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Variation in the BDNF gene interacts with age to predict mortality in a prospective, longitudinal cohort with severe TBI

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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.

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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.

Keywords

Brain Injury; BDNF; TrkB; Mortality; Age; Genetic Association Study

Introduction

In the US, ~52,000 deaths are attributed to traumatic brain injury (TBI) yearly.¹ 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 Rehabilomics² 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, GCS³), and pupillary reactivity.⁴ Further studies support extending this model by adding neurological findings (evidence of midline shift or presence of subarachnoid hemorrhage⁵). 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.⁶ 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.⁷ In experimental models, older animals show decreased neuronal survival post-injury⁸.

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.⁹ Yet in areas like the hypothalamus, BDNF can regulate metabolism^{10,11}. Evidence suggests BDNF may also affect brainstem control of cardiovascular function^{12–14}. Thus, BDNF may affect autonomic regulation and reflect the current energy state¹⁵, suggesting multiple mechanisms through which BDNF signaling could influence TBI mortality and recovery. While BDNF may affect mortality in other populations^{16–18}, 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 lifespan¹⁹ and following ischemia.²⁰ Similarly, there

are dynamic receptor expression changes following experimental TBI.²¹ 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*²² and shows an age-dependent relationship in cognition.²³ Importantly, rs6265 is in linkage disequilibrium (LD) with another *BDNF* SNP, rs7124442. The rs7124442 variant reportedly affects neuronal BDNF mRNA trafficking²⁴. While rs6265²⁵ and rs7124442²⁶ 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^{27,28} and autonomic control of heart rate²⁹, 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.

Methods

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 penetrating head injury. Subjects were consecutively recruited and consent obtained by appropriate proxy. To minimize genetic stratification effects,³⁰ 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 scores³ 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)³¹ scores for survivors was as follows: 2, n=13; 3, n=66; 4, n=49; 5, n=22.

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).³² 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.³³ 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 procedure³⁴ 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.^{22,24} 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±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.³⁶ 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 ($p < 0.2$). 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.⁵ 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).

Results

Genetic Associations with Demographics

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).

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).

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).

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 pro*BDNF*, is processed either in the soma, extracellular space (after release), or dendrites (after endocytosis).³⁷ If not cleaved, pro*BDNF* targets the pro-apoptotic $p75^{\text{NTR}}$ receptor,³⁸ 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 pro*BDNF*, shows increased activity ipsilateral to the injury³⁹ and mice lacking tPA show reduced edema and cortical lesion volume⁴⁰. Additionally, there are two truncated isoforms of *TrkB*, *TrkB.T1* and *TrkB.T2*, which lack intracellular tyrosine signaling, but are implicated in other pathways.^{20,41} In experimental TBI, there are transient increases in hippocampal *TrkB.FL*; this study also showed regionally-specific $p75^{\text{NTR}}$ increases up to 8 weeks post-TBI.²¹ Although controversial, the ratio of *TrkB.FL*/*TrkB.T* expressed has been suggested to influence cell survival during excitotoxic injury.^{20,41} Other studies report pre-incubation with *BDNF* prior to excitotoxic conditions/insults may be neuroprotective,^{42,43} but this effect is likely receptor-dependent. As studies demonstrate transient increases in hippocampal *BDNF* transcription immediately following experimental TBI,^{21,44} 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,⁴⁵ 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,²¹ 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.^{19,46} Romanczyk *et al* reports dynamic prefrontal cortex TrkB expression across the lifespan, with a peak in young to middle adulthood that decreases with age.⁴⁷ Webster *et al* reported similar TrkB expression patterns in the hippocampus and temporal lobe.⁴⁸ Compared to young adult rats, aged rats have reduced TrkB.FL, but not TrkB.T,⁴⁹ 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,²² 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,⁵⁰ 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.⁵¹ One study showed Met-carriers, despite theoretically lower activity-dependent BDNF secretion, have greater cognitive recovery following penetrating TBI decades after injury.²⁵ These data are part of a growing literature demonstrating TBI-specific risk genetic relationships in TBI outcomes.⁵²⁻⁵⁴ 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,⁵⁵ 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.⁵⁶ 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.¹²⁻¹⁴ Similarly, BDNF modulates the sympathetic/parasympathetic balance in cardiovascular function.⁵⁷ Interestingly, rs6265 is associated with differences in heart rate variability²⁹ and acute stress heart rate reactivity in healthy populations.²⁷ In fact, one study showed that local BDNF administration following surgical sympathectomy induced hippocampal vascular changes and edema⁵⁸. This study suggests BDNF effects during a

state of compromised autonomic function (e.g. immediately following TBI⁵⁹) 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 predictions.⁵ While pulmonary complications occur less frequently in survivors, subjects with pulmonary complications also were significantly younger, consistent with published studies in TBI.⁶⁰ 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.⁶¹ 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.⁵

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 TBI⁶², 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.⁶³ 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=0.787⁵). 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*⁶⁴). 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.¹⁶ Plasma BDNF levels were also related to all-cause mortality in a cohort of older women.¹⁷ 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.^{25,28} Similarly, these variants only cover the most well-studied isoform of BDNF, yet emerging research indicates that there are multiple isoforms for BDNF³⁵. 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.⁶⁵ 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 postmortem tissue. As serum BDNF is decreased acutely in clinical TBI,⁶⁶ 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.

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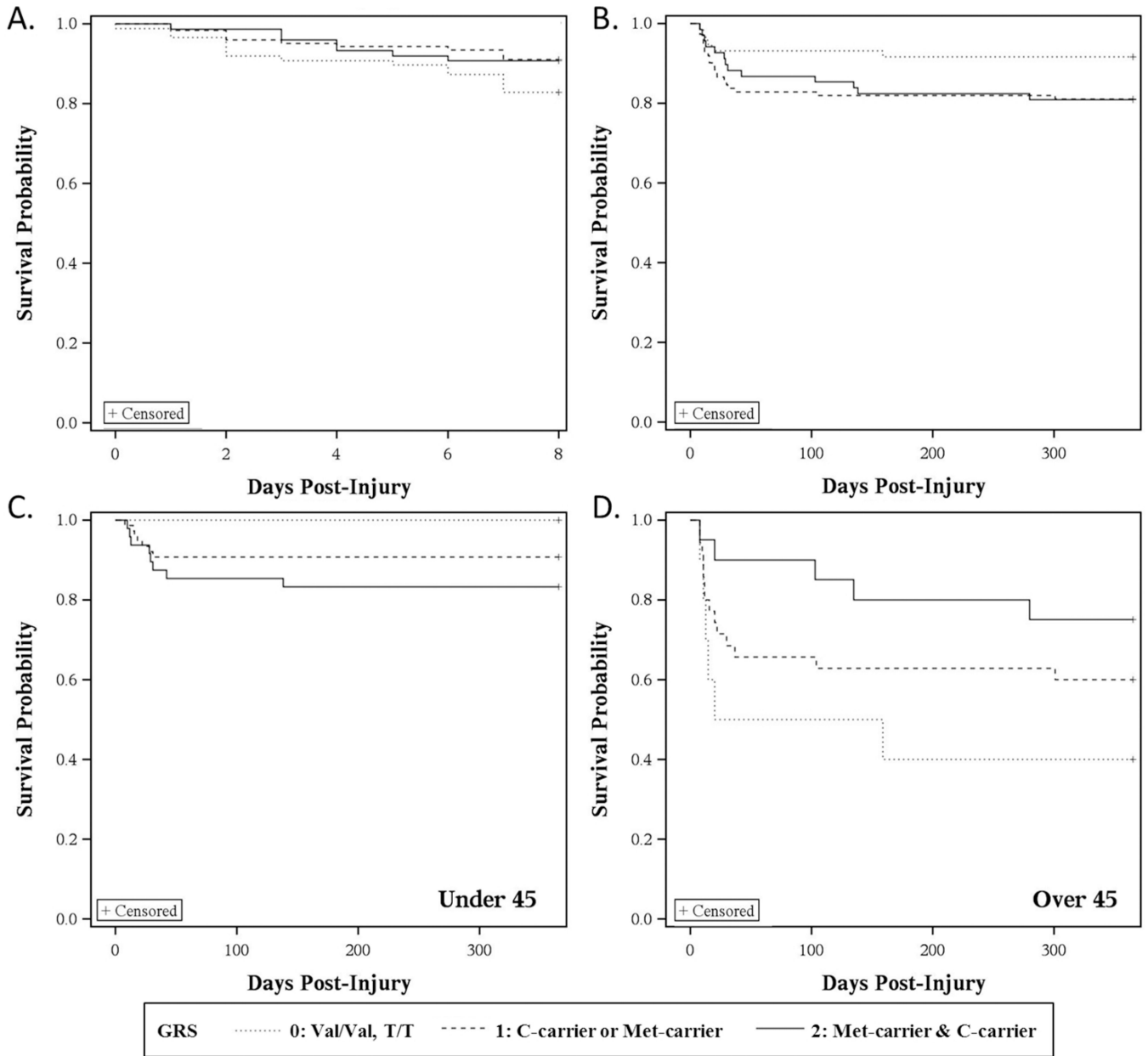


Figure 1. Kaplan-Meier curves show survivorship probabilities stratified by *BDNF* GRS
 Kaplan-Meier curves stratified by *BDNF* GRS at (A) 0–7d (GRS, $p=0.144$) and (B) 8–365d (GRS, $p=0.134$). GRS was significant in the Cox model for acute mortality, correcting for covariates. The post-acute (8–365d) 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).

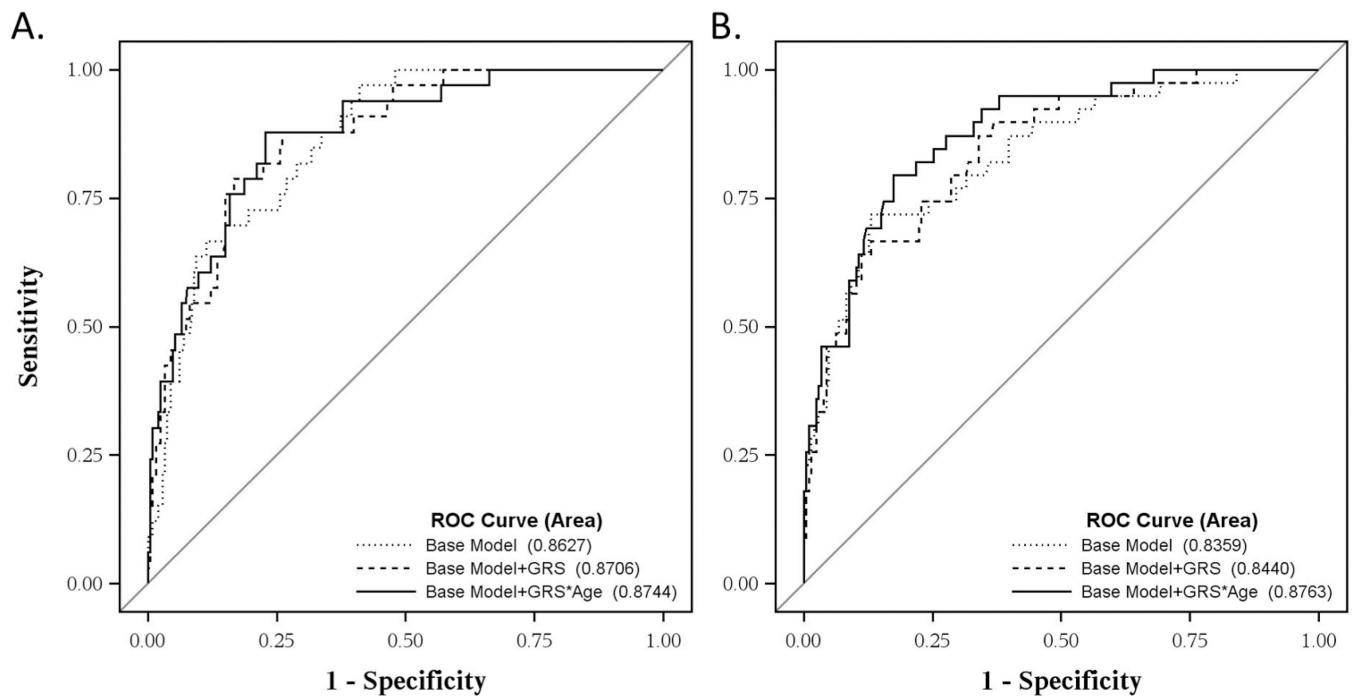


Figure 2. Receiver operating curves of GRS models in acute (0–7d) and post-acute (8–365d) mortality

(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).

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

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
Cardiovascular	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

	<i>R</i> _s 6265		<i>R</i> _s 7124442			
	Val/Val (n=170)	Val/Met Met/Met (n=114)	p value	C/C, C/T (n=126)	T/T (n=158)	p value
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

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 (pvalue)
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

Variable	Acute Mortality (0-7d, n=33)	Post-Acute Mortality (8-365d, n=40)	Survivors (at 365d n=211)	All Groups (pvalue)
ICH	12 (36.4%)	14 (35.0%)	78 (37.5%)	0.947
SAH	29 (87.9%)	30 (75.0%)	131 (62.1%)	0.005

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, 33 events)						
Age	0.02419	0.01118	4.681	0.031	1.024	(1.002–1.047)
GCS*	-0.35982	0.08939	16.204	<0.001	0.698	(0.586–0.831)
NBS#	0.47135	0.23476	4.031	0.045	1.602	(1.011–2.538)
Pulmonary Complication	-2.16656	0.43392	24.930	<0.001	0.115	(0.049–0.268)
Cardiac Complication	1.69773	0.59712	8.083	0.005	5.462	(1.695–17.603)
GRS (1 risk SNP)**	-1.04771	0.41069	6.508	0.011	0.351	(0.157–0.784)
GRS (2 risk SNP)**	-1.05395	0.48134	4.794	0.029	0.349	(0.136–0.895)

B. BDNF GRS predicting time until death, 8–365d post-injury (n=246, 39 events)						
Age	0.07065	0.01109	40.594	<0.001	1.073	(1.050–1.097)
GCS*	-0.30681	0.07779	15.556	<0.001	0.736	(0.632–0.857)
Acute PTS	0.74307	0.59263	1.572	0.210	2.102	(0.658–6.717)
Intracranial Hemorrhage	-0.74676	0.36615	4.160	0.041	0.474	(0.231–0.971)
Wound Complication	0.95654	0.46986	4.144	0.042	2.603	(1.036–6.537)
GRS 1**	0.38055	0.48137	0.625	0.429	1.463	(0.570–3.758)
GRS 2**	0.28517	0.52061	0.300	0.584	1.330	(0.479–3.690)

C. BDNF GRS x Age interaction predicting time until death, 8–365d post-injury (n=246, with 39 events)						
Age	0.15128	0.02536	35.5716	<0.001	1.163	(1.107–1.223)
GCS*	0.29814	0.07504	15.7837	<0.001	0.742	(0.641–0.860)
Acute PTS	1.44824	0.61090	5.6202	0.0178	4.256	(1.285–14.092)

C. BDNF GRS x Age interaction predicting time until death, 8–365d post-injury (n=246, with 39 events)

Intracranial Hemorrhage	-0.59617	0.36387	2.6845	0.1013	0.551	(0.270–1.124)
Wound Complication	1.10612	0.47471	5.4293	0.0198	3.023	(1.192–7.664)
GRS 1 **	3.42870	1.07292	10.2123	0.0014	30.836	(3.765–252.542)
GRS 2 **	6.25552	1.77560	12.4119	0.0004	520.881	(16.05–16909.54)
GRS x Age Interaction	-0.06964	0.01912	13.2643	0.0003	0.933	(0.898–0.968)

* GCS (Glasgow Coma Scale, Best in 24 hours)

‡ Neurological Burden Score

** GRS (0 risk SNP) was the reference category

CI: Confidence Interval