylphenidate, sertraline and placebo on neuropsychiatric sequelae in patients with traumatic brain injury. Hum. Psychopharmacol. 20, 97–104. doi:10.1002/hup.668

Lee, P., Kim, J., Williams, R., Sandhir, R., Gregory, E., Brooks, W.M., Berman, N.E.J., 2012. Effects of aging on blood brain barrier and matrix metalloproteases following controlled cortical impact in mice. Exp. Neurol. 234, 50–61. doi:10.1016/j.expneurol.2011.12.016

León-Carrión, J., De Serdio-Arias, M.L., Cabezas, F.M., Roldán, J.M., Domínguez-Morales, R., Martín, J.M., Sanchez, M.A., 2001. Neurobehavioural and cognitive profile of traumatic brain injury patients at risk for depression and suicide. Brain Inj. BI 15, 175–181. doi:10.1080/026990501458407

León-Carrión, J., Machuca-Murga, F., Solís-Marcos, I., León-Domínguez, U., Domínguez-Morales, M.D.R., 2013. The sooner patients begin neurorehabilitation, the better their functional outcome. Brain Inj. BI 27, 1119–1123. doi:10.3109/02699052.2013.804204

Lesch, K.-P., 2011. When the serotonin transporter gene meets adversity: the contribution of animal models to understanding epigenetic mechanisms in affective disorders and resilience. Curr. Top. Behav. Neurosci. 7, 251–280. doi:10.1007/7854_2010_109

Lesch, K.-P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Müller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of Anxiety-Related Traits with a Polymorphism in the Serotonin Transporter Gene Regulatory Region. Science 274, 1527–1531. doi:10.1126/science.274.5292.1527

Levin, H., Benton, A., Grossman, R.G., 1982. Neurobehavioral Consequences of Closed Head Injury. Oxford University Press, New York.

Levin, H.S., Grossman, R.G., Rose, J.E., Teasdale, G., 1979. Long-term neuropsychological outcome of closed head injury. J. Neurosurg. 50, 412–422. doi:10.3171/jns.1979.50.4.0412

Levin, R.L., Heller, W., Mohanty, A., Herrington, J.D., Miller, G.A., 2007. Cognitive deficits in depression and functional specificity of regional brain activity. Cogn. Ther. Res. 31, 211–233.

Lewis, F.D., Horn, G.J., 2013. Traumatic brain injury: analysis of functional deficits and posthospital rehabilitation outcomes. J. Spec. Oper. Med. Peer Rev. J. SOF Med. Prof. 13, 56–61.

Lindvall, O., Kokaia, Z., Bengzon, J., Elme´r, E., Kokaia, M., 1994. Neurotrophins and brain insults. Trends Neurosci. 17, 490–496. doi:10.1016/0166-2236(94)90139-2

Lipsky, R.H., Sparling, M.B., Ryan, L.M., Xu, K., Salazar, A.M., Goldman, D., Warden, D.L., 2005a. Association of COMT Val158Met Genotype With Executive Functioning Following Traumatic Brain Injury. J. Neuropsychiatry Clin. Neurosci. 17, 465–471. doi:10.1176/appi.neuropsych.17.4.465

Lipsky, R.H., Sparling, M.B., Ryan, L.M., Xu, K., Salazar, A.M., Goldman, D., Warden, D.L., 2005b. Association of COMT Val158Met Genotype With Executive Functioning Following Traumatic Brain Injury. J. Neuropsychiatry Clin. Neurosci. 17, 465–471. doi:10.1176/appi.neuropsych.17.4.465

Little, K.Y., McLaughlin, D.P., Zhang, L., Livermore, C.S., Dalack, G.W., McFinton, P.R., DelProposto, Z.S., Hill, E., Cassin, B.J., Watson, S.J., Cook, E.H., 1998. Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. Am. J. Psychiatry 155, 207–213.

Lommatzsch, M., Zingler, D., Schuhbaeck, K., Schloetcke, K., Zingler, C., Schuff-Werner, P., Virchow, J.C., 2005. The impact of age, weight and gender on BDNF levels in human platelets and plasma. Neurobiol. Aging 26, 115–123. doi:10.1016/j.neurobiolaging.2004.03.002

Lopez-Leon, S., Janssens, A.C.J.W., Gonzalez-Zuloeta Ladd, A.M., Del-Favero, J., Claes, S.J., Oostra, B.A., van Duijn, C.M., 2007. Meta-analyses of genetic studies on major depressive disorder. Mol Psychiatry 13, 772–785.

Lotrich, F.E., Pollock, B.G., Ferrell, R.E., 2003. Serotonin transporter promoter polymorphism in African Americans : allele frequencies and implications for treatment. Am. J. Pharmacogenomics Genomics-Relat. Res. Drug Dev. Clin. Pract. 3, 145–147.

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melén, K., Julkunen, I., Taskinen, J., 1995. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry (Mosc.) 34, 4202–4210.

Lu, L., Dempsey, J., Liu, S.Y., Bossert, J.M., Shaham, Y., 2004. A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. J. Neurosci. Off. J. Soc. Neurosci. 24, 1604–1611. doi:10.1523/JNEUROSCI.5124-03.2004

Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10, 434–445.

Maas, A.I.R., Murray, G.D., Roozenbeek, B., Lingsma, H.F., Butcher, I., McHugh, G.S., Weir, J., Lu, J., Steyerberg, E.W., 2013. Advancing care for traumatic brain injury: findings from the IMPACT studies and perspectives on future research. Lancet Neurol. 12, 1200–1210. doi:10.1016/S1474-4422(13)70234-5

MacKenzie, J.D., Siddiqi, F., Babb, J.S., Bagley, L.J., Mannon, L.J., Sinson, G.P., Grossman, R.I., 2002. Brain atrophy in mild or moderate traumatic brain injury: a longitudinal quantitative analysis. Am. J. Neuroradiol. 23, 1509–1515.

Mak, K.K., Kong, W.Y., Mak, A., Sharma, V.K., Ho, R.C.M., 2012. Polymorphisms of the serotonin transporter gene and post-stroke depression: a meta-analysis. J. Neurol. Neurosurg. Psychiatry. doi:10.1136/jnnp-2012-303791

Malec, J.F., Brown, A.W., Moessner, A.M., Stump, T.E., Monahan, P., 2010. A preliminary model for posttraumatic brain injury depression. Arch. Phys. Med. Rehabil. 91, 1087–1097. doi:10.1016/j.apmr.2010.04.002

Malhotra, A.K., Kestler, L.J., Mazzanti, C., Bates, J.A., Goldberg, T., Goldman, D., 2002. A Functional Polymorphism in the COMT Gene and Performance on a Test of Prefrontal Cognition. Am. J. Psychiatry 159, 652–654. doi:10.1176/appi.ajp.159.4.652

Maller, J.J., Thomson, R.H.S., Lewis, P.M., Rose, S.E., Pannek, K., Fitzgerald, P.B., 2010. Traumatic brain injury, major depression, and diffusion tensor imaging: making connections. Brain Res. Rev. 64, 213–240. doi:10.1016/j.brainresrev.2010.04.003

Maller, J.J., Thomson, R.H.S., Pannek, K., Bailey, N., Lewis, P.M., Fitzgerald, P.B., 2014. Volumetrics relate to the development of depression after traumatic brain injury. Behav. Brain Res. 271, 147–153. doi:10.1016/j.bbr.2014.05.047

Mamounas, L.A., Altar, C.A., Blue, M.E., Kaplan, D.R., Tessarollo, L., Lyons, W.E., 2000. BDNF Promotes the Regenerative Sprouting, But Not Survival, of Injured Serotonergic Axons in the Adult Rat Brain. J Neurosci 20, 771–782.

Männistö, P.T., Kaakkola, S., 1999. Catechol-O-methyltransferase (COMT): Biochemistry, Molecular Biology, Pharmacology, and Clinical Efficacy of the New Selective COMT Inhibitors. Pharmacol. Rev. 51, 593–628.

Martinowich, K., Lu, B., 2008. Interaction between BDNF and serotonin: role in mood disorders. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 33, 73–83. doi:10.1038/sj.npp.1301571

Martinowich, K., Manji, H., Lu, B., 2007. New insights into BDNF function in depression and anxiety. Nat. Neurosci. 10, 1089–1093. doi:10.1038/nn1971

Matsumoto, T., Rauskolb, S., Polack, M., Klose, J., Kolbeck, R., Korte, M., Barde, Y.-A., 2008. Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat. Neurosci. 11, 131–133. doi:10.1038/nn2038

Mattay, V.S., Goldberg, T.E., Fera, F., Hariri, A.R., Tessitore, A., Egan, M.F., Kolachana, B., Callicott, J.H., Weinberger, D.R., 2003. Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. Proc. Natl. Acad. Sci. 100, 6186–6191. doi:10.1073/pnas.0931309100

Mattson, M.P., Maudsley, S., Martin, B., 2004. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci. 27, 589–594. doi:10.1016/j.tins.2004.08.001

Mayberg, H.S., 2003. Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. Br. Med. Bull. 65, 193–207. doi:10.1093/bmb/65.1.193

McAllister, T.W., Flashman, L.A., Harker Rhodes, C., Tyler, A.L., Moore, J.H., Saykin, A.J., McDonald, B.C., Tosteson, T.D., Tsongalis, G.J., 2008. Single nucleotide polymorphisms in ANKK1 and the dopamine D2 receptor gene affect cognitive outcome shortly after traumatic brain injury: a replication and extension study. Brain Inj. BI 22, 705–714. doi:10.1080/02699050802263019

McAllister, T.W., Rhodes, C.H., Flashman, L.A., McDonald, B.C., Belloni, D., Saykin, A.J., 2005. Effect of the dopamine D2 receptor T allele on response latency after mild traumatic brain injury. Am. J. Psychiatry 162, 1749–1751. doi:10.1176/appi.ajp.162.9.1749

McCullagh, S., Feinstein, M., 2004. Cognitive impairment, in: Textbook of Traumatic Brain Injury. American Psychiatric Press, Washington, DC, p. 321—336.

McNett, M., 2007. A review of the predictive ability of Glasgow Coma Scale scores in head-injured patients. J. Neurosci. Nurs. J. Am. Assoc. Neurosci. Nurses 39, 68–75.

Mercader, J.M., Ribasés, M., Gratacòs, M., González, J.R., Bayés, M., De Cid, R., Badía, A., Fernández-Aranda, F., Estivill, X., 2007. Altered brain-derived neurotrophic factor blood levels and gene variability are associated with anorexia and bulimia. Genes Brain Behav. 6, 706–716. doi:10.1111/j.1601-183X.2007.00301.x

Mettenburg, J.M., Benzinger, T.L., Shimony, J.S., Snyder, A.Z., Sheline, Y.I., 2012. Diminished performance on neuropsychological testing in late life depression is correlated with microstructural white matter abnormalities. Neuroimage 60, 2182–2190.

Meyer, J.H., Krüger, S., Wilson, A.A., Christensen, B.K., Goulding, V.S., Schaffer, A., Minifie, C., Houle, S., Hussey, D., Kennedy, S.H., 2001. Lower dopamine transporter binding potential in striatum during depression. Neuroreport 12, 4121.

Mi, H., Thomas, P.D., Ring, H.Z., Jiang, R., Sangkuhl, K., Klein, T.E., Altman, R.B., 2011. PharmGKB summary: dopamine receptor D2. Pharmacogenet. Genomics 21, 350–356. doi:10.1097/FPC.0b013e32833ee605

Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. Biol. Psychiatry 65, 732–741. doi:10.1016/j.biopsych.2008.11.029

Miller, G., Madras, B., others, 2002. Polymorphisms in the 3'-untranslated region of human and monkey dopamine transporter genes affect reporter gene expression. Mol. Psychiatry 7, 44.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215. doi:10.1093/nar/16.3.1215

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

Mill, J., Asherson, P., Browes, C., D'Souza, U., Craig, I., 2002. Expression of the dopamine transporter gene is regulated by the 3′ UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. Am. J. Med. Genet. 114, 975–979.

Monakhov, M., Golimbet, V., Abramova, L., Kaleda, V., Karpov, V., 2008. Association study of three polymorphisms in the dopamine D2 receptor gene and schizophrenia in the Russian population. Schizophr. Res. 100, 302–307. doi:10.1016/j.schres.2008.01.007

Montag, C., Markett, S., Basten, U., Stelzel, C., Fiebach, C., Canli, T., Reuter, M., 2010. Epistasis of the DRD2/ANKK1 Taq Ia and the BDNF Val66Met polymorphism impacts novelty seeking and harm avoidance. Neuropsychopharmacology 35, 1860–1867.

Morey, R.A., Petty, C.M., Xu, Y., Hayes, J.P., Wagner, H.R., Lewis, D.V., LaBar, K.S., Styner, M., McCarthy, G., 2009. A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. NeuroImage 45, 855–866. doi:10.1016/j.neuroimage.2008.12.033

Mori, T., Wang, X., Kline, A.E., Siao, C.J., Dixon, C.E., Tsirka, S.E., Lo, E.H., 2001. Reduced cortical injury and edema in tissue plasminogen activator knockout mice after brain trauma. Neuroreport 12, 4117–4120.

Moritz, K.E., Geeck, K., Underly, R.G., Searles, M., Smith, J.S., 2014. Post-operative environmental enrichment improves spatial and motor deficits but may not ameliorate anxiety- or depression-like symptoms in rats following traumatic brain injury. Restor. Neurol. Neurosci. 32, 701–716. doi:10.3233/RNN-140414

Munafò, M.R., Matheson, I.J., Flint, J., 2007. Association of the DRD2 gene Taq1A polymorphism and alcoholism: a meta-analysis of case–control studies and evidence of publication bias. Mol. Psychiatry 12, 454–461. doi:10.1038/sj.mp.4001938

Munro, C.A., McCaul, M.E., Wong, D.F., Oswald, L.M., Zhou, Y., Brasic, J., Kuwabara, H., Kumar, A., Alexander, M., Ye, W., Wand, G.S., 2006. Sex Differences in Striatal Dopamine Release in Healthy Adults. Biol. Psychiatry 59, 966–974. doi:10.1016/j.biopsych.2006.01.008

Nagamoto-Combs, K., McNeal, D.W., Morecraft, R.J., Combs, C.K., 2007. Prolonged microgliosis in the rhesus monkey central nervous system after traumatic brain injury. J. Neurotrauma 24, 1719–1742. doi:10.1089/neu.2007.0377

Nakahashi, T., Fujimura, H., Altar, C.A., Li, J., Kambayashi, J., Tandon, N.N., Sun, B., 2000. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. FEBS Lett. 470, 113–117. doi:10.1016/S0014-5793(00)01302-8

Narayan, R.K., Michel, M.E., Ansell, B., Baethmann, A., Biegon, A., Bracken, M.B., Bullock, M.R., Choi, S.C., Clifton, G.L., Contant, C.F., Coplin, W.M., Dietrich, W.D., Ghajar, J., Grady, S.M., Grossman, R.G., Hall, E.D., Heetderks, W., Hovda, D.A., Jallo, J., Katz, R.L., Knoller, N., Kochanek, P.M., Maas, A.I., Majde, J., Marion, D.W., Marmarou, A., Marshall, L.F., McIntosh, T.K., Miller, E., Mohberg, N., Muizelaar, J.P., Pitts, L.H., Quinn, P., Riesenfeld, G., Robertson, C.S., Strauss, K.I., Teasdale, G., Temkin, N., Tuma, R., Wade, C., Walker, M.D., Weinrich, M., Whyte, J., Wilberger, J., Young, A.B., Yurkewicz, L., 2002. Clinical trials in head injury. J. Neurotrauma 19, 503–557. doi:10.1089/089771502753754037

Nettiksimmons, J., Simonsick, E.M., Harris, T., Satterfield, S., Rosano, C., Yaffe, K., for the Health ABC Study, 2014. The Associations between Serum Brain-Derived Neurotrophic Factor, Potential Confounders, and Cognitive Decline: A Longitudinal Study. PLoS ONE 9, e91339. doi:10.1371/journal.pone.0091339

Neurobehavioral Guidelines Working Group, Warden, D.L., Gordon, B., McAllister, T.W., Silver, J.M., Barth, J.T., Bruns, J., Drake, A., Gentry, T., Jagoda, A., Katz, D.I., Kraus, J., Labbate, L.A., Ryan, L.M., Sparling, M.B., Walters, B., Whyte, J., Zapata, A., Zitnay, G., 2006. Guidelines for the pharmacologic treatment of neurobehavioral sequelae of traumatic brain injury. J. Neurotrauma 23, 1468–1501. doi:10.1089/neu.2006.23.1468

Neville, M.J., Johnstone, E.C., Walton, R.T., 2004. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum. Mutat. 23, 540–545. doi:10.1002/humu.20039

Newcomer, J., Craft, S., Hershey, T., Askins, K., Bardgett, M., 1994. Glucocorticoid-induced impairment in declarative memory performance in adult humans. J. Neurosci. 14, 2047 – 2053.

Nibuya, M., Nestler, E., Duman, R., 1996. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 16, 2365–2372.

Niogi, S.N., Mukherjee, P., Ghajar, J., Johnson, C.E., Kolster, R., Lee, H., Suh, M., Zimmerman, R.D., Manley, G.T., McCandliss, B.D., 2008. Structural dissociation of attentional control

and memory in adults with and without mild traumatic brain injury. Brain 131, 3209–3221. doi:10.1093/brain/awn247

Niogi, S.N., Mukherjee, P., Ghajar, J., Johnson, C., Kolster, R.A., Sarkar, R., Lee, H., Meeker, M., Zimmerman, R.D., Manley, G.T., McCandliss, B.D., 2008. Extent of Microstructural White Matter Injury in Postconcussive Syndrome Correlates with Impaired Cognitive Reaction Time: A 3T Diffusion Tensor Imaging Study of Mild Traumatic Brain Injury. AJNR Am J Neuroradiol 29, 967–973. doi:10.3174/ajnr.A0970

Nugent, A.C., Luckenbaugh, D.A., Wood, S.E., Bogers, W., Zarate, C.A., Jr, Drevets, W.C., 2013. Automated subcortical segmentation using FIRST: Test-retest reliability, interscanner reliability, and comparison to manual segmentation. Hum. Brain Mapp. 34, 2313–2329. doi:10.1002/hbm.22068

Nutt, D.J., 2006. The role of dopamine and norepinephrine in depression and antidepressant treatment. J. Clin. Psychiatry 67 Suppl 6, 3–8.

Oakley, R., Tharakan, B., 2014. Vascular hyperpermeability and aging. Aging Dis. 5, 114–125. doi:10.14336/AD.2014.0500114

Ogilvie, A.D., Battersby, S., Bubb, V.J., Fink, G., Harmar, A.J., Goodwim, G.M., Smith, C.A., 1996. Polymorphism in serotonin transporter gene associated with susceptibility to major depression. Lancet 347, 731–733.

O'Hara, R., Miller, E., Liao, C.-P., Way, N., Lin, X., Hallmayer, J., 2006. COMT genotype, gender and cognition in community-dwelling, older adults. Neurosci. Lett. 409, 205–209. doi:10.1016/j.neulet.2006.09.047

Olvera-Cortés, M.E., Anguiano-Rodríguez, P., López-Vázquez, M.A., Alfaro, J.M.C., 2008. Serotonin/dopamine interaction in learning. Prog. Brain Res. 172, 567–602. doi:10.1016/S0079-6123(08)00927-8

Onyszchuk, G., He, Y.-Y., Berman, N.E.J., Brooks, W.M., 2008. Detrimental effects of aging on outcome from traumatic brain injury: a behavioral, magnetic resonance imaging, and histological study in mice. J. Neurotrauma 25, 153–171. doi:10.1089/neu.2007.0430

Opmeer, E.M., Kortekaas, R., Aleman, A., 2010. Depression and the role of genes involved in dopamine metabolism and signalling. Prog. Neurobiol. 92, 112–133.

Orefice, L.L., Waterhouse, E.G., Partridge, J.G., Lalchandani, R.R., Vicini, S., Xu, B., 2013. Distinct roles for somatically and dendritically synthesized brain-derived neurotrophic factor in morphogenesis of dendritic spines. J. Neurosci. Off. J. Soc. Neurosci. 33, 11618–11632. doi:10.1523/JNEUROSCI.0012-13.2013

Osterrieth, P., 1944. The Complex Figure Copy Test. Arch. Psychol. 30, 206–356.

Osterrieth, P., 1944. The Complex Figure Copy Test. Arch Psychol 206–256.

Owens, M.J., Nemeroff, C.B., 1994. Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin. Chem. 40, 288–295.

Pacheco, J., Beevers, C.G., Benavides, C., McGeary, J., Stice, E., Schnyer, D.M., 2009. Frontal-limbic white matter pathway associations with the serotonin transporter gene promoter region (5-HTTLPR) polymorphism. J. Neurosci. Off. J. Soc. Neurosci. 29, 6229–6233. doi:10.1523/JNEUROSCI.0896-09.2009

Palacios, E.M., Sala-Llonch, R., Junque, C., Fernandez-Espejo, D., Roig, T., Tormos, J.M., Bargallo, N., Vendrell, P., 2013. Long-term declarative memory deficits in diffuse TBI: correlations with cortical thickness, white matter integrity and hippocampal volume. Cortex J. Devoted Study Nerv. Syst. Behav. 49, 646–657. doi:10.1016/j.cortex.2012.02.011

Palacios, E.M., Sala-Llonch, R., Junque, C., Roig, T., Tormos, J.M., Bargallo, N., Vendrell, P., 2013. Resting-state functional magnetic resonance imaging activity and connectivity and cognitive outcome in traumatic brain injury. JAMA Neurol. 70, 845–851. doi:10.1001/jamaneurol.2013.38

Pan, W., Banks, W.A., Fasold, M.B., Bluth, J., Kastin, A.J., 1998. Transport of brain-derived neurotrophic factor across the blood-brain barrier. Neuropharmacology 37, 1553–1561.

Pardoe, H.R., Pell, G.S., Abbott, D.F., Jackson, G.D., 2009. Hippocampal volume assessment in temporal lobe epilepsy: How good is automated segmentation? Epilepsia 50, 2586–2592. doi:10.1111/j.1528-1167.2009.02243.x

Pariante, C.M., Lightman, S.L., 2008. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 31, 464–468. doi:10.1016/j.tins.2008.06.006

Park, E., Bell, J.D., Baker, A.J., 2008. Traumatic brain injury: Can the consequences be stopped? Can. Med. Assoc. J. 178, 1163–1170.

Parsey, R.V., Hastings, R.S., Oquendo, M.A., Hu, X., Goldman, D., Huang, Y., Simpson, N., Arcement, J., Huang, Y., Ogden, R.T., Van Heertum, R.L., Arango, V., Mann, J.J., 2006. Effect of a Triallelic Functional Polymorphism of the Serotonin-Transporter-Linked Promoter Region on Expression of Serotonin Transporter in the Human Brain. Am J Psychiatry 163, 48–51. doi:10.1176/appi.ajp.163.1.48

Peckham, A.D., McHugh, R.K., Otto, M.W., 2010. A meta-analysis of the magnitude of biased attention in depression. Depress. Anxiety 27, 1135–1142. doi:10.1002/da.20755

Pelleymounter, M.A., Cullen, M.J., Wellman, C.L., 1995. Characteristics of BDNF-induced weight loss. Exp. Neurol. 131, 229–238.

Perlstein, W.M., Larson, M.J., Dotson, V.M., Kelly, K.G., 2006. Temporal dissociation of components of cognitive control dysfunction in severe TBI: ERPs and the cued-Stroop task. Neuropsychologia 44, 260–274.

Perona, M.T.G., Waters, S., Hall, F.S., Sora, I., Lesch, K.P., Murphy, D.L., Caron, M., Uhl, G.R., 2008. Animal models of depression in dopamine, serotonin and norepinephrine transporter knockout mice: prominent effects of dopamine transporter deletions. Behav. Pharmacol. 19, 566.

Pessoa, L., Kastner, S., Ungerleider, L.G., 2003. Neuroimaging studies of attention: from modulation of sensory processing to top-down control. J. Neurosci. Off. J. Soc. Neurosci. 23, 3990–3998.

Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., Verchinski, B.A., Munoz, K.E., Kolachana, B.S., Egan, M.F., Mattay, V.S., Hariri, A.R., Weinberger, D.R., 2005. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. Nat Neurosci 8, 828–834. doi:10.1038/nn1463

Pezawas, L., Meyer-Lindenberg, A., Goldman, A.L., Verchinski, B.A., Chen, G., Kolachana, B.S., Egan, M.F., Mattay, V.S., Hariri, A.R., Weinberger, D.R., 2008. MET BDNF protects against morphological S allele effects of 5-HTTLPR. Mol Psychiatry 13, 654.

Phelps, T.I., Bondi, C.O., Ahmed, R.H., Olugbade, Y.T., Kline, A.E., 2014. Divergent long-term consequences of chronic treatment with haloperidol, risperidone, and bromocriptine on traumatic brain injury-induced cognitive deficits. J. Neurotrauma. doi:10.1089/neu.2014.3711

Philibert, R., Madan, A., Andersen, A., Cadoret, R., Packer, H., Sandhu, H., 2007. Serotonin transporter mRNA levels are associated with the methylation of an upstream CpG island. Am. J. Med. Genet. B Neuropsychiatr. Genet. 144B, 101–105. doi:10.1002/ajmg.b.30414

Piccinni, A., Marazziti, D., Catena, M., Domenici, L., Del Debbio, A., Bianchi, C., Mannari, C., Martini, C., Da Pozzo, E., Schiavi, E., others, 2008a. Plasma and serum brain-derived neurotrophic factor (BDNF) in depressed patients during 1 year of antidepressant treatments. J. Affect. Disord. 105, 279–283.

Piccinni, A., Marazziti, D., Del Debbio, A., Bianchi, C., Roncaglia, I., Mannari, C., Origlia, N., Catena Dell'Osso, M., Massimetti, G., Domenici, L., others, 2008b. Diurnal variation of plasma brain-derived neurotrophic factor (BDNF) in humans: an analysis of sex differences. Chronobiol. Int. 25, 819–826.

Pillai, A., Kale, A., Joshi, S., Naphade, N., Raju, M.S.V.K., Nasrallah, H., Mahadik, S.P., 2010. Decreased BDNF Levels in CSF of Drug-Naive First-Episode Psychotic Subjects: Correlation with Plasma BDNF and Psychopathology. Int. J. Neuropsychopharmacol. 13, 535–539. doi:10.1017/S1461145709991015

Pittenger, C., Duman, R.S., 2007. Stress, Depression, and Neuroplasticity: A Convergence of Mechanisms. Neuropsychopharmacology 33, 88–109.

Poduslo, J.F., Curran, G.L., 1996. Permeability at the blood-brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. Brain Res. Mol. Brain Res. 36, 280–286.

Ponce, G., Pérez-González, R., Aragüés, M., Palomo, T., Rodríguez-Jiménez, R., Jiménez-Arriero, M.A., Hoenicka, J., 2009. The ANKK1 kinase gene and psychiatric disorders. Neurotox. Res. 16, 50–59. doi:10.1007/s12640-009-9046-9

Praschak-Rieder, N., Kennedy, J., Wilson, A.A., Hussey, D., Boovariwala, A., Willeit, M., Ginovart, N., Tharmalingam, S., Masellis, M., Houle, S., Meyer, J.H., 2007. Novel 5-HTTLPR Allele Associates with Higher Serotonin Transporter Binding in Putamen: A [11C] DASB Positron Emission Tomography Study. Biol. Psychiatry 62, 327–331. doi:10.1016/j.biopsych.2006.09.022

Pruunsild, P., Kazantseva1, A., Aid, T., Palm, K., Timmusk, T., 2007. Dissecting the human BDNF locus: Bidirectional transcription, complex splicing, and multiple promoters. Genomics 90, 397–406. doi:10.1016/j.ygeno.2007.05.004

Qin, L., Jing, D., Parauda, S., Carmel, J., Ratan, R.R., Lee, F.S., Cho, S., 2014. An Adaptive Role for BDNF Val66Met Polymorphism in Motor Recovery in Chronic Stroke. J. Neurosci. 34, 2493–2502. doi:10.1523/JNEUROSCI.4140-13.2014

Radua, J., El-Hage, W., Monté, G.C., Gohier, B., Tropeano, M., Phillips, M.L., Surguladze, S.A., 2013. COMT Val158Met x SLC6A4 5-HTTLPR interaction impacts on gray matter volume of regions supporting emotion processing. Soc. Cogn. Affect. Neurosci. doi:10.1093/scan/nst089

Rapoport, M.J., McCullagh, S., Shammi, P., Feinstein, A., 2005. Cognitive Impairment Associated With Major Depression Following Mild and Moderate Traumatic Brain Injury. J Neuropsychiatry Clin Neurosci 17, 61–65. doi:10.1176/appi.neuropsych.17.1.61

Rapoport, M., McCauley, S., Levin, H., Song, J., Feinstein, A., 2002. The role of injury severity in neurobehavioral outcome 3 months after traumatic brain injury. Neuropsychiatry. Neuropsychol. Behav. Neurol. 15, 123–132.

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M.V., Leick, L., Hart, E., Secher, N.H., Pedersen, B.K., Pilegaard, H., 2009. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. Exp. Physiol. 94, 1062–1069. doi:10.1113/expphysiol.2009.048512

Rees, M., Norton, N., Jones, I., McCandless, F., Scourfield, J., Holmans, P., Moorhead, S., Feldman, E., Sadler, S., Cole, T., Redman, K., Farmer, A., McGuffin, P., Owen, M.J.,

Craddock, N., 1997. Association studies of bipolar disorder at the human serotonin transporter gene (hSERT; 5HTT). Mol. Psychiatry 2, 398–402.
Reger, M.L., Poulos, A.M., Buen, F., Giza, C.C., Hovda, D.A., Fanselow, M.S., 2012. Concussive brain injury enhances fear learning and excitatory processes in the amygdala. Biol. Psychiatry 71, 335–343. doi:10.1016/j.biopsych.2011.11.007
Reimold, M., Smolka, M.N., Schumann, G., Zimmer, A., Wrase, J., Mann, K., Hu, X.-Z., Goldman, D., Reischl, G., Solbach, C., Machulla, H.-J., Bares, R., Heinz, A., 2007. Midbrain serotonin transporter binding potential measured with [¹¹C]DASB is affected by serotonin transporter genotype. J. Neural Transm. 114, 635–639. doi:10.1007/s00702-006-0609-0
Reitan, R., Wolfson, D., 1985. The Halstead-Reitan Neuropsychological Test Battery: Theory and Clinical Interpretation. Neuropsychology Press, Tucson, AZ.
Reitan, R., Wolfson, D., 1985. The Helstead-Reitan neuropsychological test battery: Theory and clinical interpretation. Neuropsychol. Press.
Ricker, J.H., Hillary, F.G., DeLuca, J., 2001. Functionally activated brain imaging (O-15 PET and fMRI) in the study of learning and memory after traumatic brain injury. J. Head Trauma Rehabil. 16, 191–205.
Rincon, F., Ghosh, S.M., Dey, S.M., Maltenfort, M., Vibbert, M., Urtecho, J., McBride, W., Moussouttas, M., Bell, R., Ratliff, J.K., Jallo, J., 2012. Impact of Acute Lung Injury and Acute Respiratory Distress Syndrome After Traumatic Brain Injury in the United States. [Miscellaneous Article]. Neurosurg. Oct. 2012 71, 795–803. doi:10.1227/NEU.0b013e3182672ae5
Risch, N., Herrell, R., Lehner, T., Liang, K.-Y., Eaves, L., Hoh, J., Griem, A., Kovacs, M., Ott, J., Merikangas, K.R., 2009. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. JAMA J. Am. Med. Assoc. 301, 2462–2471. doi:10.1001/jama.2009.878
Ritter, C., Miranda, A.S., Giombelli, V.R., Tomasi, C.D., Comim, C.M., Teixeira, A.L., Quevedo, J., Dal-Pizzol, F., 2012. Brain-derived neurotrophic factor plasma levels are associated with mortality in critically ill patients even in the absence of brain injury. Crit. Care 16, R234. doi:10.1186/cc11902
Romanczyk, T.B., Weickert, C.S., Webster, M.J., Herman, M.M., Akil, M., Kleinman, J.E., 2002. Alterations in trkB mRNA in the human prefrontal cortex throughout the lifespan. Eur. J. Neurosci. 15, 269–280. doi:10.1046/j.0953-816x.2001.01858.x
Roozenbeek, B., Chiu, Y.-L., Lingsma, H.F., Gerber, L.M., Steyerberg, E.W., Ghajar, J., Maas, A.I.R., 2012. Predicting 14-Day Mortality after Severe Traumatic Brain Injury: Application of the IMPACT Models in the Brain Trauma Foundation TBI-trac ® New York State Database. J. Neurotrauma 29, 1306–1312. doi:10.1089/neu.2011.1988
Rosenthal, M., Christensen, B.K., Ross, T.P., 1998. Depression following traumatic brain injury. Arch. Phys. Med. Rehabil. 79, 90–103.
Rostami, E., Krueger, F., Plantman, S., Davidsson, J., Agoston, D., Grafman, J., Risling, M., 2013. Alteration in BDNF and its receptors, full-length and truncated TrkB and p75< sup> NTR</sup> following penetrating traumatic brain injury. Brain Res.
Rostami, E., Krueger, F., Zoubak, S., Dal Monte, O., Raymont, V., Pardini, M., Hodgkinson, C.A., Goldman, D., Risling, M., Grafman, J., 2011. BDNF Polymorphism Predicts General Intelligence after Penetrating Traumatic Brain Injury. PLoS ONE 6, e27389. doi:10.1371/journal.pone.0027389

Rothman, S.M., Griffioen, K.J., Wan, R., Mattson, M.P., 2012. Brain-derived neurotrophic factor as a regulator of systemic and brain energy metabolism and cardiovascular health. Ann. N. Y. Acad. Sci. 1264, 49–63. doi:10.1111/j.1749-6632.2012.06525.x

Rubia, K., Lee, F., Cleare, A.J., Tunstall, N., Fu, C.H.Y., Brammer, M., McGuire, P., 2005. Tryptophan depletion reduces right inferior prefrontal activation during response inhibition in fast, event-related fMRI. Psychopharmacology (Berl.) 179, 791–803. doi:10.1007/s00213-004-2116-z

Sabet, A.A., Christoforou, E., Zatlin, B., Genin, G.M., Bayly, P.V., 2008. Deformation of The Human Brain Induced By Mild Angular Head Acceleration. J. Biomech. 41, 307–315. doi:10.1016/j.jbiomech.2007.09.016

Saha, R.N., Liu, X., Pahan, K., 2006. Up-regulation of BDNF in astrocytes by TNF-alpha: a case for the neuroprotective role of cytokine. J. Neuroimmune Pharmacol. Off. J. Soc. NeuroImmune Pharmacol. 1, 212–222. doi:10.1007/s11481-006-9020-8

Salmond, C.H., Chatfield, D.A., Menon, D.K., Pickard, J.D., Sahakian, B.J., 2005. Cognitive sequelae of head injury: involvement of basal forebrain and associated structures. Brain J. Neurol. 128, 189–200. doi:10.1093/brain/awh352

Sánchez-Benavides, G., Gómez-Ansón, B., Sainz, A., Vives, Y., Delfino, M., Peña-Casanova, J., 2010. Manual validation of FreeSurfer's automated hippocampal segmentation in normal aging, mild cognitive impairment, and Alzheimer Disease subjects. Psychiatry Res. 181, 219–225. doi:10.1016/j.pscychresns.2009.10.011

Sánchez-Carrión, R., Gómez, P.V., Junqué, C., Fernández-Espejo, D., Falcon, C., Bargalló, N., Roig-Rovira, T., Enseñat-Cantallops, A., Bernabeu, M., 2008. Frontal hypoactivation on functional magnetic resonance imaging in working memory after severe diffuse traumatic brain injury. J. Neurotrauma 25, 479–494. doi:10.1089/neu.2007.0417

Santamaría, J., Tolosa, E., Valles, A., 1986. Parkinson's disease with depression: a possible subgroup of idiopathic parkinsonism. Neurology 36, 1130–1133.

Santarsieri, M., Niyonkuru, C., McCullough, E., Dobos, J., Dixon, C.E., Berga, S., Wagner, A.K., M.D., 2013. CSF Cortisol and Progesterone Profiles and Outcomes Prognostication after Severe TBI. J. Neurotrauma. doi:10.1089/neu.2013.3177

Sashindranath, M., Samson, A.L., Downes, C.E., Crack, P.J., Lawrence, A.J., Li, Q.-X., Ng, A.Q.P., Jones, N.C., Farrugia, J.J., Abdella, E., Vassalli, J.-D., Madani, R., Medcalf, R.L., 2011. Compartment- and context-specific changes in tissue-type plasminogen activator (tPA) activity following brain injury and pharmacological stimulation. Lab. Invest. 91, 1079–1091. doi:10.1038/labinvest.2011.67

Satz, P., Forney, D.L., Zaucha, K., Asarnow, R.R., Light, R., McCleary, C., Levin, H., Kelly, D., Bergsneider, M., Hovda, D., Martin, N., Namerow, N., Becker, D., 1998. Depression, cognition, and functional correlates of recovery outcome after traumatic brain injury. Brain Inj. BI 12, 537–553.

Sawaguchi, T., Goldman-Rakic, P.S., 1991. D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251, 947–950. doi:10.1126/science.1825731

Scanlon, J.M., Price, J., Ricker, J.H., Conley, Y.P., Becker, C., Burkhardt, J.N., Dixon, C.E., Wagner, A.K., 2009. Response to Methylphenidate Administration and Dopaminergic Function in Persons with TBI and Healthy Controls., in: 8. J. Neurotrauma, p. A–23.

Scanlon, J.M., Price, J., Ricker, J.H., Conley, Y.P., Becker, C., Lopresti, B., Drewencki, L.L., Deslouches, S., Fabio, A., Wagner, A.K., 2007. Striatal dopamine transporter and D2

receptor binding and executive functioning after TBI: an initial PET study, in: Neuroscience, 262.7/S5. Presented at the Society for Neuroscience, San Diego, CA.
Schmitter-Edgecombe, M., 2006. Implications of basic science research for brain injury rehabilitation: a focus on intact learning mechanisms. J. Head Trauma Rehabil. 21, 131–141.
Schneider, E.B., Sur, S., Raymont, V., Duckworth, J., Kowalski, R.G., Efron, D.T., Hui, X., Selvarajah, S., Hambridge, H.L., Stevens, R.D., 2014. Functional recovery after moderate/severe traumatic brain injury A role for cognitive reserve? Neurology 82, 1636–1642. doi:10.1212/WNL.0000000000000379
Seamans, J.K., Robbins, T.W., 2010. Dopamine Modulation of the Prefrontal Cortex and Cognitive Function, in: Neve, K.A. (Ed.), The Dopamine Receptors, The Receptors. Humana Press, pp. 373–398.
Seamans, J.K., Yang, C.R., 2004. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog. Neurobiol. 74, 1–58. doi:10.1016/j.pneurobio.2004.05.006
Sen, S., Duman, R., Sanacora, G., 2008. Serum Brain-Derived Neurotrophic Factor, Depression, and Antidepressant Medications: Meta-Analyses and Implications. Biol. Psychiatry 64, 527–532. doi:10.1016/j.biopsych.2008.05.005
Sesack, S.R., Hawrylak, V.A., Matus, C., Guido, M.A., Levey, A.I., 1998. Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. J. Neurosci. 18, 2697–2708.
Shalev, I., Lerer, E., Israel, S., Uzefovsky, F., Gritsenko, I., Mankuta, D., Ebstein, R.P., Kaitz, M., 2009. BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. Psychoneuroendocrinology 34, 382–388. doi:10.1016/j.psyneuen.2008.09.017
Sharp, D.J., Beckmann, C.F., Greenwood, R., Kinnunen, K.M., Bonnelle, V., De Boissezon, X., Powell, J.H., Counsell, S.J., Patel, M.C., Leech, R., 2011. Default mode network functional and structural connectivity after traumatic brain injury. Brain 134, 2233–2247.
Sheline, Y.I., Gado, M.H., Kraemer, H.C., 2003. Untreated depression and hippocampal volume loss. Am. J. Psychiatry 160, 1516–1518.
Shimada, R., Abe, K., Furutani, R., Kibayashi, K., 2014a. Changes in dopamine transporter expression in the midbrain following traumatic brain injury: an immunohistochemical and in situ hybridization study in a mouse model. Neurol. Res. 36, 239–246. doi:10.1179/1743132813Y.0000000289
Shimada, R., Abe, K., Furutani, R., Kibayashi, K., 2014b. Changes in dopamine transporter expression in the midbrain following traumatic brain injury: an immunohistochemical and in situ hybridization study in a mouse model. Neurol. Res. 36, 239–246. doi:10.1179/1743132813Y.0000000289
Sidaros, A., Engberg, A.W., Sidaros, K., Liptrot, M.G., Herning, M., Petersen, P., Paulson, O.B., Jernigan, T.L., Rostrup, E., 2008. Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. Brain 131, 559–572. doi:10.1093/brain/awm294
Siironen, J., Juvela, S., Kanarek, K., Vilkki, J., Hernesniemi, J., Lappalainen, J., 2007. The Met Allele of the BDNF Val66Met Polymorphism Predicts Poor Outcome Among Survivors of Aneurysmal Subarachnoid Hemorrhage. Stroke 38, 2858–2860. doi:10.1161/STROKEAHA.107.485441

Silhol, M., Bonnichon, V., Rage, F., Tapia-Arancibia, L., 2005. Age-related changes in brain-derived neurotrophic factor and tyrosine kinase receptor isoforms in the hippocampus and hypothalamus in male rats. Neuroscience 132, 613–624. doi:10.1016/j.neuroscience.2005.01.008

Siuciak, J.A., Lewis, D.R., Wiegand, S.J., Lindsay, R.M., 1997. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol. Biochem. Behav. 56, 131–137. doi:10.1016/S0091-3057(96)00169-4

Social Security Death Index [WWW Document], n.d. . GenealogyBank.com. URL http://www.genealogybank.com/gbnk/ssdi/ (accessed 12.9.13).

Soeiro-De-Souza, M.G., Bio, D.S., David, D.P., Missio, G., Lima, B., Fernandes, F., Machado-Vieira, R., Moreno, R.A., 2013. Gender effects of the COMT Val 158 Met genotype on verbal fluency in healthy adults. Mol. Med. Rep. 8, 837–844. doi:10.3892/mmr.2013.1564

Stroop, J., 1935. Studies of interference in serial verbal reactions. J. Exp. Psychol. 18, 643–662. doi:10.1037/h0054651

Stroop, J.R., 1935. Studies of interference in serial verbal reactions. J. Exp. Psychol. Exp. Psychol. 18, 643–662. doi:10.1037/h0054651

Susman, M., DiRusso, S.M., Sullivan, T., Risucci, D., Nealon, P., Cuff, S., Haider, A., Benzil, D., 2002. Traumatic brain injury in the elderly: increased mortality and worse functional outcome at discharge despite lower injury severity. J. Trauma 53, 219–223; discussion 223–224.

Tang, L., Todd, R.D., O'Malley, K.L., 1994. Dopamine D2 and D3 receptors inhibit dopamine release. J. Pharmacol. Exp. Ther. 270, 475.

Tanriverdi, F., Senyurek, H., Unluhizarci, K., Selcuklu, A., Casanueva, F.F., Kelestimur, F., 2006. High Risk of Hypopituitarism after Traumatic Brain Injury: A Prospective Investigation of Anterior Pituitary Function in the Acute Phase and 12 Months after Trauma. J. Clin. Endocrinol. Metab. 91, 2105–2111. doi:10.1210/jc.2005-2476

Tao, X., Finkbeiner, S., Arnold, D.B., Shaywitz, A.J., Greenberg, M.E., 1998. Ca2+ Influx Regulates BDNF Transcription by a CREB Family Transcription Factor-Dependent Mechanism. Neuron 20, 709–726. doi:10.1016/S0896-6273(00)81010-7

Tapia-Arancibia, L., Aliaga, E., Silhol, M., Arancibia, S., 2008. New insights into brain BDNF function in normal aging and Alzheimer disease. Brain Res. Rev. 59, 201–220. doi:10.1016/j.brainresrev.2008.07.007

Tate, D.F., Bigler, E.D., 2000. Fornix and Hippocampal Atrophy in Traumatic Brain Injury. Learn. Mem. 7, 442 –446. doi:10.1101/lm.33000

Tate, D.F., Bigler, E.D., 2000. Fornix and hippocampal atrophy in traumatic brain injury. Learn. Mem. 7, 442–446.

Teasdale, G., Jennett, B., 1974a. Assessment of coma and impaired consciousness. A practical scale. Lancet 2, 81–84.

Teasdale, G., Jennett, B., 1974b. Assessment of coma and impaired consciousness. A practical scale. Lancet 2, 81–84.

Teasdale, G.M., Murray, G.D., Nicoll, J.A.R., 2005. The association between APOE epsilon4, age and outcome after head injury: a prospective cohort study. Brain J. Neurol. 128, 2556–2561. doi:10.1093/brain/awh595

Temkin, N.R., Dikmen, S.S., Wilensky, A.J., Keihm, J., Chabal, S., Winn, H.R., 1990. A randomized, double-blind study of phenytoin for the prevention of post-traumatic seizures. N. Engl. J. Med. 323, 497–502. doi:10.1056/NEJM199008233230801

Terracciano, A., Piras, M.G., Lobina, M., Mulas, A., Meirelles, O., Sutin, A.R., Chan, W., Sanna, S., Uda, M., Crisponi, L., Schlessinger, D., 2013. Genetics of serum BDNF: Meta-analysis of the Val66Met and genome-wide association study. World J. Biol. Psychiatry Off. J. World Fed. Soc. Biol. Psychiatry 14. doi:10.3109/15622975.2011.616533

Thompson, J., Thomas, N., Singleton, A., Piggott, M., Lloyd, S., Perry, E.K., Morris, C.M., Perry, R.H., Ferrier, I.N., Court, J.A., 1997. D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. Pharmacogenetics 7, 479–484.

Toker, L., Amar, S., Bersudsky, Y., Benjamin, J., Klein, E., 2010. The biology of tryptophan depletion and mood disorders. Isr. J. Psychiatry Relat. Sci. 47, 46–55.

Tomaiuolo, F., Carlesimo, G.A., Paola, M.D., Petrides, M., Fera, F., Bonanni, R., Formisano, R., Pasqualetti, P., Caltagirone, C., 2004. Gross morphology and morphometric sequelae in the hippocampus, fornix, and corpus callosum of patients with severe non-missile traumatic brain injury without macroscopically detectable lesions: a T1 weighted MRI study. J. Neurol. Neurosurg. Psychiatry 75, 1314–1322. doi:10.1136/jnnp.2003.017046

Torres, G.E., Carneiro, A., Seamans, K., Fiorentini, C., Sweeney, A., Yao, W.D., Caron, M.G., 2003. Oligomerization and trafficking of the human dopamine transporter. J. Biol. Chem. 278, 2731.

Tunbridge, E.M., Harrison, P.J., Weinberger, D.R., 2006. Catechol-o-Methyltransferase, Cognition, and Psychosis: Val158Met and Beyond. Biol. Psychiatry 60, 141–151. doi:10.1016/j.biopsych.2005.10.024

Turken, A.U., Whitfield-Gabrieli, S., Bammer, R., Baldo, J., Dronkers, N.F., Gabrieli, J.D.E., 2008. COGNITIVE PROCESSING SPEED AND THE STRUCTURE OF WHITE MATTER PATHWAYS: CONVERGENT EVIDENCE FROM NORMAL VARIATION AND LESION STUDIES. NeuroImage 42, 1032–1044. doi:10.1016/j.neuroimage.2008.03.057

Udekwu, P., Kromhout-Schiro, S., Vaslef, S., Baker, C., Oller, D., 2004a. Glasgow Coma Scale score, mortality, and functional outcome in head-injured patients. J. Trauma 56, 1084–1089.

Udekwu, P., Kromhout-Schiro, S., Vaslef, S., Baker, C., Oller, D., 2004b. Glasgow Coma Scale score, mortality, and functional outcome in head-injured patients. J. Trauma 56, 1084–1089.

Vakil, E., 2005. The effect of moderate to severe traumatic brain injury (TBI) on different aspects of memory: a selective review. J. Clin. Exp. Neuropsychol. 27, 977–1021. doi:10.1080/13803390490919245

Van Dyck, C.H., Malison, R.T., Staley, J.K., Jacobsen, L.K., Seibyl, J.P., Laruelle, M., Baldwin, R.M., Innis, R.B., Gelernter, J., 2004. Central serotonin transporter availability measured with [123I]beta-CIT SPECT in relation to serotonin transporter genotype. Am. J. Psychiatry 161, 525–531.

Van IJzendoorn, M.H., Caspers, K., Bakermans-Kranenburg, M.J., Beach, S.R.H., Philibert, R., 2010. Methylation matters: interaction between methylation density and serotonin transporter genotype predicts unresolved loss or trauma. Biol. Psychiatry 68, 405–407. doi:10.1016/j.biopsych.2010.05.008

VanNess, S.H., Owens, M.J., Kilts, C.D., 2005. The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. BMC Genet. 6, 55. doi:10.1186/1471-2156-6-55

Vidaurre, O.G., Gascón, S., Deogracias, R., Sobrado, M., Cuadrado, E., Montaner, J., Rodríguez-Peña, A., Díaz-Guerra, M., 2012. Imbalance of neurotrophin receptor isoforms TrkB-FL/TrkB-T1 induces neuronal death in excitotoxicity. Cell Death Dis. 3, e256. doi:10.1038/cddis.2011.143

Vijayraghavan, S., Wang, M., Birnbaum, S.G., Williams, G.V., Arnsten, A.F.T., 2007. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nat. Neurosci. 10, 376–384. doi:10.1038/nn1846

Vinogradov, S., Fisher, M., Holland, C., Shelly, W., Wolkowitz, O., Mellon, S.H., 2009. Is serum brain-derived neurotrophic factor a biomarker for cognitive enhancement in schizophrenia? Biol. Psychiatry 66, 549–553. doi:10.1016/j.biopsych.2009.02.017

Voisey, J., Swagell, C.D., Hughes, I.P., Morris, C.P., van Daal, A., Noble, E.P., Kann, B., Heslop, K.A., Young, R.M., Lawford, B.R., 2009. The DRD2 gene 957C>T polymorphism is associated with posttraumatic stress disorder in war veterans. Depress. Anxiety 26, 28–33. doi:10.1002/da.20517

Wagner, A.K., 2010. TBI translational rehabilitation research in the 21st Century: exploring a Rehabilomics research model. Eur. J. Phys. Rehabil. Med. 46, 549–556.

Wagner, A.K., 2010. TBI translational rehabilitation research in the 21st Century: exploring a Rehabilomics research model. Eur. J. Phys. Rehabil. Med. 46, 549–556.

Wagner, A.K., 2011. Rehabilomics: a conceptual framework to drive biologics research. PM R 3, S28–30. doi:10.1016/j.pmrj.2011.04.013

Wagner, A.K., Amin, K.B., Niyonkuru, C., Postal, B.A., McCullough, E.H., Ozawa, H., Dixon, C.E., Bayir, H., Clark, R.S., Kochanek, P.M., Fabio, A., 2011a. CSF Bcl-2 and cytochrome C temporal profiles in outcome prediction for adults with severe TBI. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 31, 1886–1896. doi:10.1038/jcbfm.2011.31

Wagner, A.K., Bayir, H., Ren, D., Puccio, A., Zafonte, R.D., Kochanek, P.M., 2004. Relationships between cerebrospinal fluid markers of excitotoxicity, ischemia, and oxidative damage after severe TBI: the impact of gender, age, and hypothermia. J. Neurotrauma 21, 125–136.

Wagner, A.K., Chen, X., Kline, A.E., Li, Y., Zafonte, R.D., Dixon, C.E., 2005. Gender and environmental enrichment impact dopamine transporter expression after experimental traumatic brain injury. Exp. Neurol. 195, 475–483. doi:10.1016/j.expneurol.2005.06.009

Wagner, A.K., Drewencki, L.L., Chen, X., Santos, F.R., Khan, A.S., Harun, R., Torres, G.E., Michael, A.C., Dixon, C.E., 2009. Chronic methylphenidate treatment enhances striatal dopamine neurotransmission after experimental traumatic brain injury. J. Neurochem. 108, 986–997. doi:10.1111/j.1471-4159.2008.05840.x

Wagner, A.K., Hatz, L.E., Scanlon, J.M., Niyonkuru, C., Miller, M.A., Ricker, J.H., Conley, Y.P., Ferrell, R.E., 2012. Association of KIBRA rs17070145 polymorphism and episodic memory in individuals with severe TBI. Brain Inj. BI. doi:10.3109/02699052.2012.700089

Wagner, A.K., Kline, A.E., Ren, D., Willard, L.A., Wenger, M.K., Zafonte, R.D., Dixon, C.E., 2007a. Gender associations with chronic methylphenidate treatment and behavioral performance following experimental traumatic brain injury. Behav. Brain Res. 181, 200–209. doi:10.1016/j.bbr.2007.04.006

Wagner, A.K., McCullough, E.H., Niyonkuru, C., Ozawa, H., Loucks, T.L., Dobos, J.A., Brett, C.A., Santarsieri, M., Dixon, C.E., Berga, S.L., Fabio, A., 2011b. Acute serum hormone levels: characterization and prognosis after severe traumatic brain injury. J. Neurotrauma 28, 871–888. doi:10.1089/neu.2010.1586

Wagner, A.K., McCullough, E.H., Niyonkuru, C., Ozawa, H., Loucks, T.L., Dobos, J.A., Brett, C.A., Santarsieri, M., Dixon, C.E., Berga, S.L., Fabio, A., 2011c. Acute serum hormone levels: characterization and prognosis after severe traumatic brain injury. J. Neurotrauma 28, 871–888. doi:10.1089/neu.2010.1586

Wagner, A.K., Miller, M.A., Scanlon, J., Ren, D., Kochanek, P.M., Conley, Y.P., 2010. Adenosine A1 receptor gene variants associated with post-traumatic seizures after severe TBI. Epilepsy Res. 90, 259–272. doi:10.1016/j.eplepsyres.2010.06.001

Wagner, A.K., Ren, D., Conley, Y.P., Ma, X., Kerr, M.E., Zafonte, R.D., Puccio, A.M., Marion, D.W., Dixon, C.E., 2007. Sex and genetic associations with cerebrospinal fluid dopamine and metabolite production after severe traumatic brain injury. J. Neurosurg. 106, 538–547. doi:10.3171/jns.2007.106.4.538

Wagner, A.K., Ren, D., Conley, Y.P., Ma, X., Kerr, M.E., Zafonte, R.D., Puccio, A.M., Marion, D.W., Dixon, C.E., 2007b. Sex and genetic associations with cerebrospinal fluid dopamine and metabolite production after severe traumatic brain injury. J. Neurosurg. 106, 538–547. doi:10.3171/jns.2007.106.4.538

Wagner, A.K., Scanlon, J.M., Becker, C.R., Ritter, A.C., Niyonkuru, C., Dixon, C.E., Conley, Y.P., Price, J.C., 2014. The influence of genetic variants on striatal dopamine transporter and D2 receptor binding after TBI. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. doi:10.1038/jcbfm.2014.87

Wagner, A.K., Scanlon, J.M., Becker, C.R., Ritter, A.C., Niyonkuru, C., Dixon, C.E., Conley, Y.P., Price, J.C., 2014. The influence of genetic variants on striatal dopamine transporter and D2 receptor binding after TBI. J. Cereb. Blood Flow Metab. 34, 1328–1339. doi:10.1038/jcbfm.2014.87

Wagner, A.K., Zitelli, K.T., 2012. A Rehabilomics focused perspective on molecular mechanisms underlying neurological injury, complications, and recovery after severe TBI. Pathophysiol. Off. J. Int. Soc. Pathophysiol. ISP. doi:10.1016/j.pathophys.2012.02.007

Wagner, A.K., Zitelli, K.T., 2013. A Rehabilomics focused perspective on molecular mechanisms underlying neurological injury, complications, and recovery after severe TBI. Pathophysiol. Off. J. Int. Soc. Pathophysiol. ISP 20, 39–48. doi:10.1016/j.pathophys.2012.02.007

Walton, M.R., Dragunow, M., 2000. Is CREB a key to neuronal survival? Trends Neurosci. 23, 48–53. doi:10.1016/S0166-2236(99)01500-3

Wang, H., Zhou, X.-F., 2002. Injection of brain-derived neurotrophic factor in the rostral ventrolateral medulla increases arterial blood pressure in anaesthetized rats. Neuroscience 112, 967–975.

Wang, Y., Neumann, M., Hansen, K., Hong, S.M., Kim, S., Noble-Haeusslein, L.J., Liu, J., 2011. Fluoxetine increases hippocampal neurogenesis and induces epigenetic factors but does not improve functional recovery after traumatic brain injury. J. Neurotrauma 28, 259–268. doi:10.1089/neu.2010.1648

Wan, R., Weigand, L.A., Bateman, R., Griffioen, K., Mendelowitz, D., Mattson, M.P., 2014. Evidence that BDNF regulates heart rate by a mechanism involving increased brainstem parasympathetic neuron excitability. J. Neurochem. doi:10.1111/jnc.12656

Warden, D.L., Gordon, B., McAllister, T.W., Silver, J.M., Barth, J.T., Bruns, J., Drake, A., Gentry, T., Jagoda, A., Katz, D.I., Kraus, J., Labbate, L.A., Ryan, L.M., Sparling, M.B., Walters, B., Whyte, J., Zapata, A., Zitnay, G., 2006. Guidelines for the Pharmacologic Treatment

of Neurobehavioral Sequelae of Traumatic Brain Injury. J. Neurotrauma 23, 1468–1501. doi:10.1089/neu.2006.23.1468

Warner, M.A., de la Plata, C.M., Spence, J., Wang, J.Y., Harper, C., Moore, C., Devous, M., Diaz-Arrastia, R., 2010. Assessing spatial relationships between axonal integrity, regional brain volumes, and neuropsychological outcomes after traumatic axonal injury. J. Neurotrauma 27, 2121–2130.

Warner-Schmidt, J.L., Duman, R.S., 2006. Hippocampal neurogenesis: Opposing effects of stress and antidepressant treatment. Hippocampus 16, 239–249. doi:10.1002/hipo.20156

Washington, P.M., Forcelli, P.A., Wilkins, T., Zapple, D.N., Parsadanian, M., Burns, M.P., 2012. The Effect of Injury Severity on Behavior: A Phenotypic Study of Cognitive and Emotional Deficits after Mild, Moderate, and Severe Controlled Cortical Impact Injury in Mice. J. Neurotrauma 29, 2283–2296. doi:10.1089/neu.2012.2456

Webster, M.J., Herman, M.M., Kleinman, J.E., Shannon Weickert, C., 2006. BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan. Gene Expr. Patterns 6, 941–951.

Wendland, J.R., Martin, B.J., Kruse, M.R., Lesch, K.-P., Murphy, D.L., 2006. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. Mol Psychiatry 11, 224–226.

Whitnall, L., McMillan, T.M., Murray, G.D., Teasdale, G.M., 2006. Disability in young people and adults after head injury: 5-7 year follow up of a prospective cohort study. J. Neurol. Neurosurg. Psychiatry 77, 640–645. doi:10.1136/jnnp.2005.078246

Whyte, J., Hart, T., Vaccaro, M., Grieb-Neff, P., Risser, A., Polansky, M., Coslett, H.B., 2004. Effects of methylphenidate on attention deficits after traumatic brain injury: a multidimensional, randomized, controlled trial. Am. J. Phys. Med. Rehabil. Assoc. Acad. Physiatr. 83, 401–420.

Willmott, C., Ponsford, J., McAllister, T.W., Burke, R., 2013. Effect of COMT Val158Met genotype on attention and response to methylphenidate following traumatic brain injury. Brain Inj. 27, 1281–1286. doi:10.3109/02699052.2013.809553

Willmott, C., Withiel, T., Ponsford, J., Burke, R., 2014a. COMT Val158Met and Cognitive and Functional Outcomes after Traumatic Brain Injury. J. Neurotrauma. doi:10.1089/neu.2013.3308

Willmott, C., Withiel, T., Ponsford, J., Burke, R., 2014b. COMT Val158Met and cognitive and functional outcomes after traumatic brain injury. J. Neurotrauma. doi:10.1089/neu.2013.3308

Willmott, C., Withiel, T., Ponsford, J., Burke, R., 2014c. COMT Val(158)Met and Cognitive and Functional Outcomes after Traumatic Brain Injury. J. Neurotrauma 31, 1507–1514. doi:10.1089/neu.2013.3308

Wilson, J.T., Hadley, D.M., Wiedmann, K.D., Teasdale, G.M., 1995. Neuropsychological consequences of two patterns of brain damage shown by MRI in survivors of severe head injury. J. Neurol. Neurosurg. Psychiatry 59, 328–331.

Wilson, J.T., Pettigrew, L.E., Teasdale, G.M., 1998. Structured interviews for the Glasgow Outcome Scale and the extended Glasgow Outcome Scale: guidelines for their use. J. Neurotrauma 15, 573–585.

Wilson, M.S., Hamm, R.J., 2002. Effects of fluoxetine on the 5-HT1A receptor and recovery of cognitive function after traumatic brain injury in rats. Am. J. Phys. Med. Rehabil. Assoc. Acad. Physiatr. 81, 364–372.

Wise, E.K., Hoffman, J.M., Powell, J.M., Bombardier, C.H., Bell, K.R., 2012. Benefits of exercise maintenance after traumatic brain injury. Arch. Phys. Med. Rehabil. 93, 1319–1323. doi:10.1016/j.apmr.2012.05.009

Woolf, P.D., 1992. Hormonal responses to trauma. Crit. Care Med. 20, 216–226.

Wozniak, G., Georgoulias, P., Iliadis, C., Valotasiou, V., Papadopoulos, G., Bagiatis, V., Tsougos, I., Paterakis, K.N., Fountas, K.N., 2010. Serotonin and Neuron-specific Enolase. Neurosurg. Q. 20, 297–303. doi:10.1097/WNQ.0b013e3181732399

Xiao, L., Becker, J.B., 1994. Quantitative microdialysis determination of extracellular striatal dopamine concentration in male and female rats: effects of estrous cycle and gonadectomy. Neurosci. Lett. 180, 155–158.

Xie, P., Kranzler, H.R., Poling, J., Stein, M.B., Anton, R.F., Brady, K., Weiss, R.D., Farrer, L., Gelernter, J., 2009. Interactive effect of stressful life events and the serotonin transporter 5-HTTLPR genotype on posttraumatic stress disorder diagnosis in 2 independent populations. Arch. Gen. Psychiatry 66, 1201–1209. doi:10.1001/archgenpsychiatry.2009.153

Xie, T., Ho, S.L., Ramsden, D., 1999. Characterization and implications of estrogenic down-regulation of human catechol-O-methyltransferase gene transcription. Mol. Pharmacol. 56, 31–38.

Yang, A.C., Chen, T.-J., Tsai, S.-J., Hong, C.-J., Kuo, C.-H., Yang, C.-H., Kao, K.-P., 2010. BDNF Val66Met polymorphism alters sympathovagal balance in healthy subjects. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 153B, 1024–1030. doi:10.1002/ajmg.b.31069

Yang, B., Slonimsky, J.D., Birren, S.J., 2002. A rapid switch in sympathetic neurotransmitter release properties mediated by the p75 receptor. Nat. Neurosci. 5, 539–545. doi:10.1038/nn853

Yang, L., Zhang, Z., Sun, D., Xu, Z., Yuan, Y., Zhang, X., Li, L., 2010. Low serum BDNF may indicate the development of PSD in patients with acute ischemic stroke. Int. J. Geriatr. Psychiatry. doi:10.1002/gps.2552

Yan, H.Q., Kline, A.E., Ma, X., Hooghe-Peters, E.L., Marion, D.W., Dixon, C.E., 2001. Tyrosine hydroxylase, but not dopamine beta-hydroxylase, is increased in rat frontal cortex after traumatic brain injury. Neuroreport 12, 2323.

Yan, H.Q., Kline, A.E., Ma, X., Li, Y., Dixon, C.E., 2002. Traumatic brain injury reduces dopamine transporter protein expression in the rat frontal cortex. Neuroreport 13, 1899.

Yan, H.Q., Kline, A.E., Ma, X., Li, Y., Dixon, C.E., 2002. Traumatic brain injury reduces dopamine transporter protein expression in the rat frontal cortex. Neuroreport 13, 1899–1901.

Yasuda, S., Wehman, P., Targett, P., Cifu, D., West, M., 2001. Return to work for persons with traumatic brain injury. Am. J. Phys. Med. Rehabil. Assoc. Acad. Physiatr. 80, 852–864.

You, Z., Luo, C., Zhang, W., Chen, Y., He, J., Zhao, Q., Zuo, R., Wu, Y., 2011. Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: involvement in depression. Behav. Brain Res. 225, 135–141. doi:10.1016/j.bbr.2011.07.006

Zafonte, R.D., Cullen, N., Lexell, J., 2002. Serotonin agents in the treatment of acquired brain injury. J. Head Trauma Rehabil. 17, 322.

Zalsman, G., Huang, Y., Oquendo, M.A., Burke, A.K., Hu, X., Brent, D.A., Ellis, S.P., Goldman, D., Mann, J.J., 2006. Association of a Triallelic Serotonin Transporter Gene Promoter

Region (5-HTTLPR) Polymorphism With Stressful Life Events and Severity of Depression. Am J Psychiatry 163, 1588–1593. doi: 10.1176/appi.ajp.163.9.1588
Zawarynski, P., Tallerico, T., Seeman, P., Lee, S.P., O'Dowd, B.F., George, S.R., 1998. Dopamine D2 receptor dimers in human and rat brain. FEBS Lett. 441, 383–386.
Zeng, L.-L., Shen, H., Liu, L., Wang, L., Li, B., Fang, P., Zhou, Z., Li, Y., Hu, D., 2012. Identifying major depression using whole-brain functional connectivity: a multivariate pattern analysis. Brain 135, 1498–1507. doi:10.1093/brain/aws059
Zhan C, Miller MR, 2003. EXcess length of stay, charges, and mortality attributable to medical injuries during hospitalization. JAMA 290, 1868–1874. doi:10.1001/jama.290.14.1868
Zhang, Y., Bertolino, A., Fazio, L., Blasi, G., Rampino, A., Romano, R., Lee, M.-L.T., Xiao, T., Papp, A., Wang, D., Sadée, W., 2007. Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. Proc. Natl. Acad. Sci. 104, 20552–20557. doi:10.1073/pnas.0707106104
Zhang, Z.-Y., Zhang, Z., Fauser, U., Schluesener, H.J., 2007. Global hypomethylation defines a sub-population of reactive microglia/macrophages in experimental traumatic brain injury. Neurosci. Lett. 429, 1–6. doi:10.1016/j.neulet.2007.09.061
Zhu, X., Wang, X., Xiao, J., Liao, J., Zhong, M., Wang, W., Yao, S., 2012. Evidence of a dissociation pattern in resting-state default mode network connectivity in first-episode, treatment-naive major depression patients. Biol. Psychiatry 71, 611–617. doi:10.1016/j.biopsych.2011.10.035