Downstream *TRPM4* Polymorphisms Are Associated with Intracranial Hypertension and Statistically Interact with *ABCC8* Polymorphisms in a Prospective Cohort of Severe Traumatic Brain Injury

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Abstract

Sulfonylurea-receptor-1(SUR1) and its associated transient-receptor-potential cation channel subfamily-M (TRPM4) channel are key contributors to cerebral edema and intracranial hypertension in traumatic brain injury (TBI) and other neurological disorders. Channel inhibition by glyburide is clinically promising. *ABCC8* (encoding SUR1) single-nucleotide polymorphisms (SNPs) are reported as predictors of raised intracranial pressure (ICP). This project evaluated whether *TRPM4* SNPs predicted ICP and TBI outcome. DNA was extracted from 435 consecutively enrolled severe TBI patients. Without *a priori* selection, all 11 *TRPM4* SNPs available on the multiplex platform (Illumina:Human-Core-Exome v1.0) were genotyped spanning the 25 exon gene. A total of 385 patients were analyzed after quality control. Outcomes included ICP and 6 month Glasgow Outcome Scale (GOS) score. Proxy SNPs, spatial modeling, and functional predictions were determined using established software programs. rs8104571 (intron-20) and rs150391806 (exon-24) were predictors of ICP. rs8104571 heterozygotes predicted higher average ICP ($\beta = 10.3$ mm Hg, $p = 0.00000029$), peak ICP ($\beta = 19.6$ mm Hg, $p = 0.0007$), and proportion ICP >25 mm Hg ($\beta = 0.16$ $p = 0.004$). rs150391806 heterozygotes had higher mean ($\beta = 7.2$ mm Hg, $p = 0.042$) and peak ($\beta = 28.9$ mm Hg, $p = 0.0015$) ICPs. rs8104571, rs150391806, and 34 associated proxy SNPs in linkage-disequilibrium clustered downstream. This region encodes TRPM4's channel pore and a region postulated to juxtapose SUR1 sequences encoded by an *ABCC8* DNA segment containing previously identified relevant SNPs. There was an interaction effect on ICP between rs8104571 and a cluster of predictive *ABCC8* SNPs (rs2237982, rs2283261, rs11024286). Although not significant in univariable or a basic multivariable model, in an expanded model additionally accounting for injury pattern, computed tomographic (CT) appearance, and intracranial hypertension, heterozygous rs8104571 was associated with favorable 6 month GOS (odds ratio [OR] = 16.7, $p = 0.007951$). This trend persisted in a survivor-only subcohort (OR = 20.67, $p = 0.0168$). In this cohort, two *TRPM4* SNPs predicted increased ICP with large effect sizes. Both clustered downstream, spanning a region encoding the channel pore and interacting with SUR1. If validated, this may guide risk stratification and eventually inform treatment-responder classification for SUR1-TRPM4 inhibition in TBI. Larger studies are warranted.

Keywords: ABCC8/ SUR1; cerebral edema; SNPs; TBI; TRPM4

Introduction

SIGNIFICANT FINANCIAL AND CLINICAL RESOURCES are dedicated to the evaluation and management of intracranial hypertension after severe traumatic brain injury (sTBI). Nonetheless, no pre-ventative or targeted treatments are available.[1] Intracranial hypertension is a complex product of many variables including injury characteristics (mechanism, severity, velocity, force, pattern, extent), patient characteristics (age, sex, race, comorbidities, intracranial compliance, autoregulation, genetics), and an expanding

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network of identified mechanisms underlying cerebral edema. Heterogeneity in these factors has rendered development of targeted treatments and success of clinical trials challenging; this underscores the importance of granular individualized phenotyping.

As with many diseases in this era of precision medicine, there is a growing recognition of the importance of genetic contributions to TBI variability including secondary-injury processes such as cerebral edema and intracranial hypertension.[2–6] Identifying genetic variants in key molecular pathways known to contribute to underlying cerebral edema may be valuable for both predictive and prognostic enrichment. These may affect transcriptional regulation, protein expression, structure, or function, thereby directing individual differences in timing, degree, and type of cerebral edema, relative predominance of specific pathways, and response to therapy. Eventually, this may guide patient selection for clinical trials and have implications for targeted therapy.

Seminal research has identified a central pathway in cerebral edema development in many acute neurological disorders including TBI, involving the transmembrane receptor sulfonylurea-receptor 1 (SUR1) and its associated non-selective cation channel-transient receptor potential cation channel subfamily M-4 (TRPM4).[7,8] Historically, SUR1 has been known for its regulation of a potassium-channel modulating insulin secretion (Kir6.2), and TRPM4 has been predominantly studied in cardiac-conductance disorders.[9] The SUR1-TRPM4 channel is now emerging as an exciting potential target and biomarker in brain injury and cerebral edema; the channel is not normally expressed in the central nervous system (CNS), but upregulated after injury in multiple cell types including neurons, astrocytes, and glia.[7,8,10,11] Channel opening occurs when upregulated SUR1 undergoes obligate association with TRPM4 resulting in depolarization, water influx, oncotic edema, and cell death. An existing antidiabetes medication (glibenclamide) inhibits SUR1-TRPM4 and has shown promising results against cerebral edema in recent clinical trials of stroke.[12,13] This pathway and potential benefits of its inhibition have been implicated in both pre-clinical and human TBI.[10,11,14–18] Regionally clustered single nucleotide polymorphisms (SNPs) in *ABCC8* (the gene encoding SUR1) have been associated with intracranial pressure (ICP) in TBI.[4,5] However, the impact of *TRPM4* genetic variation on cerebral edema is currently unknown.

We undertook an exploratory candidate-gene association approach to examining effects of *TRPM4* sequence variability on intracranial hypertension in TBI. We hypothesized that a subgroup of coding and non-coding *TRPM4* SNPs would be associated with ICP and outcome. We further explored the possibility of an interaction between *TRPM4* SNPs and previously reported *ABCC8* SNPs associated with ICP and outcome.[4,5]

Methods

Study design

Subjects were prospectively enrolled through the University of Pittsburgh Brain-Trauma Research Center protocol. Informed consent was obtained from legal authorized representatives. Inclusion criteria were: being between 16 and 80 years of age, having sTBI with admission Glasgow Coma Scale (GCS) score <9 and an external ventricular drain (EVD) per standard institutional clinical protocol. Exclusion criteria were imminent brain death and pregnancy. A total of 435 subjects were consecutively enrolled between 2006 and 2014. The University of Pittsburgh Institutional Review Board approved the study.

DNA collection and genotyping

Per our previously published protocol, DNA was obtained and extracted from white blood cells by the simple-salting out method.[5] Genotyping was performed using Illumina (Human-Core Exome v1.0) multiplex platform by the Center for Inherited Disease Research (CIDR) and included 11 SNPs distributed across *TRPM4*: rs11667393, rs3760666, rs113984787, rs146564314, rs1477363, rs10410857, rs56355369, rs909010, rs145847114, rs8104571, and rs150391806. SNPs were not identified *a priori*. SNP selection was unbiased and included all available TRPM4 SNPs covered by the Illumina Human-Core Exome v1.0 chip. Data cleaning and quality control included blind technical duplicates, Hardy–Weinberg Equilibrium (HWE) testing, and exclusion of SNPs that did not have a minimum 95% call rate. Principal-component analysis demonstrated that most participants clustered as Caucasian with the small numbers outside of that cluster being too few to analyze. After quality control exclusion of 50 samples, genotypes for 385 patients were included in the analysis. *ABCC8* SNPs in this TBI cohort had previously been genotyped per our published protocol and evaluated for associations with ICP and outcome; significant *ABCC8* SNPs were evaluated for interactions with *TRPM4* SNPs in this analysis.[4,5] CIDR research assistants involved in genotyping SNPs were blinded to demographic and outcome data.

Clinical data collection

Two previously established outcome measures were investigated.[5,14]

1. Hourly ICP (continuous), subcategorized into average and peak ICP (typically 5 days of neuromonitoring at the University of Pittsburgh Medical Center), as well as proportion of ICP spikes >25 mm Hg. Each hourly measurement was a point value obtained at the end of the hour. Proportion of ICP >25 mm Hg per patient was calculated by total number of ICP measurements >25 mm Hg for the specific patient divided by their total number of ICP measurements recorded over 5 days. The threshold of 25 mm Hg was selected because it evaluates the presence of intracranial hypertension regardless of the Brain Trauma Foundation guidelines defining intracranial hypertension as either 22 (4th edition) or 20 (3rd edition) mm Hg.[19]

2. Six-month Glasgow Outcome Scale (GOS) score (binary): favorable (GOS ≥ 4) versus unfavorable (GOS <4).

Secondary analyses censoring ICP recordings based on timing of craniectomy (if present) were also performed. Because exact matches for hourly ICP recording relative to timing of surgery were unavailable, standard censors were applied as follows. For patients who received their craniectomy on the day of presentation, ICP recordings after the first 6 h were censored, for patients who underwent craniectomy on post-TBI day 1, ICP recordings after the first 24 h were censored, for patients who underwent craniectomy on post-TBI day 2, ICP recordings after the first 48 h were censored, for patients who underwent craniectomy on post-TBI day 3, ICP recordings after the first 72 h were censored, for patients who underwent craniectomy on post-TBI day 4, ICP recordings after the first 96 h were censored, and for patients who underwent craniectomy on or after post-TBI day 5, or did not undergo craniectomy, all 120 h of ICP recordings were retained.

Spatial relationship modeling between *ABCC8* and *TRPM4* (gene) regions and SUR1-TRPM4 (protein) structure

Chromosomal locations were determined using the University of California, Santa Cruz (UCSC) genome browser, human genome assembly-38. Putative and canonical topology for human TRPM4

amino-acid sequence was obtained from PROTTER version-1.0, UniProt ID Q8TD43.[20] Peptide sequences encoded by specific exons were identified by Ensembl (release-92) and confirmed by the UCSC genome browser.[21] ProtAnnot within the Integrated-Genome Browser identified TRPM4 protein annotations in the context of *TRPM4* genomic sequence.[22] The established TRPM4 3-dimensional electron microscopy structure (6BQV) was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank.[23,24] University of California, San Francisco Chimera was used to generate three-dimensional structures of TRPM4 with its regulatory associated subunit SUR1 (5WUA).[25,26]

Functional category of TRPM4 SNPs

Predicted functional effects for exonic-SNPs were investigated using PolyPhen, SIFT/PROVEAN, and MutPred2.[27–29] RegulomeDB and Ensembl's Varient Effect Predictor were used for intronic-SNPs.[30,31] Significant and proxy-SNPs were evaluated for established clinical significance via PubMed, Embase, and ClinVar searches (see Supplementary Methods at at http://www.liebertpub.com).

Statistical analysis

Outcome variables between SNP genotypes were compared using analysis of variance (F-test, ANOVA) and Fisher's exact test. Between-group comparisons were adjusted using Bonferroni's method. Pairwise linkage-disequilibrium (LD) between SNPs was determined using LDlink for proxy-SNPs.[32] Univariable linear and logistical regression models evaluated independent relationships between *TRPM4* SNPs and continuous or categorical outcomes. Basic multivariable regression models were developed with clinically relevant variables (age, gender, initial GCS score, presence of craniectomy) to control for confounders that could influence ICP and/or outcome. Expanded multivariable models also included pattern of primary injury, computed tomographic (CT) appearances (either collectively in expanded model-1, or with individual components separated out as independent predictors in expanded model-2; that is, cistern effacement, sulcal effacement, ventricular compression, midline shift, herniation), and (for clinical outcome), proportion of intracranial hypertension recordings >25 mm Hg. Multiple imputations were used for missing data; models with and without multiple imputations are presented. Odds ratios (OR) and β-coefficients were based on genotype: homozygous-wild-type (AA), heterozygous (Ab), or homozygous-variant (bb). Multiple comparisons for the regression models were adjusted using the established Benjamin–Yekutieli (B–Y) method[5,33,34] yielding a significance threshold of $p = 0.01656$. The likelihood ratio test compared full (with SNP) versus reduced (without SNP) models to test whether the inclusion of the respective SNP significantly added to model fit. The exact test assessed HWE. Weighted and un-weighted gene risk scores were calculated for *TRPM4* and *ABCC8* SNPs using a similar method as previously described by Lu and coworkers (see Supplementary Methods at http://www.liebertpub.com).[35,36] Analyses were performed using Stata 15.0 (StataCorp, TX) and RStudio Version 1.1.453.

Results

Patient characteristics are summarized in Table 1. Of 11 analyzed SNPs distributed across the length of the *TRPM4* gene, two downstream SNPs, rs8104571 (intron 20) and rs150391806 (exon 24), were significantly associated with average ICP, peak ICP, and proportion of ICP spikes >25 mm Hg in univariable and multivariable single-locus analysis (Fig. 1A). Table 2 describes genotype frequencies and descriptive statistics of ICP variation and 6 month GOS between genotypes. There were no homozygous variants.

TABLE 1. PATIENT CHARACTERISTICS

Variable (n = 385)		Mean (SD, Range)
Age		37.9 (16.8, 16–77)
Initial GCS		5.83 (1.51, 3–8)
Average ICP (*n* = 191)		10.4 (5.0, 4–45)
Peak ICP (*n* = 191)		25.9 (9–96)
		Frequency n (%)
Gender (M)		304 (79.0%)
Proportion of ICP >25 mmHg		5.9%
Decompressive craniotomy (Y)		128/385 (0.33)
Day 0		87/128 (0.68)
Day 1		18/128 (0.14)
Days 2, 3, 4 or 5		14/128 (0.11)
Pattern of injury	*Primary*	*Additional/Mixed* (n = 257)
No radiographic lesion	9 (2.4%)	0
Epidural	22 (6.0%)	6 (2.3%)
Subdural	118 (32%)	53 (20.6%)
Subarachnoid	60 (16.3%)	98 (38.1%)
Contusion	107 (29%)	74 (28.8%)
Intraventricular	11 (3%)	22 (8.6%)
Diffuse axonal injury	42 (11.3%)	4 (1.6%)

Summarizes the patient characteristics of the severe TBI population overall (*n* = 385). ICP measurements were available for 191 patients.

GCS, Glasgow Coma Scale; ICP, intracranial pressure; TBI, traumatic brain injury.

rs8104571 and rs150391806 are associated with ICP

Average ICP was increased by >100% in rs8104571 heterozygotes (20.4 ± 6.5 mm Hg) versus homozygous wild-type (10.1 ± 0.3 mm Hg, $p = 0.0000$, Table 2). Concordantly, heterozygotes had significantly higher peak ICP (45.0 ± 10.7 mm Hg vs. 25.4 ± 0.90 mm Hg, $p = 0.0007$) and proportion of ICP spikes >25 mm Hg (21.3 ± 13.1% vs. 5.5 ± 0.77%, $p = 0.004$). Univariable and multivariable regression analysis demonstrated that heterozygous rs8104571 was an independent predictor of all three measures including higher average ICP (univariable, $\beta = 10.3$ mm Hg, multivariable, $\beta = 9.11$ mm Hg [basic model], 9.55 mm Hg [expanded model 1]), peak ICP (univariable, $\beta = 19.6$ mm Hg, multivariable, $\beta = 16.8$ mm Hg [basic model], 17.6 mm Hg [expanded model 1]), and proportion of ICP spikes >25 mm Hg (univariable, $\beta = 0.16$, multivariable, $\beta = 0.15$ [basic model], 0.13 [expanded-model 1]) surviving the B–Y correction for multiple comparisons (Table 3). Effects were large. Inclusion of rs8104571 genotype in the basic multivariable regression model significantly increased the model's R^2 from 0.1204 to 0.205, and further to 0.2483 in the expanded multivariable regression model that also included pattern of primary injury and acute CT findings with edema.

rs150391806 heterozygotes had significantly higher peak ICP (54.5 ± 17.5 mm Hg vs. 25.6 ± 0.91 mm Hg, $p = 0.0015$) and average ICP (17.5 ± 3.5 mm Hg vs. 10.3 ± 0.36 mm Hg, $p = 0.041$) versus homozygous wild-type (Table 2). In univariable and multivariable analyses, heterozygous rs150391806 remained an independent predictor of peak ICP (univariable, $\beta = 28.9$ mm Hg, multivariable, $\beta = 25.7$ mm Hg, Table 3).

All results were robust to multiple imputations for missing values (Table S1) as well as expanded multivariable models that
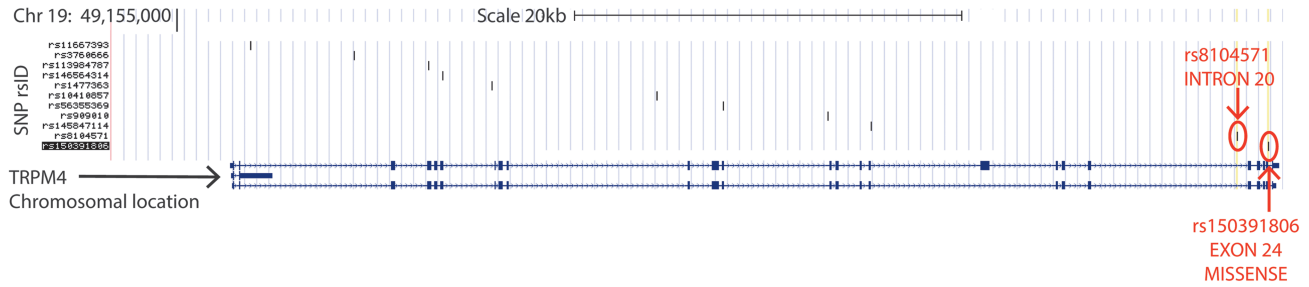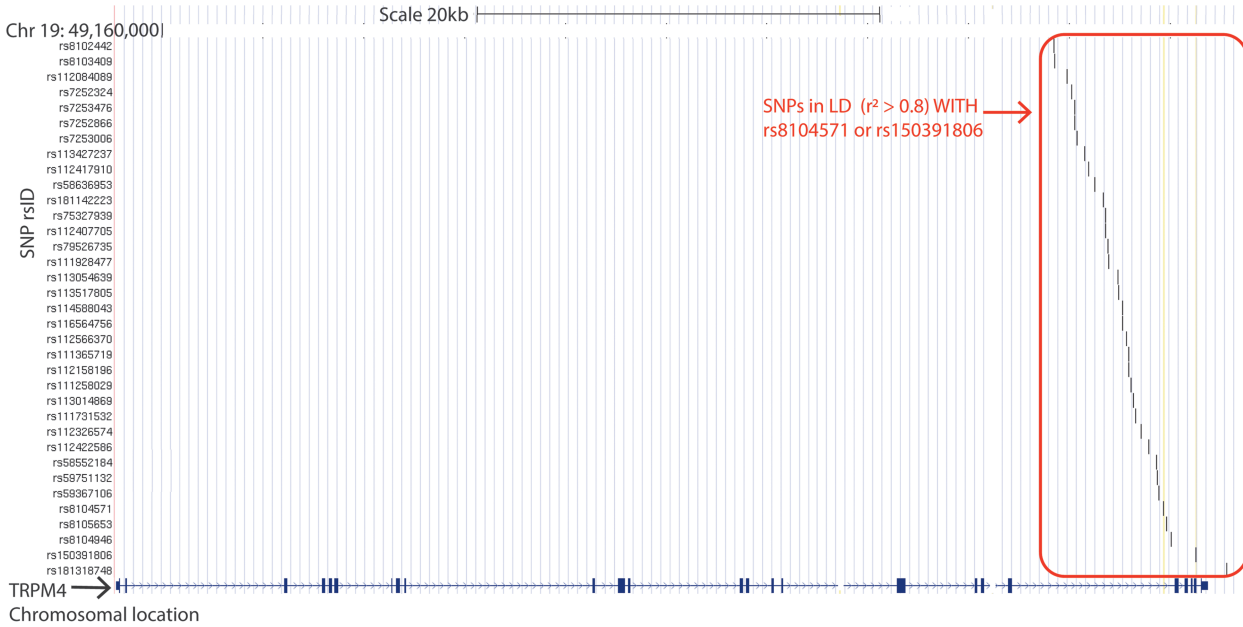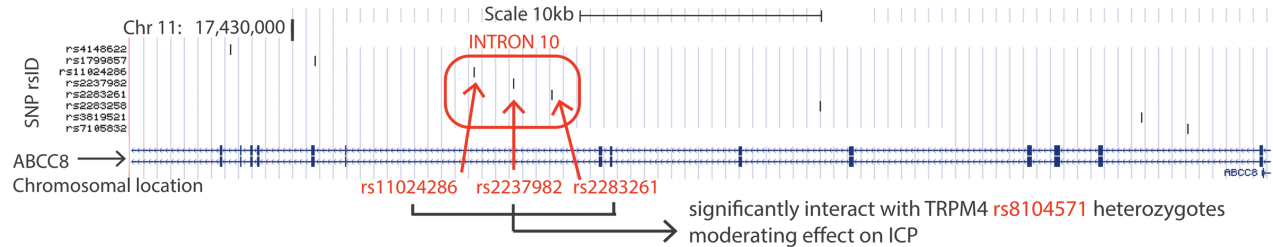
FIG. 1. Transient-receptor-potential cation channel subfamily-M (*TRPM4*) single nucleotide polymorphisms (SNPs) associated with intracranial pressure (ICP) in severe traumatic brain injury (sTBI). **(A)** Chromosomal location on chromosome 19 of all sequenced *TRPM4* polymorphisms in this study plotted on the University of California, Santa Cruz (UCSC) genome browser (assembly version GRCh38/hg38, scale bar for 20 kb, shown). The schematic of the 34-exon *TRPM4* gene is on the x axis where exons (separated by introns) are marked with navy blue vertical lines/blocks. Because *TRPM4* is encoded on the forward strand, exon #1 is on the left extreme and exon #25 is on the right extreme of the x axis gene schematic. The vertical axis lists all SNPs by reference-SNP cluster identification (rsid) sequenced in this study. Locations of the two significant SNPs rs8104571 (intron 20) and rs150391806 (exon 24) are indicated by the red arrow. **(B)** Chromosomal location for all proxy SNPs in linkage-disequilibrium (LD) with either rs8104571 or rs150391806 shown on the UCSC genome browser as mentioned. The vertical axis lists all these proxy SNPs by rsid. Their corresponding locations are indicated by the red box. **(C)** Chromosomal location for the three *ABCC8* SNPs (rs11024286, rs2237982, and rs2283261) located in *ABCC8*'s intron 10 (on chromosome 11) that interact with rs8104571 (*TRPM4*) are plotted on the UCSC genome browser. The vertical axis labels rsids for all eight significant ABCC8 SNPs previously identified as important predictors of cerebral edema ± outcome after TBI. Their corresponding locations on a zoomed in version of the *ABCC8* chromosome are demarcated by vertical black lines (scale bar 10 kb). The red box encircles all three significant *ABCC8* SNPs that interact with rs8104571 in terms of their effects on ICP, and the red arrows label each of these SNPs individually. Chr, chromosome; hg, human genome; kb, kilo-basepair.

TABLE 2. POLYMORPHISM DISTRIBUTION AND ASSOCIATED CHARACTERISTICS IN THE SEVERE TBI COHORT

SNP	Variable measured	Homozygous wild-type	Heterozygous	p-value (ANOVA/Fisher)
rs8104571 (Intron 20) wild-type C	SNP Frequency in sTBI population (%)	377/385 (97.9%)	8/385 (2.1%)	
	Average ICP, mean (SE)	10.1±0.31 mmHg	20.4±6.5 mmHg	*0.0000**
	Peak ICP, mean (SE)	25.4±0.90 mmHg	45.0±10.7 mmHg	*0.0007**
	Average proportion ICP >25 mmHg (SE)	0.055 (0.0077)	0.213 (0.131)	*0.004**
	% Craniectomy Y	32.9%	50%	0.45
	% Favorable 6 month GOS ≥ 4, frequency (%)	32.8%	62.5%	0.087
	% Favorable 6 month GOS ≥4 in survivors, frequency(%)	45.0%	83.3%	0.073
rs150391806 (Exon 23) Wild-type C	SNP Frequency in sTBI population (%)	382/385 (99.2%)	3/385 (0.8%)	
	Average ICP, mean (SE)	10.3±0.36 mmHg	17.5±3.5 mmHg	*0.041**
	Peak ICP, mean (SE)	25.6±0.91 mmHg	54.5±17.5 mmHg	*0.0015**
	Average proportion ICP >25 mmHg (SE)	0.058 (0.008)	0.129 (0.103)	0.49
	% Craniectomy Y	33.2%	33.3%	1.0
	% Favorable 6 month GOS ≥ 4, frequency (%)	33.5%	33.3%	1.0
	% Favorable 6 month GOS ≥4 in survivors, frequency(%)	45.9%	50%	1.0

Summarizes the relative frequencies of the SNP genotypes in the severe TBI population, as well as for measures of cerebral edema (average ICP, peak ICP, proportion of ICP spikes >25 mm Hg), need for craniectomy, and outcome.
Boldface, italics, and asterisks indicate significant p-value after B–Y correction for multiple comparisons.
TBI, traumatic brain injury; SNP, single-nucleotide polymorphism; ANOVA, analysis of variance; ICP, intracranial pressure; SE, standard error of the mean; GOS, Glasgow Outcome Scale.

included primary injury pattern and acute CT characteristics as covariates (Table 3) (see online supplementary material at http://www.liebertpub.com). Despite exclusion of post-craniectomy ICP values in secondary sensitivity analyses resulting in an inevitable loss of data points, rs8104571 (not rs150391806) remained an independent univariable predictor of average ICP ($\beta = 4.74$, $p = 0.0342$), peak ICP ($\beta = 8.89$, $p = 0.0256$), and proportion of ICP spikes >25 mm Hg ($\beta = 098$, $p = 0.0193$); however, it did not withstand the B–Y correction (Table S2)(see online supplementary material at http://www.liebertpub.com). The same trend was observed in the multivariable analyses for average and peak ICP ($p = 0.0625$ and $p = 0.0855$ respectively), as well as increased proportion of ICP spikes >25 mm Hg ($\beta = 0.095$, $p = 0.02391$) which also did not withstand the B–Y correction.

rs8104571 and GOS

Neither rs8104571 nor rs150391806 were associated with GOS or mortality in univariable or basic multivariable models (Tables 3 and S3)(see online supplementary material at http://www.liebertpub.com). However, as proportion of intracranial hypertension and CT appearances (compression of basal cisterns, sulcal effacement, ventricular effacement, herniation, midline shift) are known to influence clinical outcome after TBI, expanded multivariable models were created to include these covariates. These expanded models suggest that variant rs8104571 but not rs150391806 may be associated with improved 6 month GOS (Table 3). Patients with variant rs8104571 in expanded model-1 had an OR of 16.7 for favorable outcome ($p = 0.007951$). Variant rs8104571 in expanded model-2 had a trend for favorable GOS that did not survive the B–Y correction (OR = 10.91, $p = 0.027$). This association with favorable GOS persisted in a subcohort of *survivors-only* ($p = 0.0168$, Table 3) and came close to surviving the B–Y correction of 0.01656.

The likelihood ratio test comparing expanded model-1 versus a reduced version of this model without *TRPM4* rs8104571 (but with the same covariates) was significant ($p = 0.0064$), suggesting that this additional predictor with genetic information added to model fit. The pseudo-R^2 for the reduced model increased from 0.2946 (without *TRPM4* rs8104571) to 0.3191 with inclusion of the SNP in expanded model-1 indicating that with this information, ~31.91% of the variation in 6 month GOS could be explained by the model. Area under the receiver operating characteristic (ROC) curve (AUC) for the multivariable model without any SNP information was 0.8069, demonstrating excellent discrimination for 6 month GOS (Fig. 2). This AUC was further slightly improved by addition of rs8104571 in the basic model (0.8165), and even more so in expanded model-1 (0.8565) and expanded model-2 (0.8558). Weighted/unweighted gene risk scores in small random training and testing subsets did not significantly improve model prediction (Fig. S1) (see online supplementary material at http://www.liebertpub.com); however, the analysis was limited by the small number of subjects. Finally, including the three *ABCC8* SNPs (rs2237982, rs2283261, rs11024286, see next subsection) that statistically significantly interacted with *TRPM4* rs4108571 in expanded-model 1 further increased the AUC to 0.8738. These AUCs were significantly different ($p = 0.011$).

Interaction between rs8104571 and a regional cluster of ABCC8 SNPs reported to predict ICP

There was a significant interaction between rs8104571 genotypes and a regional cluster of *ABCC8* SNPs located in intron-10

TABLE 3. TRPM4 SNP PREDICTION OF INTRACRANIAL PRESSURE AND OUTCOME IN SEVERE TBI

	Average ICP β-coefficient (95% CI, p)	Peak ICP β-coefficient (95% CI, p)	Proportion of ICP >25 mmHg β-coefficient (95% CI, p)	Favorable 6 month GOS Odds ratio (95% CI, p)
Univariable regression analyses				
rs8104571 (TC)	10.3 mmHg (6.1–14.5, **0.0000029***)	19.6 mmHg (8.4–30.9, **0.00071***)	0.16 (0.05–0.26, **0.0042***)	3.4 (0.8–14.5, 0.097)
rs150391806 (TC)	7.19 mmHg (0.28–14.1, **0.042**)	28.9 mmHg (11.2–46.6, **0.0015***)	0.07 (−0.13–0.27, 0.5)	0.99 (0.09–11.0, 0.99)
Multivariable regression analyses				
rs8104571 (TC)				
Basic model	9.11 mmHg (5.0–13.2, **0.000021***)	16.8 mmHg (5.6–28.1, **0.0035***)	0.15 (0.04–0.25, **0.0095***)	4.0 (0.74–21.6, 0.1)
Expanded model 1	9.55 mmHg (5.22–13.89, **0.000025***)	17.6 mmHg (6.25–28.99, **0.0026***)	0.13 (0.021–0.25, **0.021**)	16.7 (2.23–165.21, **0.007951***)
Expanded model 2	9.92 mmHg (5.52–14.31, **0.000016***)	18.5 mmHg (6.94–30.08, **0.001912***)	0.14 (0.022–0.26, **0.0197**)	10.97 (1.32–91.23, **0.027**)
Survivors only	NA	NA	NA	20.67 (2.20–480.5, **0.016819**)
rs150391806 (TC)				
Basic model	5.91 mmHg (−0.77–12.6, 0.082)	25.7 mmHg (8.2–43.3, **0.004***)	0.05 (−0.15–0.26, 0.6)	0.79 (0.06–10.6, 0.86)
Expanded model 1	4.34 mmHg (−2.6–11.3, 0.21)	22.6 mmHg (5.26–39.9, **0.011***)	0.027 (−0.18–0.24, 0.79)	1.6e-6 (NA–1.01e61, 0.99)
Expanded model 2	5.74 mmHg (−1.2–12.7, 0.10)	26.6 mmHg (9.2–44.1, **0.003***)	0.06 (−0.15–0.27, 0.59)	1.01e-6 (NA–9.98e58, 0.99)
Survivors only	NA	NA	NA	2.9e-6 (NA–2.98e72, 0.99)

Provides the regression analysis results of each significant TRPM4 single nucleotide polymorphism (SNP) effect on the outcome measures including average ICP, peak ICP, proportion of ICP spikes >25 mmHg, and odds ratio for favorable 6 month Glasgow Outcome Scale (GOS). Univariate regression results are provided first, followed by multivariate regression analysis results. For the multivariable regression analyses for ICP outcomes, basic model = SNP, sex, initial Glasgow Coma Scale (GCS) score, and presence/absence of craniectomy; expanded model 1 = SNP, age, sex, initial GCS, craniectomy, pattern of primary injury, edema on acute CT; expanded model 2 = SNP, age, sex, initial GCS, craniectomy, pattern of primary injury, sulcal effacement, cistern effacement, ventricular effacement, herniation, midline shift. For favorable 6 month GOS, basic model = multivariable model including age, sex, initial GCS; expanded model 1 = multivariable model including age, sex, initial GCS, craniectomy, pattern of primary injury, edema on acute CT, proportion of intracranial hypertension >25 mm Hg; expanded model 2 = multivariable model including age, sex, initial GCS, craniectomy, pattern of primary injury, sulcal effacement, cistern effacement, ventricular effacement, herniation, midline shift, proportion of intracranial hypertension >25 mm Hg. Survivors only subcohort = expanded model 1 in patients who were alive at discharge.

Boldface, italics, and asterisks indicate significant p-value after B-Y correction for multiple comparisons. Boldface and italics, but no asterisks, indicate p-value <0.05 standard threshold for significance but does not withstand B-Y correction for multiple comparisons.

TBI, traumatic brain injury; ICP, intracranial pressure; TC, thymine-cytosine.
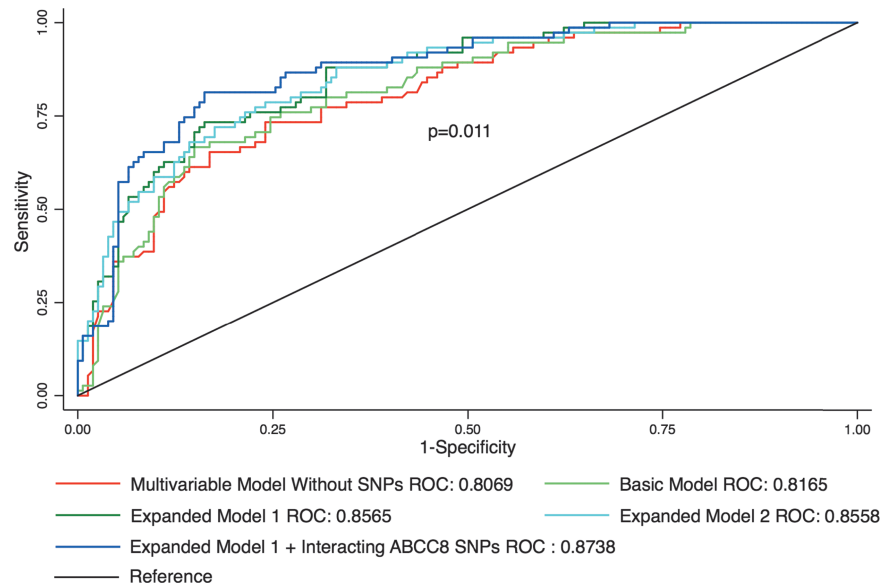
FIG. 2. Receiver operating characteristic curves for 6 month Glasgow Outcome Scale (GOS). Graph of receiver operating characteristic (ROC) curves for different multivariable models used to predict 6 month GOS score. The multivariable model without any single nucleotide polymorphism (SNP) information (red) provides excellent discrimination with an area under the curve (AUC) of 0.8069. The basic multivariable model (light green, containing *TRPM4* rs8104571, age, sex, and initial Glasgow Coma Scale [GCS] score) had an AUC of 0.8165. Expanded model-1 (dark green, containing transient-receptor-potential cation channel subfamily-M [*TRPM4*] rs8104571, age, sex, initial GCS, pattern of primary injury, computed tomographic [CT] characteristics of edema, and proportion of intracranial hypertension >25 mm Hg) had an AUC of 0.8565. Expanded model-2 (turquoise, containing *TRPM4* rs8104571, age, sex, initial GCS, pattern of primary injury, distinct CT characteristics of edema [separated into individual components of sulcal effacement, cistern effacement, ventricular compression, midline shift, and herniation], and proportion of intracranial hypertension >25 mm Hg) had an AUC of 0.8558. Addition of *ABCC8* SNPs (rs2237982, rs2283261, and rs11024286 that statistically significantly interacted with TRPM4 rs8104571 in this cohort) to expanded model-1 (blue) further improved the AUC to 0.8738. These AUCs were significantly different from one another ($p = 0.011$).

previously reported to predict ICP and/or TBI outcome (rs2237982, rs2283261, rs11024286, Fig. 1C).[4,5] Effects of rs2237982, rs2283261, and rs11024286 *ABCC8* genotypes on all measures of ICP (average ICP, peak ICP, and proportion of ICP spikes >25 mm Hg) were significantly different based on the

TRPM4 rs8104571 genotype and vice versa (Table 4). The magnitude and direction of interactions between rs8104571 and each of the three *ABCC8* SNPs on ICP is illustrated in Figure 3A–C. Patients heterozygous for rs8104571 had markedly increased average ICP, peak ICP, and proportion of ICP >25 mm Hg if they

TABLE 4. *TRPM4* AND *ABCC8* POLYMORPHISM INTERACTIONS IN MULTIVARIABLE REGRESSION MODEL

|	*TRM4 SNP interactions*					
	TRPM4 rs8104571			*TRPM4 rs150391806*		
ABCC8 SNP	*Average ICP*	*Peak ICP*	*Proportion ICP >25 mmHg*	*Average ICP*	*Peak ICP*	*Proportion ICP >25 mmHg*
Significant interactions	Interaction *p* value			Non-significant interactions		
rs2237982	*0.0000034**	*0.00079**	*0.00023**	rs2237982		
rs2283261	*0.0000016**	*0.0004**	*0.0008**	rs2283261		
rs11024286	*0.000021**	*0.00032**	*0.0003**	rs11024286		
Non- significant interactions				rs7105832		
				rs3819521		
				rs2283258		
rs7105832				rs1799857		
rs3819521				rs4148622		
rs2283258						
rs1799857						
rs4148622						

Demonstrates the interactions between the two significant TRPM4 SNPs (rs8104571and rs150391806) found to predict measures of intracranial pressure (ICP) in this study, and previously reported significant *ABCC8* SNPs (rs2237982, rs2283261, rs11024286, rs7105832, rs3819521, rs2283258, rs1799857, and rs4148622). There were significant interactions between rs8104571 and a cluster of significant ABCC8 SNPs located in intron-10 (rs2237982, rs2283261, and rs11024286). The direction and magnitude of these interactions on outcome measures (average, peak, and proportion of ICP spikes) is demonstrated in Figure 3.

Boldface, italics, and asterisks indicate significant p-value after B-Y correction for multiple comparisons.

were also homozygous-*variant* for *ABCC8* SNPs rs2237982 or rs228326, potentially indicating dose-dependent interactions (Fig. 3A and B). This maintains directional consistency with previous reports in which homozygous-variant rs2237982 and rs2283261 both independently predicted increased ICP.[4,5] rs8104571 heterozygotes had markedly increased average ICP, peak ICP, and proportion of ICP >25 mm Hg if they were also homozygous *wild-type* for *ABCC8* SNP rs1102428 (Fig. 3C), congruent with prior reports that the variant rs1102428 allele is protective.

There were no patients both heterozygous for *TRPM4* rs8104571 *and* homozygous variant for *ABCC8* rs11024286. For average and peak ICP, there was collinearity between heterozygous *TRPM4* rs8104571 and homozygous wild-type *ABCC8* rs2237982, as well as between heterozygous *TRPM4* rs8104571 and homozygous wild-type *ABCC8* rs2283261. There were no statistically significant interactions between rs8104571 or rs150391806 and any of the previously reported significant *ABCC8* SNPs in terms of effect on GOS or mortality (Table S4)(see online supplementary material at http://www.liebertpub.com).
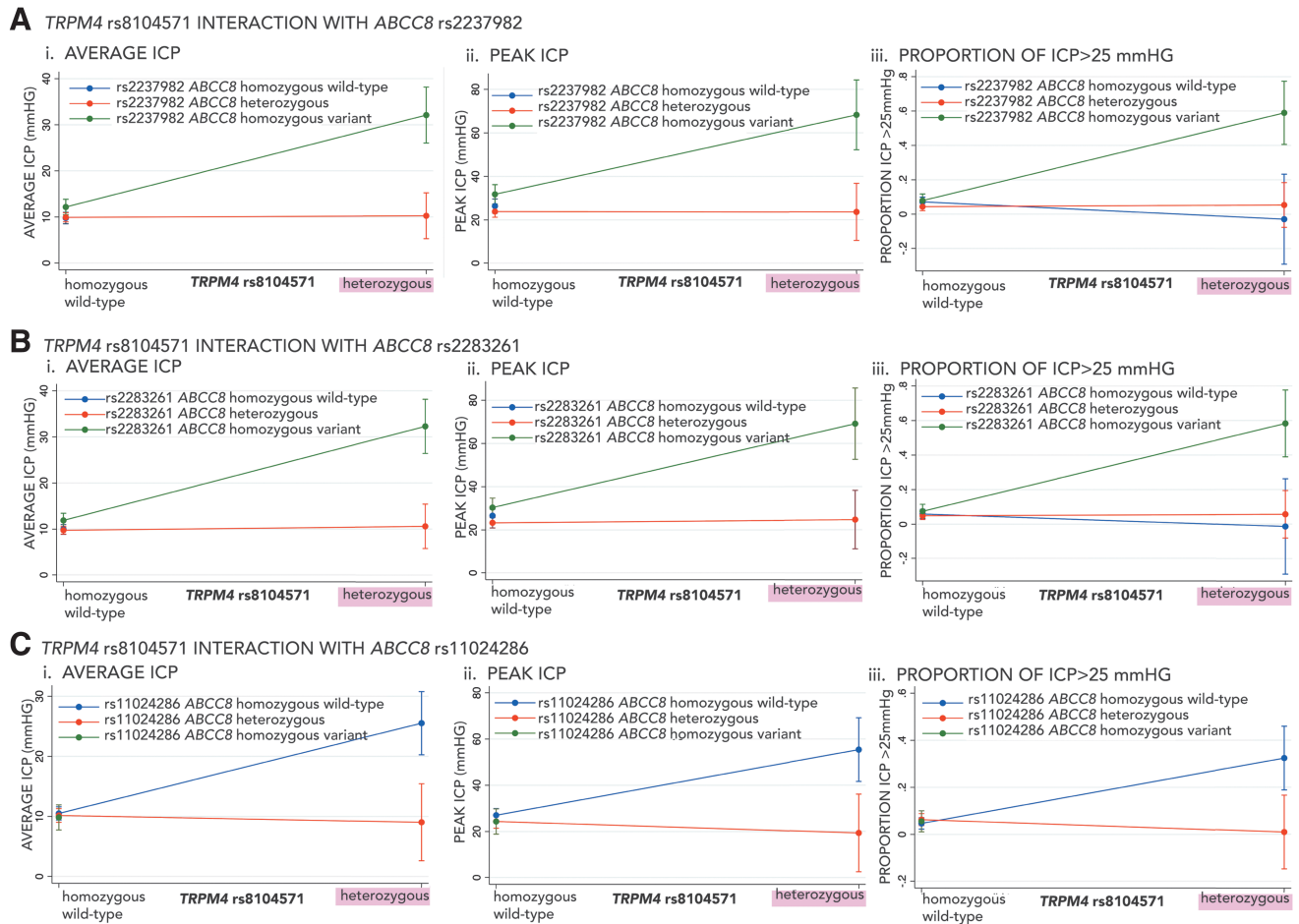
FIG. 3. Interaction effects of transient-receptor-potential cation channel subfamily-M (*TRPM4*) rs8104571 with previously reported significant *ABCC8* single nucleotide polymorphisms (SNPs) on intracranial pressure (ICP). Graphs demonstrating the direction and magnitude of the interaction effect on ICP between *TRPM4* rs8104571and each of the three *ABCC8* SNPs previously implicated as significant in traumatic brain injury (TBI) organized into the following panels: **(A)** *ABCC8*- rs2237982, **(B)** *ABCC8*-rs2283261, and **(C)** *ABCC8*-rs11024286. Panel subgraphs show the interaction effects between these SNPs on various measures of ICP including **(i)** average-ICP, **(ii)** peak = ICP, and **(iii)** proportion of ICP spikes >25 mm Hg. The x axes for all graphs are *TRPM4* rs8104571 genotypes: homozygous wild-type and heterozygous. The y axes for subgraphs **A-i, B-I,** and **C-i** are average ICP (mm Hg). The y axes for subgraphs **A-ii, B-ii,** and **C-ii** are peak ICP (mm Hg). The y axes for subgraphs **A-iii, B-iii,** and **C-iii** are proportion of ICP >25 mm Hg. Each individual graph shows the interaction between *TRPM4* rs8104571 genotypes and the respective *ABCC8* SNP genotypes where homozygous wild-type *ABCC8* genotypes are in blue, heterozygous *ABCC8* genotypes are in red, and homozygous-variant *ABCC8* genotypes are in green. Error bars are 95% confidence intervals. For example, in Panel A-subgraph i, the average ICP in patients who are homozygous-wild type for *TRPM4* rs8104571 is ~10 mm Hg regardless of the *ABCC8* rs2237982 genotype. However, in patients heterozygous for *TRPM4* rs8104571, the average ICP is significantly higher (i.e., ~30 mm Hg) if the patients are also homozygous-variant for *ABCC8* rs2237982. In panels **A-i, A-ii, B-i,** and **B-ii** (depicting interaction effects on average and peak ICP), there are no observations for homozygous wild-type *ABCC8* SNPs (blue) in any of these panels, because the interaction coefficient was omitted by the regression model as a result of collinearity between heterozygous *TRPM4* rs8104571 and homozygous wild type *ABCC8* rs2237982 (**A-i, A-ii**) as well as between *TRPM4* rs8104571 and homozygous wild-type *ABCC8* rs2283261 (**B-i, B-ii**). For Panel C (**i, ii, iii**) there were no patients who were both heterozygous for *TRPM4* rs8104571 and homozygous variant for *ABCC8* rs11024286.

*Predicted functional implications of significant TRPM4
and proxy-SNPs*

rs8104571 and rs150391806 were not in LD with each
other ($r^2 \leq 0.8$) thus precluding haplotype generation. Thirty-
four additional *TRPM4*-SNPs were in LD ($r^2 \geq 0.8$) with ei-
ther rs8104571 or rs150391806 (Fig. 1B). This region of LD
extended across intron 20-exon 24 (Fig. S2). Table S5 and
Figure S3 summarize association strengths, LD, and pre-
dicted functional implications of these SNPs including a mis-
sense mutation, regulatory region variants, upstream gene
variants, non-coding transcript variants, and nonsense-mediated
decay (see online supplementary material at http://www
.liebertpub.com).

Gene-protein spatial model of significant TRPM4 SNPs

TRPM4 has 25 exons. rs8104571, rs150391806, and all 34
proxy-SNPs in this region of LD were downstream (intron 20-exon
24; Figs. 1B and S2) (see online supplementary material at http://
www.liebertpub.com). Combining human TRPM4 putative and
canonical amino-acid topology with *TRPM4* exons, we identified
protein regions encoded by exon 21 (closely flanking rs8104571 in
intron 20 and other proxy SNPs in LD), and exons 23–24 (the latter
containing rs150391806, Fig. 4A). A three-dimensional model
demonstrates that these sequences spatially contribute to the
channel-pore and a SUR1-TRPM4 binding interface (Fig. 4B). The
involved binding site region juxtaposes a part of SUR1(the asso-
ciated regulatory protein) encoded by a section of *ABCC8* con-
taining previously identified SNPs associated with cerebral edema
and ICP after TBI.[4] This may conceptually support the earlier result
of interactions between genetic variations in *ABCC8* and *TRPM4*
influencing cerebral edema and ICP.

Discussion

In a cohort of consecutive sTBI patients, two *TRPM4* SNPs
spanning a region encoding the channel pore and believed to in-
teract with SUR1, predicted increased ICP, thus adding to a
growing body of research supporting the potential importance of
genetic variation in this pathway and its phenotypic influence after
brain injury.[4,5] SUR1-TRPM4 plays a key role in cerebral edema
development and intracranial hypertension in a spectrum of acute
neurological disorders, the consequences of which are often life
threatening.[5,8,12,13,18,37] To date, no targeted preventive therapies
are available. Glibenclamide inhibition of SUR1-TRPM4 in human
and pre-clinical TBI has been independently linked with various
favorable outcomes including reduced cerebral edema, hemorrhage
progression, tissue loss, preserved white matter, and improved
cognition.[8,10,11,15–17,38] Results from phase I and II stroke trials of
glibenclamide are encouraging, and a phase III trial is enrolling
participants.[12,13] Given the promising nature of this target for
clinical inhibition in the context of many failed TBI trials attributed
to disease heterogeneity and scarcity of biomarkers, granular
phenotyping (including genetic information) could be valuable for
enriching patient selection.[14,39] Genetic variation may influence the
cellular location, timing, and degree to which SUR1-TRPM4
contributes to edema in individual patients, and whether (or how)
these patients differentially respond to targeted inhibition.

This study provides a foundation to further develop the predic-
tive and prognostic enrichment utility of *ABCC8-TRPM4* genetic
variability. We report three central findings. (1) Two *TRPM4* SNPs
(rs8104571 and rs150391806) were independent predictors of ICP

and in LD with 34 additional proxy-SNPs located between intron
20 and exon 24. (2) There was a significant interaction effect on ICP
between rs8104571 and three regionally clustered *ABCC8* poly-
morphisms (rs2237982, rs2283261, and rs11024286) previously
reported as predictors of cerebral edema and ICP in TBI. (3) The
implicated genetic region of *TRPM4* encodes sequences contrib-
uting to the channel pore and a TRPM4-SUR1 binding interface.
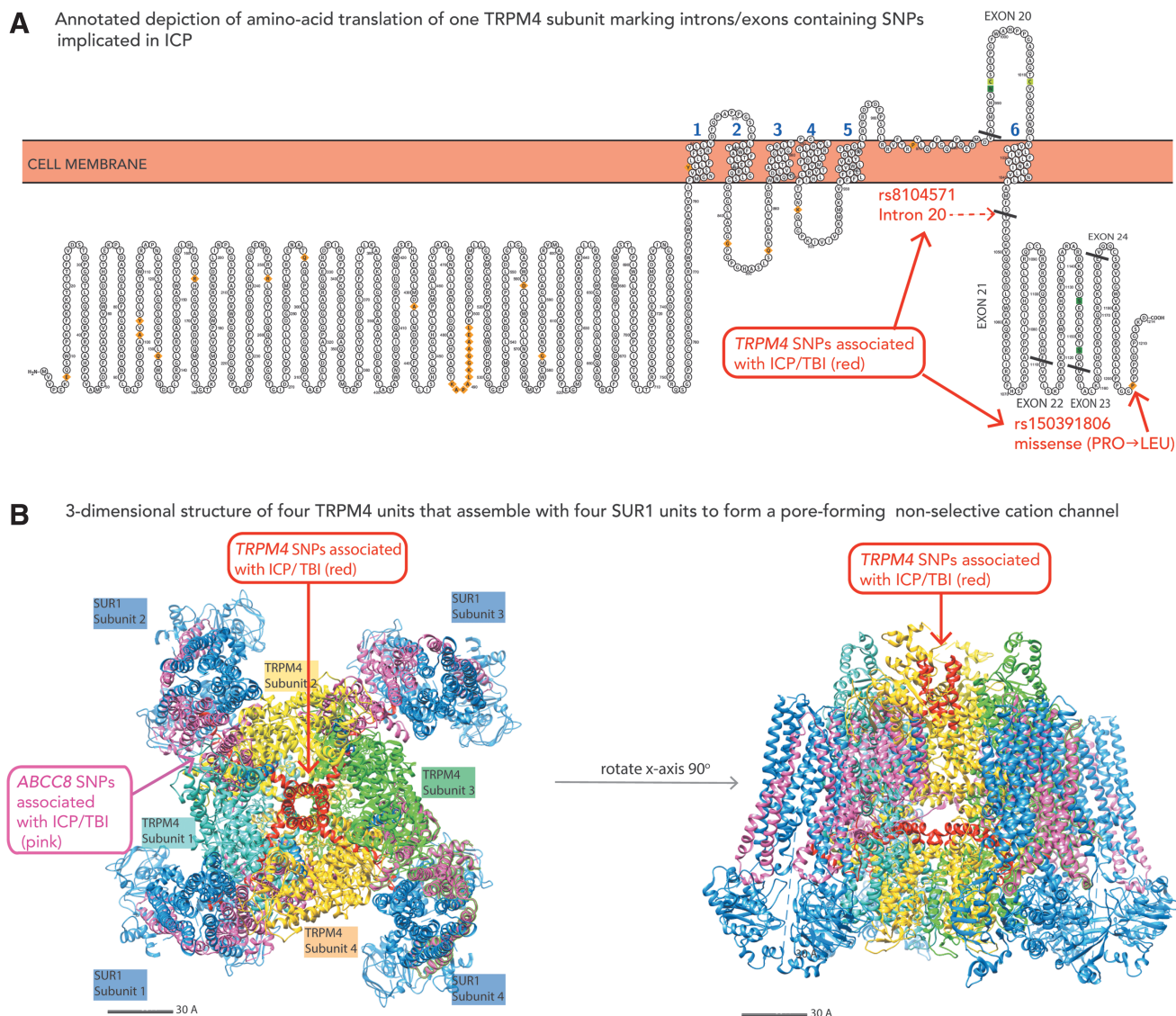
TRPM4 polymorphisms and ICP

Both rs8104571 and rs150391806 were independent predictors
of ICP measures in univariable and multivariable models. The
predictive effect of rs8104571 was robust to multiple measures
including average ICP, peak ICP, and proportion of ICP mea-
surements >25 mm Hg. rs8105471 heterozygotes had average ICPs
in the concerning range of ~ 20 mm Hg (intracranial hypertension),
relative to ~ 10 mm Hg in homozygous wild-types. Degree of in-
tracranial hypertension reflected by peak ICP was also significantly
greater in these heterozygotes (~ 45 mm Hg vs. 25 mm Hg).
Proportion of intracranial hypertension episodes (i.e., dose) was
four times greater in rs8105471 heterozygotes (21.3% vs. 5.5%).
These large effects are not surprising given the low allele frequency
of rs8104571 and rs150391806 variants. An inverse relationship
between a variant's effect size and frequency in the population is
expected/attributed to differential selection/evolutionary pres-
sures.[40] Although common variants are more frequently identified
in genome-wide association studies, rare variants have a higher
likelihood of informing genetic underpinnings of complex diseases,
providing insight into functional consequences of the variation, and
identifying causal mechanisms; all of which may eventually im-
prove prioritization of biological therapeutic targets.[40] Therefore,
further study exploring the effects of rs8104571 and rs150391806
variants in biological models *in vitro* and *in vivo* may be infor-
mative.

Of the 36 genotyped and proxy SNPs, only rs8104571 and
rs150391806 are reported.[9,41,42] In principal-component analysis of
cofactor transporters that potentially influence micronutrient re-
quirements and individual variability in response to nutritional
intervention, rs8104571 showed evidence of positive selection in
an African hunter-gatherer population.[42] rs150391806 causes a
missense mutation (CCG→CTG) resulting in the substitution of
leucine for proline. This has been associated with cardiac con-
duction disorders including progressive familial heart block-type-
1B (ClinVar 381692) and Brugada syndrome.[41] It is predicted to be
''likely benign''; however, the true functional consequence is un-
known.

TRPM4 polymorphisms and outcome

Consistent with the literature, in our cohort, ICP measures in-
cluding average, peak, and proportion of ICP spikes >25 mm Hg
predicted outcome and mortality after TBI (Table S6).
rs150391806 did not predict clinical outcome. Although not sta-
tistically significant, in univariable ($p = 0.097$) and most multi-
variate models (basic model $p = 0.10$, expanded-model 2 $p = 0.027$),
heterozygote rs8104571 had a trend toward favorable 6 month
outcome versus homozygous wild-type. This finding was signifi-
cant in expanded-model 1 ($p = 0.007951$) controlling for propor-
tion of ICP >25 mm Hg and acute-CT findings in addition to the
other covariates. These results are in the opposite anticipated di-
rection of the significant and large effect on ICP in which hetero-
zygotes had increased odds of intracranial hypertension measures.
Given the small sample size and lack of consistent statistical

FIG. 4. Spatial relationships between transient-receptor-potential cation channel subfamily-M (*TRPM4*) and *ABCC8* polymorphism regions and sulfonylurea-receptor-1 (SUR1)-TRPM4 protein structure. **(A)** This is an annotated depiction of amino acid translation of one *TRPM4* subunit marking locations/amino acid sequences encoded by corresponding DNA regions of linkage-disequilibrium (LD) containing *TRPM4* single nucleotide polymorphisms (SNPs) implicated in intracranial pressure (ICP) after traumatic brain injury (TBI). The diagrammatic representation of TRPM4 was generated using PROTTER software (UniProt ID Q8TD43). Exons 20–24 are numbered and separated by solid black lines. The specific amino acid location of the missense mutation caused by rs150391806 is indicated by a red arrow and results in substitution of leucine for proline. The dashed red arrow marks the location where intron 20 (containing rs8104571) separates exons 20 and 21. **(B)** The three-dimensional structure of four TRPM4 subunits (inner channel) that assemble with four regulatory SUR1 subunits to form an octameric non-selective cation channel. The TRPM4 three-dimensional structure was obtained from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB) based on work by Autzen and coworkers[23] using electron microscopy to elucidate the structure of the human TRPM4 channel in a calcium-bound state. The SUR1 three-dimensional structure was also obtained from RCSB-PDB based on work by Li and coworkers[26] using cryoelectron microscopy to elucidate the structure of the pancreatic Kir6.2-SUR1 complex. University of California, San Francisco Chimera automated software was used to combine these three-dimensional structures of SUR1 and TRPM4 into the predicted octameric channel. The panel on the left is an aerial view illustrating four SUR1 subunits binding with four inner TRPM4 subunits. The SUR1 subunits are blue and pink: the pink reflecting amino acid sequences encoded by regions of DNA containing/in LD with previously reported significant *ABCC8* SNPs in TBI. Each TRPM4 subunit is a different color: subunit 1 is turquoise, subunit 2 is yellow, subunit 3 is green, and subunit 4 is peach. The TRPM4 amino acid sequences in red are those encoded by regions of *TRPM4* DNA in spatial proximity to rs8104571 and rs150391806. The aerial view of this octameric protein is rotated 90 degrees around the x axis to provide a coronal view. As evident from the illustration, the two protein regions (red) captured by the implicated region of TRPM4 DNA involve the channel pore, as well as interface with key regions of SUR1 (pink) associated with significant *ABCC8* polymorphisms.

significance surviving corrections for multiple comparisons, it is difficult to interpret the implications of this finding, which may be the result of a biological effect, consequence of sample size, or patient heterogeneity. Nonetheless, it is interesting that prior studies of *ABCC8* also report different polymorphisms associated with measures of cerebral edema/ICP versus those that predict outcome. In these studies, variant alleles were also typically associated with favorable outcome; however, wild-type polymorphisms were associated with improved/reduced odds of cerebral edema measures.[4,5]

There are multiple speculative explanations for these findings. It is possible that to an extent, cerebral edema and intracranial hypertension may have an evolutionary function and, in patients for whom it is not fatal, it may play a role in longer-term neuroprotection. For example, astrocytic swelling may protect neurons; endothelial cell leakage may allow recruitment of important cytokines and cells involved in repair, regeneration, and neuroplasticity (akin to the dual edged sword of neuroinflammation). Intracranial hypertension may also, in part, reflect increased cerebral blood flow in attempts to improve perfusion to ischemic penumbra of the injured brain. Indeed, TRPM4 channels are thought to be important in cerebral artery myocyte depolarization, vasoconstriction, and cerebral blood flow autoregulation.[43] Many prior studies have established that although intracranial hypertension is strongly associated with morbidity and mortality after severe TBI, it is not independently predictive of functional outcome in survivor-only cohorts.[44–51] A recent study evaluating longitudinal ICP trajectories in severe TBI showed that groups of patients with longitudinal ICP trajectories containing multiple episodes of ICP >20 or 25 mm Hg had increased odds of *favorable* outcome versus other groups with either persistently low ICP trajectories or recalcitrant/severe intracranial hypertension, a finding that persisted in a survivors-only subcohort.[51] The strong trend of heterozygote *TRPM4* rs8104571 increasing odds of favorable GOS (OR = 20.67, *p* = 0.0168) in our subcohort of survivors only, although inconclusive, is therefore intriguing and potentially consistent with the hypothesis that polymorphisms increasing the risk of intracranial hypertension may also be protective in terms of clinical outcome if the patient survives.

Although the SUR1-TRPM4 channel is uniquely upregulated in CNS injury, *ABCC8* also influences other targets in the CNS that may contribute to outcome independent of its association with TRPM4.[52] TRPM4 *in vivo* has been associated with astrocytic swelling but not death.[53] *TRPM4* and *ABCC8* polymorphisms may also have different local effects based on cell type, timing, and spatial distribution. It is worth emphasizing that the association between these genes and ICP is extremely complex, as is the relationship between ICP and outcome. *TRPM4* genetic variation is one piece of a complex phenotype. Although inclusion of this single predictor (*TRPM4* genetic variation) in a multivariable regression model of intracranial hypertension significantly increased the model's R^2 by 8% (e.g., from 0.1204 to 0.205 [basic] or 0.2483 [expanded] for rs8104571) with concordantly large effect sizes, overall only 20.5–24.83% of the variation in ICP was explained by the model. Similarly although inclusion of rs8104571 in the multivariable logistic regression model for 6 month GOS resulted in an increase in pseudo R^2 from 0.2946 to 0.3191, only ∼31% of the variation in GOS is explained by the model.

It is intuitive that ICP and the impact on outcome cannot solely be explained by *TRPM4* genetic variation and our limited multivariable models given the underlying complexity of this process and its effect on outcome. ICP is impacted by innumerable other factors including intracranial compliance, autoregulation, and presence and degree of secondary insults such as hypoxia, as well as unmeasured genetic influences of other pathways (e.g., aquaporin-4 [AQP-4], toll-like-receptor-4 [TLR4], and matrix-metalloproteinase-9 [MMP9]).[54–57] There are also potentially alternate mechanisms for neuroprotection/outcome versus edema via SUR1-TRPM4 that have been posited.[4,5,8,11,58] This may relate to temporal and spatial cell-type distributions in which this pathway is upregulated after TBI (e.g., neurons vs. astrocytes vs. endothelial cells).

TRPM4–ABCC8 *Interactions*

Identification of ICP-predictive *ABCC8* SNPs after TBI, combined with the recognized role of SUR1-TRPM4 in cerebral edema,[4,5] led to the hypothesis that *TRPM4* may also harbor genetic variants associated with ICP, and that these may interact with *ABCC8*. Analogous to *ABCC8*, the two significant genotyped *TRPM4* SNPs predictive of ICP and their associated proxy SNPs regionally clustered in a downstream region of the gene that encoded sequences contained within the TRPM4-pore and the putative SUR1-TRPM4 binding interface. This spatial interaction supports the finding that the effect of rs8104571 on ICP was moderated by an interaction with three clustered *ABCC8* SNPs: rs2237982, rs2283261, and rs11024286. The directional effect of these interactions on ICP reinforces the robustness of this result: variant rs11024286 has been reported as neuroprotective; however, variants rs2237982 and rs2283261 are associated with increased ICP.[4,5] Concordantly, rs8104571 interactions with *variant* rs2237982 and rs2283261 but *wild-type* rs11024286 had dose-dependent increases in ICP measures.

If validated in larger studies, these SNPs may provide prognostic enrichment as predictors of intracranial hypertension as well as predictive enrichment for patients likely to need/benefit from targeted therapy. However, the functional consequences of these variations and the phenotypic effects of their interactions are currently unknown. Biologic causality between *ABCC8* and *TRPM4* genetic variation and intracranial hypertension in sTBI remains to be elucidated. Although beyond the scope of this study, this is an important avenue for future research, as it may partly explain the pathophysiology underlying differential cerebral edema development among individuals and potentially identify treatment responders.

Additional limitations

This study has several limitations. ICP measurements were point values recorded at the end of each hour and, therefore, do not integrate to provide a true proportion of time spent in intracranial hypertension. The complexity and heterogeneity of all possible variables influencing ICP and outcome after TBI could not be captured by our models. Further, although the SUR1-TRPM4 pathway is a key contributor to edema and intracranial hypertension, it is one of several known (and unknown) mechanisms. Investigation of potentially relevant genes either related to this pathway (e.g., *TNFA*, *KCJN11, MMP-9* and *CASP3)* or other known mechanisms of cerebral edema (e.g. *AQP-4, TLR4*) may be valuable.[57] Dedicated tag-SNP sequencing may provide better coverage across coding and non-coding regions of the *TRPM4* gene. Another approach that would capture genetic variations in established and unknown contributors to cerebral edema is a genome-wide association study. This would require an extremely large sample size and multi-center collaborations from initiatives

such as Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) or Collaborative European NeuroTrauma Effectiveness Research in TBI (CENTER-TBI). To place our cohort in context, after 4 years with 11 enrolling sites, recent genetic-association studies published in the TRACK-TBI cohort have included between 93 and 220 subjects, and most have had mild rather than sTBI.[2,3,59,60] There are currently ∼230 sTBI patients enrolled in TRACK-TBI; although this number is expected to grow over several years, data such as ours may importantly support development and funding of these vital multi-center resources.

Our work needs to be validated with other cohorts. Although this report represents a large polymorphism study in sTBI, it is small for a genetics study.[5,60–62] Nonetheless, it is a candidate-gene targeted approach, reducing sample-size requirements. Our findings had large effect sizes, retained significance in multivariable analyses after correcting for multiple comparisons, and had a strong pathophysiological basis.[34]

Results from our single-center cohort are prone to selection bias. Variables such as ''therapeutic intensity level'' or secondary insults that could influence ICP while potentially useful, were unavailable. Historically at the University of Pittsburgh Medical Center during the study time period, treatment thresholds of 20 mm Hg have been used to target therapy, with sustained values >25 mm Hg triggering a neurosurgical (re)assessment. Our ICP calculations included patients after craniectomy. Importantly, our models withstood controlling for surgical treatment (craniectomy), which has one of the most powerful therapeutic influences on ICP; however, we recognize that ICP values in this context may no longer reflect biology that can be modulated by genetic variance. Although secondary analyses censoring post-craniectomy ICP values suggested similar trends, these models did not withstand the B–Y correction, likely because of imperfect censoring and the inevitable loss of data points. Subgroup analyses by injury pattern may also have been informative; however, meaningful statistical comparisons were precluded by insufficient sample sizes within each subgroup.

Conclusions

The impact of genetic polymorphisms on guiding precision medicine has been demonstrated across various medical subspecialties, and is increasingly informing clinical practice.[63–67] We report two *TRPM4* polymorphisms, rs8104571 and rs150391806, associated with intracranial hypertension after TBI. These polymorphisms and associated proxy SNPs spatially clustered around critical regions of DNA encoding sequences in the channel pore and the SUR1-TRPM4 binding interface. rs8104571's effect on ICP was moderated by three previously reported *ABCC8* SNPs clustered in intron-10, further adding credence to the hypothesis that there may be important interactions between the two genes (*ABCC8, TRPM4*) and resultant associated proteins (SUR1, TRPM4). Our findings are consistent with SUR1-TRPM4's pathophysiological basis in cerebral edema development. Although the functional consequences of these SNPs are unknown and need exploration in biological models, our results support the hypothesis that genetic variability in this pathway may play a role in phenotyping intracranial hypertension after TBI. Identifying effects of *ABCC8* and *TRPM4* genetic variability on intracranial hypertension is a first step toward understanding their functional significance and causal mechanisms. In addition to clinical prognostication and risk stratification, this may help characterize treatment responders and inform patient selection for precision medicine based trials. This is particularly important in the context of optimally evaluating glibenclamide, a targeted therapy inhibiting SUR1-TRPM4 that has shown promise in early clinical and preclinical TBI studies but is yet to have proven benefit in large randomized trials.

Author Disclosure Statement

RMJ has provided consulting services for Biogen.

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