Supplementary Data

Supplementary Methods A:Identifying Functional Category of Transient-Receptor-Potential Cation Channel Subfamily-M (*TRPM4*) Single-Nucleotide Polymorphisms SNPs

All significant SNPs were evaluated for established clinical significance via PubMed, Embase, and ClinVar searches. The following search terms were used without restrictions: *TRPM4* polymorphisms, *TRPM4* SNPs, transient receptor potential cation channel subfamily M polymorphisms, and transient receptor potential cation channel subfamily M polymorphisms SNPs. Reference lists for major reviews were also scrutinized. References were individually examined for SNPs (identified by their reference-SNP cluster identification, [rsid]). For variants reported using human genome variation society (HGVS) nomenclature, ClinVar was searched using the HGVS identification to obtain the equivalent rsid whenever available. Finally, *TRPM4* was searched in ClinVar to identify any additional SNPs.

Supplementary Methods B: Exploratory Genetic Risk Scores

Exploratory genetic risk scores were created as independent variables for logistical regression models evaluating association with 6 month Glasgow Outcome Score (GOS). SNPs included in the construction of the genetic risk score were *TRPM4* rs8104571 (because this had a trend toward significant association with 6 month GOS in a univariable model ($p = 0.09$), and met significance in expanded multivariable model-1 ($p = 0.0079$)), as well as three ABCC8 SNPs given previously reported significant associations with clinical outcome after severe traumatic brain injury (TBI) (rs2237982, rs11024286, and rs4148622).

Unweighted gene risk score (U-GRS) construction

This additive genetic model assigned linear genotypes for each SNP as 0, 1, or 2 depending on the number of risk alleles with 0 = homozygous wild-type, 1 = heterozygous, and 2 = homozygous variant. The U-GRS was the sum of the total number of risk alleles in a patient, resulting in a maximal U-GRS of 8.

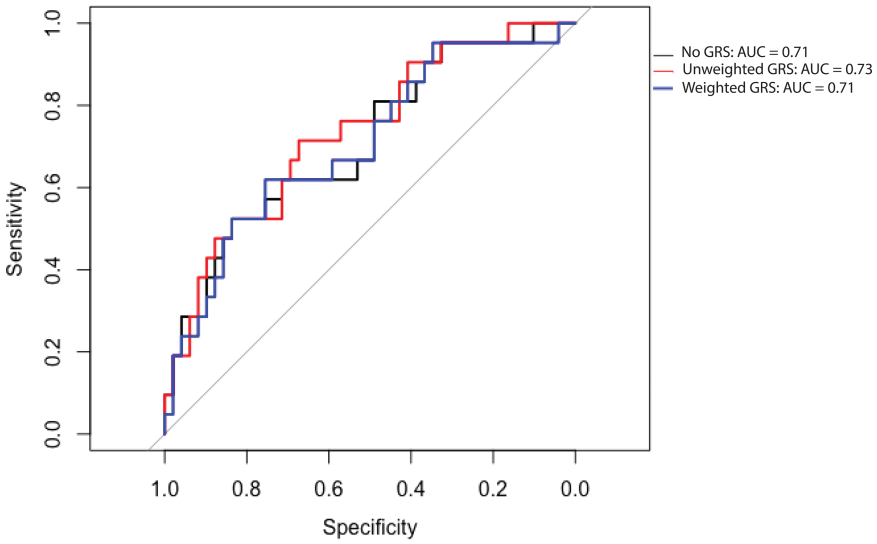
Weighted gene risk score (W-GRS) construction

The b-coefficient for each SNP (obtained from univariable regression models of the respective SNP with GOS) was multiplied by the number of risk alleles present for that SNP. The W-GRS was sum of these products for all four SNPs.

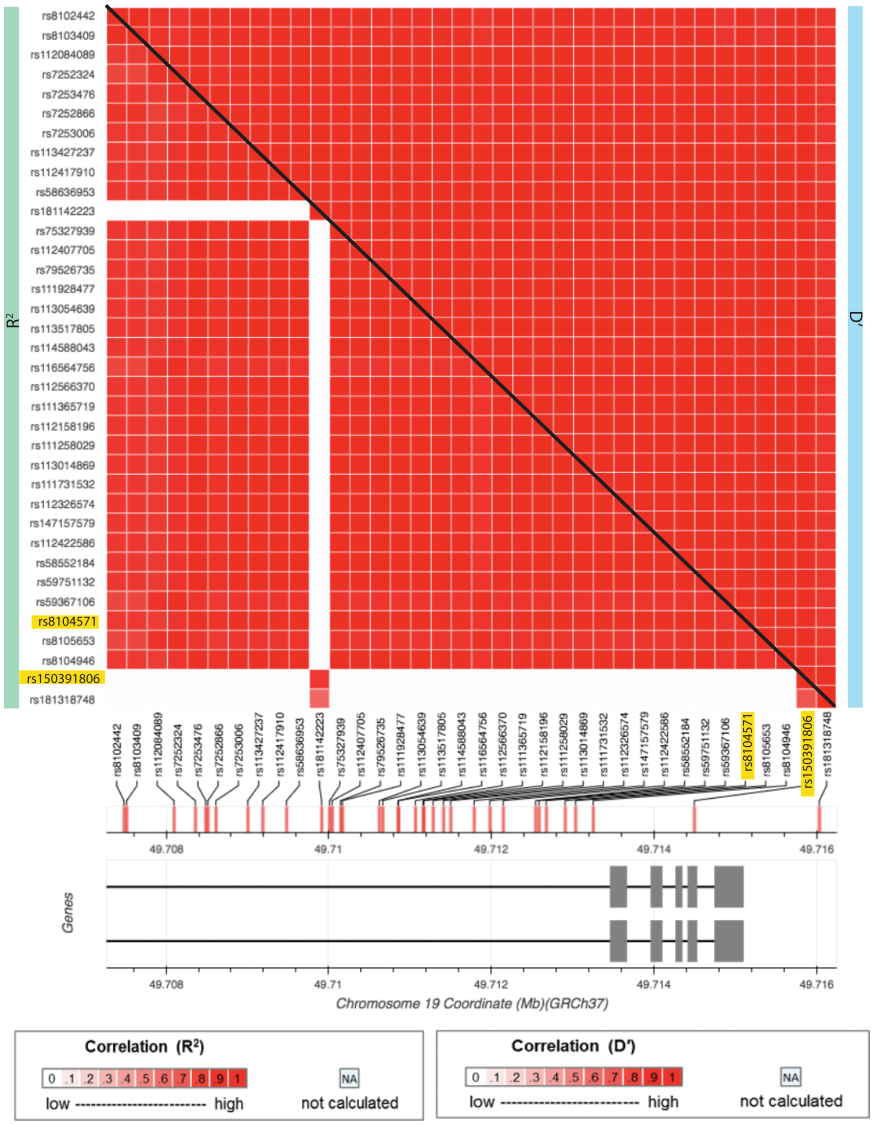
For both the exploratory U-GRS and W-GRS, a training set of subjects (comprising a random selection of half the total number of subjects without missing values) was used to generate multivariable logistical regression models evaluating the effect of these gene risk scores on clinical outcome. The performance of the prediction probabilities was subsequently tested in the remaining half of the cohort. The potential additional value of the U/W-GRS in outcome prediction was evaluated by areas under the receiver operating characteristic (ROC) curves (AUCs). These were created comparing multivariable regression models containing conventional covariates (age, sex, initial Glasgow Coma Scale [GCS] score, craniectomy, primary injury pattern, proportion of intracranial pressure [ICP] >25 mm Hg, and acute computed tomographic [CT] characteristics) versus models that additionally included U-GRS or W-GRS. R package pROC was used for calculating and displaying ROC curves for outcome models evaluating these gene risk scores.[1]

Reference

1. Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J.-C., and Müller, M. (2011). pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 12, 77.

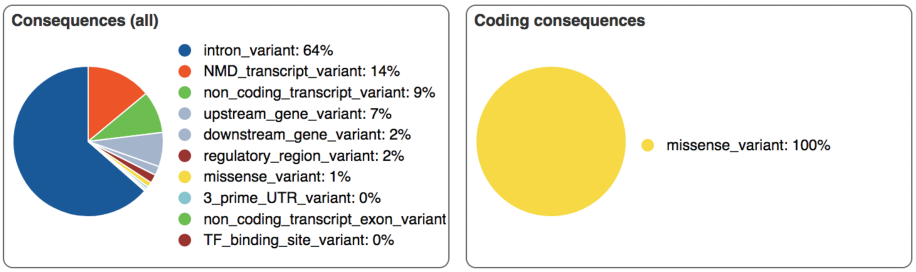


SUPPLEMENTARY FIG. S1. Graph of receiver operating characteristic (ROC) curves for multivariable models used to predict 6 month Glasgow Outcome Scale (GOS) score using weighted and unweighted genetic risk scores (GOS). The multivariable model without any genetic information (black) has an area under the curve (AUC) of 0.71 (which is lower than the AUC in the full cohort set likely because of a smaller sample size used in the test set from which these AUCs were generated, $n = 70$). The multivariable models containing the unweighted (red) and weighted (blue) gene risk scores of all transient-receptor-potential cation channel subfamily-M (*TRPM4*) and *ABCC8* single nucleotide polymorphisms (SNPs) currently and previously reported to associate with outcome (TRPM4 rs8104571 and ABCC8 rs2237982, rs11024286, and rs4148622) were not significantly different, with AUCs of 0.73 and 0.71 respectively.



SUPPLEMENTARY FIG. S2. Proxy single nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD) plot of proxy SNPs. LD plot of proxy SNPs demonstrating that although the rs8104571 and rs150391806 have $r^2 < 0.8$ between them, the associated proxy SNPs are in significant LD ($r^2 > 0.8$) with either rs8104571 or rs150391806. This region of LD encompasses interspersed regions of coding sequences/exons as shown by the zoomed in schematic of transient-receptor-potential cation channel subfamily-M (*TRPM4*) chromosomal locations/coordinates. High LD is indicated by heavily red-shaded squares (both for r^2 associations shown on the bottom triangle to the left of the diagonal line, and for D' associations shown to the right of the diagonal line).

Category	Count
Variants processed	36
Variants filtered out	0
Novel / existing variants	0 (0.0) / 36 (100.0)
Overlapped genes	2
Overlapped transcripts	16
Overlapped regulatory features	4



SUPPLEMENTARY FIG. S3. Ensembl variant effect predictor results for transient-receptor-potential cation channel subfamily-M (*TRPM4*) genotyped and proxy single nucleotide polymorphisms (SNPs). Pie graph showing the predicted consequences of the 36 *TRPM4* SNPs (2 genotyped rs8104571 and rs150391806, 34 proxy) using Ensembl's variant effect predictor software. The majority of the variants are described as intron variants (64%), followed by nuclear mediated decay variants (14%). The remaining are distributed among non-coding transcript variants, downstream variants, and regulatory-region variants. rs150391806 was the only variant with a coding consequence; it is a missense variant (CCG→ CTG) resulting in the substitution of leucine for proline.

Supplementary Table S1. *TRPM4* SNP Prediction of Intracranial Pressure and Outcome (Multiple Imputations Adjusting for Missing Values)

	Average ICP *β-coefficient (*p*)*	*Peak ICP* *β-coefficient (*p*)*	*Proportion of ICP >25 mmHg* *β-coefficient (*p*)*	*Favorable 6 month GOS* *Odds ratio (*p*)*
Univariate regression analysis				
rs8104571 (TC)	10.2 mmHg (***0.0000086****)	19.3 mmHg ***(0.0013*)***	0.15 (***0.0067****)	3.4 (0.097)
rs150391806 (TC)	6.08 mmHg (*0.1*)	26.0 mmHg (***0.0096****)	0.08 (0.4)	–0.03 (0.98)
Multivariate regression analysis				
rs8104571 (TC)	9.62 mmHg (***0.000018****)	17.6 mmHg ***(0.003*)***	0.14 (***0.01****)	1.2 (0.1)
rs150391806 (TC)	5.88 mmHg (*0.1*)	25.4 mmHg (***0.010****)	0.08 (0.4)	–0.23 (0.86)

Boldface, italics, and asterisks indicate significant p-value after B-Y correction for multiple comparisons.
SNP, single-nucleotide polymorphism; ICP, intracranial pressure; GOS, Glasgow Outcome Scale; TC, thymine-cytosine.

Supplementary Table S2. *TRPM4* SNP Prediction of Intracranial Pressure (ICP) and Outcome in Severe TBI, Censoring ICP Values After Day of Craniectomy

	Average ICP *β-coefficient (Standard error,* p*)*	*Peak ICP* *β-coefficient (95% CI,* p*)*	*Proportion of ICP >25 mmHg* *β-coefficient (95% CI,* p*)*
Univariate regression analysis			
rs8104571 (TC)	4.74 mmHg (2.23, ***0.0342****)	8.89 mmHg (3.96, ***0.0256*)***	0.098 (0.006, ***0.0193****)
rs150391806 (TC)	4.6 mmHg (4.16, 0.264)	10.9 mmHg (7.39, 0.141)	0.043 0.068, 0.527)
Multivariate regression analysis			
rs8104571 (TC)	4.06 mmHg (2.17**,** *0.0625*)	6.53 mmHg (3.78, *0.0855)*	0.095 (0.0418, ***0.02391****)
rs150391806 (TC)	4.28 mmHg (3.98, 0.283)	8.45 mmHg (86.99, 0.227)	0.044 (0.067, 0.516)

Provides the regression analysis results of each significant TRPM-4 single nucleotide polymorphism (SNP) effect on average ICP, peak ICP, proportion of ICP spikes >25 mm Hg, using censored ICP measurements depending on the timing of craniectomy (if present); where ICP values after day of craniectomy are excluded. Univariate regression results are provided first, followed by multivariate regression analysis results.
Boldface, italics, and asterisks indicate significant p-value after B-Y correction for multiple comparisons.
TBI, traumatic brain injury; TC, thymine-cytosine.

Supplementary Table S3. *TRPM4* SNP Prediction of Mortality in Severe TBI

	Univariate 3 month mortality Odds ratio (95% CI, p*)*	*Multivariate 3 month mortality Odds ratio (95% CI,* p*)*
rs8104571 (TC)	0.83 (0.16–4.18, 0.82)	0.95 (0.13–6.75, 0.96)
rs150391806 (TC)	1.25 (0.11–14.0, 0.85)	2.24 (0.18– 28.6, 0.54)

SNP, single-nucleotide polymorphism; TBI, traumatic brain injury; CI, confidence interval; TC, thymine-cytosine.

Supplementary Table S4. *TRPM4* and *ABCC8* Polymorphism Interactions in Multivariable Regression Models Evaluating Clinical Outcomes

TRM4 SNP interactions					
TRPM4 rs8104571			*TRPM4 rs150391806*		
Discharge mortality	*3 month mortality*	*Favorable 6 month GOS*	*Discharge mortality*	*3 month mortality*	*Favorable 6 month GOS*
Non-significant interactions w ABCC8 SNPs			Non-significant interactions w ABCC8 SNPs		
rs2237982			rs2237982		
rs2283261			rs2283261		
rs11024286			rs11024286		
rs7105832			rs7105832		
rs3819521			rs3819521		
rs2283258			rs2283258		
rs1799857			rs1799857		
rs4148622			rs4148622		

Reports no significant interactions with respect to clinical outcome between the two *TRPM4* SNPs (rs8104571, rs150391806) found to predict measures of intracranial pressure (ICP) in this study, and previously reported significant *ABCC8* SNPs in TBI (rs2237982, rs2283261, rs11024286, rs7105832, rs3819521, rs2283258, rs1799857, rs4148622).

GOS, Glasgow Outcome Scale; SNP, single-nucleotide polymorphism.

SUPPLEMENTARY TABLE S5. PREDICTED ± KNOWN FUNCTIONAL IMPLICATIONS OF TRPM4 WHOLE EXOME GENOTYPED SNPS AND ASSOCIATED SNPS IN LINKAGE DISEQUILIBRIUM

Exonic SNPs

No.	*SNP ID*	*G/P*	R^2 *with genotyped SNP*	*PubMed or ClinVar Reports*	*Functional change and prediction PolyPhen, SIFT/PROVEAN, MutPred*
1	rs150391806	G	NA	Progressive familial heart block (ClinVar ID 381692)	Missense → P1204L PolyPhen score 0.643 → possibly damaging SIFT/PROVEAN score 0.212/–1.81 → tolerated MutPred2 score 0.210 Ensembl variant effect predictor → Moderate

Intronic SNPs

No.	*SNP ID*	*G/P*	R^2 *with genotyped SNP*	*PubMed or ClinVar Reports*	*Functional Prediction* *1. Regulome DB score/prediction* *2. Ensembl Variant Effect Predictor*
2	rs8104571	G	NA	Positive selection in cofactor transporter (PMID 29043008)	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein-coding Consequence: IV, NMD, NCTV
3	rs8102442	P	0.875	None	1. 2b likely to affect TF binding, DNase footprint, DNase peak 2. Impact: modifier; biotype: protein-coding, retained intron, NMD Consequence: IV, NMD, NCTV
4	rs8103409	P	0.875	None	1. 2b likely to affect TF binding, DNase footprint, DNase peak 2. Impact: modifier; biotype: protein-coding, retained intron, NMD open chromatin region Consequence: IV, NMD, NCTV, RRV
5	rs112084089	P	0.854	None	1. No data 2. Impact: modifier; biotype: protein-coding Consequence: IV, NMD, NCTV
6	rs7252324	P	0.905	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein-coding, retained intron, NMD open chromatin region Consequence: IV, NMD, NCTV, RRV
7	rs7253476	P	0.993	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein-coding, retained intron, NMD open chromatin region Consequence: IV, NMD, NCTV, RRV
8	rs7252866	P	0.955	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein-coding, retained intron, NMD open chromatin region Consequence: IV, NMD, NCTV, RRV
9	rs7253006	P	0.986	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein-coding, retained intron, NMD open chromatin region Consequence: IV, NMD, NCTV, RRV
10	rs113427237	P	0.989	None	1. 6 minimal binding evidence 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
11	rs112417910	P	0.993	None	1. No data 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
12	rs58636953	P	0.993	None	1. 6 minimal binding evidence 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
13	rs75327939	P	0.993	None	1. No data 2. Impact: modifier; biotype: protein coding, retained intron, NMD; Consequence: IV, NMD, NCTV, UGV

(*continued*)

SUPPLEMENTARY TABLE 5. (CONTINUED)

Intronic SNPs

No.	*SNP ID*	*G/P*	R^2 *with genotyped SNP*	*PubMed or ClinVar Reports*	*Functional Prediction* *1. Regulome DB score/prediction* *2. Ensembl Variant Effect Predictor*
14	rs112407705	P	0.989	None	1. No data 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
15	rs79526735	P	0.993	None	1. No data 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
16	rs111928477	P	0.993	None	1. 5 Minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
17	rs113054639	P	0.993	None	1. No data 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
18	rs113517805	P	0.993	None	1. No data 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
19	rs114588043	P	0.993	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
20	rs116564756	P	0.902	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; Biotype: protein coding, retained intron, NMD; Consequence: IV, NMD, NCTV, UGV
21	rs112566370	P	0.993	None	1. 3a less likely to affect TF binding + DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD, promotor flanking region Consequence: IV, NMD, NCTV, UGV, RRV
22	rs111365719	P	0.993	None	1. 4 minimal binding evidence, DNase peak 2. Impact modifier; biotype: protein coding, retained intron, NMD, promotor flanking region Consequence: IV, NMD, NCTV, UGV, RRV
23	rs112158196	P	0.993	None	1. 4 minimal binding evidence, DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD, promotor flanking region Consequence: IV, NMD, NCTV, UGV, RRV
24	rs111258029	P	0.993	None	1. 4 minimal binding evidence, DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD, promotor flanking region Consequence: IV, NMD, NCTV, UGV, RRV
25	rs113014869	P	0.993	None	1. 4 minimal binding evidence, DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD, promotor flanking region Consequence: IV, NMD, NCTV, UGV, RRV, transcription factor binding site variant
26	rs111731532	P	0.993	None	1. 4 minimal binding evidence, DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD, promotor flanking region Consequence: IV, NMD, NCTV, UGV, RRV
27	rs112326574	P	0.993	None	1. 4 Minimal binding evidence, DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD, promotor flanking region Consequence: IV, NMD, NCTV, UGV, RRV
28	rs112422586	P	0.993	None	1. 6 minimal binding evidence 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
29	rs61304201	P	0.993	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
30	rs58552184	P	0.993	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
31	rs59751132	P	0.993	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV

(*continued*)

SUPPLEMENTARY TABLE 5. (CONTINUED)

Intronic SNPs					
No.	*SNP ID*	*G/P*	R^2 *with genotyped SNP*	*PubMed or ClinVar Reports*	*Functional Prediction* *1. Regulome DB score/prediction* *2. Ensembl Variant Effect Predictor*
32	rs59367106	P	0.993	None	1. No data 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
33	rs8105653	P	0.908	None	1. No data 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
34	rs8104946	P	0.908	None	1. 5 minimal binding evidence: TF binding or DNase peak 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
35	rs181142223	P	0.874	None	1. No data 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: IV, NMD, NCTV, UGV
36	rs181318748	P	0.727	None	1. 6 minimal binding evidence 2. Impact: modifier; biotype: protein coding, retained intron, NMD Consequence: downstream gene variant

G, genotyped; IV, intron variant; NMD, nonsense-mediated decay; NCTV, non-coding transcript variant; P, proxy; RRV, regulatory region variant; SNP, single-nucleotide polymorphism; TF, transcription factor; UGV, upstream gene variant.

Supplementary Table S6. Intracranial Pressure (ICP) and Outcome in Severe TBI

	*Discharge mortality Odds ratio (*p*)*	*3-month mortality Odds ratio (*p*)*	*Favorable 6 month GOS Odds ratio (*p*)*
Predictor			
Average ICP	1.2 (***0.0019****)	1.2 (***0.0026****)	0.87 (***0.023****)
Peak ICP	1.04 (***0.007****)	1.04 (***0.01****)	0.95 (***0.015****)
Proportion ICP >25 mmHg	118.1 (***0.00032****)	307.1 (***0.00036****)	0.0005706 (***0.0056****)

Potential confounders included in the model: age, sex, initial GCS score, craniectomy.
Boldface, italics, and asterisks indicate significant p-value after B-Y correction for multiple comparisons.
TBI, Traumatic brain injury; GOS, Glasgow Outcome Score.