Supplementary Online Content

Jha RM, Zusman BE, Puccio AM, et al. Genetic variants associated with intraparenchymal hemorrhage progression after traumatic brain injury. *JAMA Netw Open*. 2021;4(7):e2116839. doi:10.1001/jamanetworkopen.2021.16839

eMethods. Outcomes, Measures, and Analysis

eTable 1. List of All Genotyped SNPs in ABCC8 and TRPM4

eTable 2. Cohort Demographics, Clinical Characteristics and Association With Hemorrhage Progression in TBI

eTable 3. Clinical Characteristics and Outcomes of All Patients With Severe TBI During Enrollment Period

eTable 4. Cohort Demographics and Clinical Characteristics of Progressors and Nonprogressors

eTable 5. Association of Intraparenchymal Hemorrhage Progression With Outcome After Severe TBI

eTable 6. Genotype Frequencies of ABCC8 and TRPM4 SNPs Associated With Hemorrhage Progression in TBI

eTable 7. ABCC8 and TRPM4 Single-Nucleotide Polymorphisms Associated With Quantitative Change in Intraparenchymal Hemorrhage Volumes in Severe TBI

eTable 8. ABCC8 and TRPM4 Single-Nucleotide Polymorphisms Associated With Intraparenchymal Hemorrhage Progression in Severe TBI (Full Cohort)

eTable 9. ABCC8 and TRPM4 Haplotypes Associated With Contusion Expansion in Severe TBI

eTable 10. Haplotype Distribution in the Cohort of Severe TBI

eTable 11. Intraparenchymal Hemorrhage Progression Risk Polymorphisms Associated With 6-mo Glasgow Outcome Scale (GOS) Score

eTable 12. Sample Size Calculations for Genotype-Based Patient Selection in Clinical Trial

eTable 13. Regulatory Annotations of ABCC8 and TRPM4 Single-Nucleotide Polymorphisms Associated With Hemorrhage Progression in Severe TBI

eTable 14. Association of ABCC8 and TRPM4 Single-Nucleotide Polymorphisms With IPH Progression (Quantitative Definition Only)

eFigure 1. Opposite Associations of ABCC8 vs TRPM4 variant SNPs with Intraparenchymal Hemorrhage Progression in TBI

eFigure 2. Spatial Distribution of TRPM4 SNPs in Hemorrhage Progression After TBI

eFigure 3. Variant TRPM4 SNPs Associated With Decreased Hemorrhage Progression in TBI are eQTL With Decreased TRPM4 Expression in Cerebellum

eFigure 4. Sequence Logos Demonstrating Impact of ABCC8 and TRPM4 SNPs on Transcription Factor Motifs

eFigure 5. Brain Chromatin States of ABCC8 and TRPM4 Genomic Loci

eFigure 6. Linkage Disequilibrium Maps for ABCC8 and TRPM4 SNPs Regional Loci

eReferences

© 2021 Jha RM et al. *JAMA Network Open.*
This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods. Outcomes, Measures, and Analysis

DNA collection and Genotyping

DNA was extracted and genotyped per established methods[1–4]. Briefly, ABCC8 and TRPM4 exonic SNPs were genotyped using Illumina (Human-Core Exome v1.0) multiplex platform by the Center for Inherited Disease Research. Selection was unbiased and included all ABCC8 and TRPM4 SNPs covered by the chip (n=25). For ABCC8 intron coverage, 15 tag-SNPs were identified using HapMap with the tagger-algorithm pairwise approach, $r^2 \geq 0.8$ and minor allele frequency $>0.20$ and genotyped using iPLEX-Gold with an Agena Compact MassArray with Nanodispenser. Genotype analysis was performed using Typer-4.0. Assay. 40 SNPs were interrogated (Supplemental-Table 1). Genotyping researchers were blinded to demographic and outcome data. Data cleaning and quality control included blind technical duplicates, Hardy-Weinberg Equilibrium (HWE) testing, and excluding SNPs that did not have a minimum 95% call-rate. Principal-component analysis used for ancestry filtering assessed for population stratification and identified a single cluster, with the small numbers outside of that cluster being too few to analyze.

Hemorrhage Progression

Serial CT scans were assessed for intraparenchymal hemorrhage progression at presentation, 6h, 24h and 120h. Hemorrhage progression was determined using two criteria: quantitative estimation of traumatic intraparenchymal hemorrhage (IPH) volumes, and official neuroradiologist interpretations. Both criteria had to be fulfilled for a scan to qualify as demonstrating progression. We selected this two-part criteria to identify hemorrhage progression to prioritize the specificity rather than the sensitivity of the definition. This conservative approach was used to minimize the likelihood of false positive associations.

- Hemorrhage volume quantification: total IPH volumes were estimated using the standard ABC/2 calculation[5, 6]. Based on previous literature, hemorrhage progression was defined as an increase of 6 mL in ICH volume from admission CT, an increase of IPH volume by 30% from admission CT, or the appearance of a new intra-axial hemorrhage from the admission CT[6]. First appearance of catheter-tract hemorrhages were not counted towards progression, however subsequent enlargement of catheter tract hemorrhages qualified for inclusion in volumetric calculations. 10% of all CTs were independently reviewed in a blinded fashion, and yielded excellent interclass correlation (0.917).
- Neuroradiologist interpretation: All final neuroradiologist reports were manually read by trained research staff blind to patient genotype for phrasing indicating progression of the hemorrhagic component of the
IPH specifically. Reports were searched for the terms including “expanded”, “blossomed”, “increased” or “new” in reference to the hemorrhagic component of the IPH only while terms such as “evolved” or “matured” were felt to be more representative of the natural course of disease rather than progression. To minimize likelihood of false positive results, reports where expansion was not mentioned were interpreted as stability.

- Comparison of the two independent metrics of hemorrhage progression yielded a percent agreement of 88.9% and a Cohen’s kappa of 0.78, suggesting substantial agreement between the two metrics.

**SNP Functional Potential Determination**

SNPs were evaluated for impact on gene expression using the Genotype Tissue Expression (GTEx) Project data portal (www.gtexportal.org, 06/23/2020) [7]. They were interrogated for brain-specific gene expression quantitative trait loci (eQTL) in the hippocampus, non-specified cortex, frontal cortex, putamen, and cerebellum to assess breadth and consistency of impact on gene expression across cortical vs. deep brain structures. Regulatory potential was evaluated using RegulomeDB v2.0 and HaploReg V4.1[8–10]. SNPs loci were explored for the ~200 bp regional chromatin state, transcription start sites, promoter histone marks, enhancer histone marks, DNAse, and protein binding (via Chromatin-ImmunoPrecipitation, ChIP, reports) in brain vs. all reported tissues. Individual SNPs were interrogated for impact on altering regulatory motifs for transcription factors using sequence logos (RegulomeDB) as well as position-weighted matrix (PWM) scores (HaploReg). PWM scores on Haploreg (determined using experimental data on JASAPR, TRANSFAC, and protein binding microarray experiments) are available as log-odds, and account for motif lengths and base-pair compositions; they reflect transcription factor binding affinity[9]. Log-odds score differences between variant allele vs reference alleles evaluate change in binding affinity[9] (positive values reflect an increase in log-odds score for variant alleles, and suggests increased transcription factor binding strength). SNPs were evaluated for reported clinical significance via systematic PubMed, Embase, and ClinVar searches.

**Spatial relationship modeling between ABCC8 and TRPM4 loci and channel structure**

Chromosomai locations were identified using the University of California, Santa Cruz genome browser, human-genome assembly(hg-38). Linkage disequilibrium (LD), distance from the proximal exon, peptide sequences encoded by specific exons and residue overlap splice sites were identified via Ensembl-100[11]. Established SUR1 (5WUA) and TRPM4 (6BQV) 3-dimensional electron microscopy structures were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank[12–15]. University of California, San Francisco Chimera was used to generate the octameric SUR1-TRPM4 channel[16].

© 2021 Jha RM et al. JAMA Network Open.
Statistical Analysis: Sample Size Impact Simulation

We also simulated an example to demonstrate the potential impact of a priori knowledge of patients’ ABCC8 and/or TRPM4 genotypes on patient selection for clinical-trial design. We stratified our sample by genotypes, and, based on the genotype-subgroup, looked at both the sample size required and the number of patients who would need to be genotyped in order to have a 90% power to find a 30% relative risk reduction (RRR) in the risk of intra-axial hemorrhage progression within 120 hours. For this, we first calculated the probability of belonging to a specific genotype subgroup and the probability of hemorrhage progression within that subgroup for each of the following categories: 1) all comers (current trial designs), 2) at least one ABCC8 SNP with homozygous variant (risk) genotype, 3) at least one TRPM4 SNP with homozygous wildtype (risk) genotype, 4) at least one ABCC8 SNP with homozygous variant (risk) phenotype AND at least one TRPM4 SNP with homozygous wildtype (risk) genotype, 5) and at least one ABCC8 SNP with homozygous variant (risk) phenotype OR at least one TRPM4 SNP with homozygous wildtype (risk) genotype, 6) patients predicted to have a >50% probability of hemorrhage progression by the full clinical model without genotypes used for creating the ROC curves, 7) and patients predicted to have a >50% probability of hemorrhage progression by the full clinical plus genotypes model used for creating the ROC curves. The probability of hemorrhage progression for each subgroup was then used in sample size calculations to design different iterations of the hypothetical study where a treatment is expected to result in a RRR of hemorrhage progression by 30% with a power of 0.9. The obtained sample size was divided by the proportion of patients within that subgroup to determine how many patients would need to be genotyped to reach the necessary enrollment sample size. This procedure was followed for all 5 subgroups to identify the degree to which genotype-based patient selection may impact sample size based on varying risk of hemorrhage progression.

table 1. List of All Genotyped SNPs in ABCC8 and TRPM4

| Gene | SNPs genotyped |
|------|----------------|
| **ABCC8** | rs7105832, rs2237982, rs11024286, rs4148622, rs2283261, rs381952, rs2283258, rs1799857, rs8192695, rs11024273, rs2074311, rs2237991, rs2299639, rs3758953, rs4148618, rs4148641, rs4757517, rs7119439, rs1048099 |
| **TRPM4** | rs8104571, rs150391806, rs11667393, rs3760666, rs113984787, rs1477363, rs10410857, rs56355369, rs909010, rs145847114 |
| Characteristic | Entire TBI Cohort | No-Craniectomy Primary Cohort | Entire TBI Cohort | No-Craniectomy Primary Cohort |
|---------------|------------------|-------------------------------|------------------|-------------------------------|
|               | (n=416)          | (n=321)                       |                  |                               |
| Age in years: mean (SD) | 38.6 (16.9) | 37.0 (16.3) | 1.02 (1.01-1.03)* | 1.03 (1.01-1.04)* |
| Sex: n (%)    |                  |                               |                  |                               |
| Male          | 324 (77.9)       | 247 (76.9)                    | Ref              | Ref                           |
| Female        | 92 (22.1)        | 74 (23.1)                     | 0.74 (0.44-1.23) | 0.79 (0.44-1.43)              |
| Admission GCS: median (IQR) | 6 (5-7) | 7 (5-7) | 0.87 (0.76-1.01) | 0.98 (0.83-1.15) |
| ISS: mean (SD) | 31.8 (11.1) | 32.6 (11.4) | 1.0 (0.98-1.02) | 1.0 (0.98-1.02) |
| Race: n (%)   |                  |                               |                  |                               |
| African American | 30 (7.2) | 23 (7.2) | 0.75 (0.32-1.76) | 0.76 (0.29-1.99) |
| Southeast Asian | 4 (1)     | 4 (1.3)     | 1.25 (0.14-7.36) | 1.30 (0.18-9.35) |
| Unknown       | 1 (0.2)         | 1 (0.3)                      | -                | -                             |
| Mechanism of Injury: n (%) |              |                               |                  |                               |
| Enclosed Motor Vehicle Accident | 216 (51.9%) | 185 (57.6%) | Ref              | Ref                           |
| Motor Cycle Crash | 67 (16.1%) | 58 (18.1%) | 1.08 (0.60-1.94) | 1.37 (0.73-2.57)              |
| Fall          | 87 (20.9%)       | 50 (15.6%)                    | 2.10 (1.19-3.72)* | 2.04 (1.02-4.09)*             |
| Assault       | 17 (4.1%)        | 10 (3.2%)                     | 1.34 (0.50-3.63) | 0.66 (0.16-2.64)              |
| Gun Shot Wound | 2 (0.5%)    | 1 (0.3%)                      | 0.55 (0.20-1.51) | 1.53 (0.09-24.98)             |
| Other a       | 23 (5.5%)        | 15 (4.7%)                     | 0.55 (0.20-1.51) | 0.58 (0.15-2.25)              |
| Unknown       | 4 (1%)           | 2 (0.6%)                      | 3.58 (0.37-35.05) | -                             |
| Primary Injury Pattern: n (%) |              |                               |                  |                               |
| None          | 9 (2.5%)         | 9 (3.2%)                      | -                | -                             |
| Subdural Hematoma | 115 (31.9%) | 55 (19.8%) | Ref              | Ref                           |
| Epidural Hematoma | 21 (5.8%)  | 16 (5.8%)                     | 0.73 (0.27-2.00) | 0.76 (0.23-2.47)              |
| Subarachnoid Hemorrhage | 60 (16.6%) | 55 (19.8%) | 0.39 (0.19-0.81)* | 0.59 (0.23-1.40) |
| Intraparenchymal Hemorrhage | 105 (29.9%) | 92 (33.1%) | 0.46 (0.26-0.82)* | 0.85 (0.18-11.1) |
| Intraventricular Hemorrhage | 10 (2.8%)  | 10 (3.6%)                     | -                | -                             |
| Diffuse Axonal Injury | 41 (11.4%) | 41 (14.8%) | 0.28 (0.12-0.61)* | 0.45 (0.18-2.09) |
| Admission Platelet Count, 10^9/L: mean (SD) | 228.8 (71.4) | 229.9 (66.0) | 1.00 (1.00-1.00) | 1.00 (0.99-1.00) |
| Admission Thrombocytopenia <100x10^9/L: n(%) | 14 (3.4)     | 7 (2.2)                       | 1.46 (0.45-4.69) | 1.31 (0.26-6.62) |
| Admission PTT, sec: mean (SD) | 27.6 (5.6)     | 27.5 (5.7)                    | 1.04 (0.99-1.08) | 1.01 (0.97-1.06) |
| Admission INR: median (IQR) | 1.2 (1.1-1.3) | 1.1 (1.1-1.3) | 1.72 (0.72-4.15) | 1.22 (0.51-2.93) |
| Admission Anticoagulant Use, Yes: n (%) | 9 (2.2%)    | 4 (1.3%)                      | -                | -                             |
| Admission Antitplatelet Use, Yes: n (%) | 28 (7.0%)    | 21 (6.9%)                     | 0.89 (0.39-2.05) | 0.86 (0.32-3.30) |
| Craniectomy, Yes: n (%) | 124 (29.8%) | 30 (9.4%)                     | 5.13 (3.10-8.52)* | 22.6 (5.24-97.06) |
| Early Craniectomy, Yes: n (%) | 95 (22.8) | 0 (0%)                      | 2.92 (1.72-4.96)* | -                             |
| Admission IPH Volume, mL: median (IQR) | 1.1 (0.2-6.7) | 1 (0.2-4.4) | 1.01 (0.99-1.02) | 1.03 (1.00-1.05)* |
| Admission IPH Volume >1.5mL, Yes: n (%) | 181(43.5%) | 133 (41.4%) | 1.81(1.19-2.77)* | 2.89 (1.76-4.75)* |

CI= confidence interval; GCS= Glasgow Coma Scale Score; OR= Odds Ratio; PTT= Partial Thromboplastin Time; INR= International Normalized Ratio; IQR=inter quartile range; n=number; %= percentage; IPH=intraparenchymal hemorrhage; SD= standard deviation

*Indicates statistical significance at p<0.05.

Only one patient had unknown race, so effect could not be estimated.

Other includes: Hit by falling object (n = 6), explosion (n = 1), recreational sports (n = 1), bicycle vs vehicle (n = 7), pedestrian vs vehicle (n = 4), other not otherwise specified (n = 4).

Patients with no primary injury pattern had no blood on their CT, and therefore could not have effect estimates.

No patient with a primary injury pattern of intraventricular hemorrhage progressed.

All patients using anticoagulants in the cohort were taking coumadin.

All patients using anticoagulants in the cohort progressed.
Early craniectomy was performed in 70 patients for SDH, 8 patients for EDH, 14 patients for intraparenchymal hematoma evacuation, and 3 patients for intractable intracranial pressure.
**Table 3. Clinical Characteristics and Outcomes of All Patients With Severe TBI During Enrollment Period**

|                       | All Severe TBI Patients (n = 541) | Primary analysis sample (n = 321) |
|-----------------------|-----------------------------------|-----------------------------------|
| Age, years (mean±SD)  | 39.9±17.1                         | 37.0±16.3                         |
| Sex, n (%)            |                                   |                                   |
| Male                  | 424 (78.4%)                       | 247 (76.9%)                       |
| Female                | 117 (21.6%)                       | 74 (23.1%)                        |
| Admission GCS (median [IQR]) | 6 (4-7)                          | 7 (5-7)                           |
| 6-month GOS score (median [IQR]) | 3 (1-4)                          | 3 (1-4)                           |

SD: Standard Deviation; GCS: Glasgow Coma Scale score; GOS: Glasgow Outcome Scale score; IQR: interquartile range
eTable 4. Cohort Demographics and Clinical Characteristics of Progressors and Nonprogressors

| Characteristic                              | No progression within 5d (n=204) | Progression within 5d (n=117) | p-value |
|---------------------------------------------|----------------------------------|--------------------------------|---------|
| Age in years: mean (SD)                     | 33.8 (15.4)                      | 40.6 (16.2)                    | <0.001* |
| Sex: n (%)                                  |                                  |                                | 0.52    |
| Male                                        | 153 (75.0)                       | 94 (80.3)                      |         |
| Female                                       | 51 (25.0)                        | 23 (19.7)                      |         |
| Admission GCS: median (IQR)                 | 7 (5-7)                          | 7 (5-7)                        | 0.88    |
| ISS: mean (SD)                              | 32.6 (11.4)                      | 32.5 (11.9)                    | 0.97    |
| Race: n (%)                                 |                                  |                                | 0.82    |
| White                                       | 186 (91.2)                       | 107 (91.5)                     |         |
| African American                            | 16 (7.8)                         | 7 (6.0)                        |         |
| Southeast Asian                             | 2 (1)                            | 2 (1.7)                        |         |
| Unknown                                     | 0 (0%)                           | 1 (0.9)                        |         |
| Mechanism of Injury: n (%)                  |                                  |                                | 0.12    |
| Enclosed Motor Vehicle Accident             | 126 (61.8%)                      | 60 (51.3%)                     |         |
| Motor Cycle Crash                           | 32 (15.7%)                       | 25 (21.4%)                     |         |
| Fall                                        | 26 (12.8%)                       | 24 (20.5%)                     |         |
| Assault                                     | 7 (3.4%)                         | 2 (1.7%)                       |         |
| Gun Shot Wound                              | 1 (0.5%)                         | 1 (0.9%)                       |         |
| Other                                       | 12 (5.9%)                        | 3 (2.6%)                       |         |
| Unknown                                     | 0 (0%)                           | 2 (1.7%)                       |         |
| Primary Injury Pattern: n (%)               |                                  |                                | 0.02*   |
| None                                        | 9 (5.1%)                         | 0 (0%)                         |         |
| Subdural Hematoma                           | 32 (18.1%)                       | 23 (22.3%)                     |         |
| Epidural Hematoma                           | 9 (5.1%)                         | 7 (6.8%)                       |         |
| Subarachnoid Hemorrhage                     | 38 (21.5%)                       | 17 (16.5%)                     |         |
| Intraparenchymal Hemorrhage                 | 51 (28.8%)                       | 43 (41.8%)                     |         |
| Intraventricular Hemorrhage                 | 10 (5.7%)                        | 0 (0%)                         |         |
| Diffuse Axonal Injury                       | 28 (15.8%)                       | 13 (12.6%)                     |         |
| Admission Platelet Count, 10^9/L: mean (SD) | 231.2 (62.2)                     | 225.7 (71.2)                   | 0.49    |
| Admission Thrombocytopenia <100x10^9/L: n(%)| 4 (2.0)                          | 2 (1.7)                        | 0.62    |
| Admission INR: median (IQR)                 | 1.1 (1.1-1.2)                    | 1.1 (1.1-1.3)                  | 0.94    |
| Admission Anticoagulant Use, Yes: n (%)     | 0 (0.0%)                         | 3 (2.6%)                       | 0.08    |
| Admission Antiplatelet Use, Yes: n (%)      | 14 (6.8%)                        | 7 (6.5%)                       | 0.77    |
| Admission IPH Volume, mL: median (IQR)      | 0.8 (0.3-2.8)                    | 2.9 (0.7-9.5)                  | <0.001* |
| Admission IPH Volume >1.5mL, Yes: n (%)     | 60 (29.7%)                       | 72 (61.5%)                     | <0.001* |

GCS= Glasgow Coma Scale Score; OR= Odds Ratio; PTT= Partial Thromboplastin Time; INR= International Normalized Ratio; IQR= inter quartile range; n=number; %= percentage; IPH=intraparenchymal hemorrhage; SD= standard deviation

*Indicates statistical significance at p<0.05

Other includes: Hit by falling object, explosion, recreational sports, bicycle vs vehicle, pedestrian vs vehicle, other not otherwise specified.

Patients with no primary injury pattern had no blood on their CT, and therefore could not have effect estimates.

No patient with a primary injury pattern of intraventricular hemorrhage progressed.

All patients using anticoagulants in the cohort were taking coumadin.
## eTable 5. Association of Intraparenchymal Hemorrhage Progression With Outcome After Severe TBI

|                | Univariable associations, Odds Ratio (95% CI) | Multivariable<sup>a</sup> Association Odds Ratio (95% CI) |
|----------------|-----------------------------------------------|----------------------------------------------------------|
|                | 6h <sup>p-value</sup> | 24h <sup>p-value</sup> | 120h <sup>p-value</sup> | 6h <sup>p-value</sup> | 24h <sup>p-value</sup> | 120h <sup>p-value</sup> |
| Discharge Mortality | 3.37 (1.78-6.38) | <0.001 | 2.57 (1.44-4.57) | 0.001 | 3.31 (1.80-6.08) | <0.001 | 1.98 (0.92-4.27) | 0.08 | 1.58 (0.81-3.10) | 0.18 | 2.27 (1.14-4.55) | 0.02 |
| 3 months        | 0.41 (0.20-0.84) | 0.02 | 0.45 (0.24-0.82) | 0.01 | 0.48 (0.27-0.87) | 0.02 | 0.35 (0.15-0.83) | 0.02 | 0.42 (0.20-0.85) | 0.02 | 0.48 (0.24-0.96) | 0.04 |
| 6 months        | 0.31 (0.16-0.59) | <0.001 | 0.31 (0.18-0.54) | <0.001 | 0.30 (0.17-0.51) | <0.001 | 0.31 (0.14-0.69) | 0.004 | 0.30 (0.15-0.60) | 0.001 | 0.30 (0.15-0.57) | <0.001 |
| 12 months       | 0.41 (0.22-0.75) | 0.004 | 0.35 (0.20-0.60) | <0.001 | 0.34 (0.19-0.58) | <0.001 | 0.49 (0.23-1.04) | 0.06 | 0.40 (0.21-0.77) | 0.006 | 0.37 (0.20-0.70) | 0.002 |

<sup>a</sup> Backwards elimination models including age, sex, initial GCS-score, injury severity score, coagulation factors, thrombocytopenia, and initial hemorrhage volume.
### eTable 6. Genotype Frequencies of ABCC8 and TRPM4 SNPs Associated With Hemorrhage Progression in TBI

| SNP Details | Sample | Homozygous Wild-type n (%) | Heterozygous n (%) | Homozygous Variant n (%) | p-value (Fisher) |
|-------------|--------|----------------------------|-------------------|--------------------------|-----------------|
| **ABCC8**   |        |                            |                   |                          |                 |
| rs2237982   | Full Cohort | 106 (32.7%)               | 161 (49.7%)      | 57 (17.6%)               | -               |
| rs2283261   | No-Cranectomy subcohort | 73 (31.2%)               | 120 (51.3%)      | 41 (17.5%)               | -               |
|             | - Hemorrhage Progression-Yes | 30/73 (41.1%)             | 45/120 (37.5%)   | 27/61 (65.9%)            | 0.006           |
| rs3819521   | Full Cohort | 97 (33.0%)               | 157 (53.4%)      | 40 (13.6%)               | -               |
| rs8192695   | No-Cranectomy subcohort | 64 (30.8%)               | 117 (56.3%)      | 27 (13.0%)               | -               |
|             | - Hemorrhage Progression-Yes | 26/61 (40.6%)             | 46/117 (39.3%)   | 19/27 (70.4%)            | 0.012           |
| rs1477363   | Full Cohort | 123 (41.8%)               | 142 (48.3%)      | 29 (9.9%)                | -               |
| rs10410857  | No-Cranectomy subcohort | 82 (39.4%)               | 105 (50.5%)      | 21 (10.1%)               | -               |
|             | - Hemorrhage Progression-Yes | 35/82 (42.7%)             | 42/105 (40.0%)   | 14/21 (66.7%)            | 0.084           |
| rs909010    | Full Cohort | 266 (90.5%)               | 27 (9.2%)        | 1 (0.3%)                 | -               |
| rs100010    | No-Cranectomy subcohort | 170 (90.4%)               | 17 (9.0%)        | 1 (0.5%)                 | -               |
|             | - Hemorrhage Progression-Yes | 69/170 (40.6%)             | 11/17 (64.7%)    | 1/1 (100%)               | .052            |
| **TRPM4**   |        |                            |                   |                          |                 |
| rs3760666   | Full Cohort | 126 (42.9%)               | 139 (47.3%)      | 29 (9.9%)                | -               |
| rs1477363   | No-Cranectomy subcohort | 88 (46.8%)               | 88 (46.8%)       | 12 (6.4%)                | -               |
|             | - Hemorrhage Progression-Yes | 46/88 (52.3%)             | 31/88 (35.2%)    | 4/12 (33.3%)             | 0.060           |
| rs10410857  | Full Cohort | 151 (51.4%)               | 124 (42.2%)      | 19 (6.5%)                | -               |
| rs909010    | No-Cranectomy subcohort | 104 (55.3%)               | 74 (39.4%)       | 10 (5.3%)                | -               |
|             | - Hemorrhage Progression-Yes | 53/104 (51.0%)             | 24/74 (32.4%)    | 4/10 (40%)               | 0.042           |
| rs1090010   | Full Cohort | 129 (43.9%)               | 134 (45.6%)      | 31 (10.5%)               | -               |
| rs100010    | No-Cranectomy subcohort | 90 (47.9%)               | 84 (44.7%)       | 14 (7.5%)                | -               |
|             | - Hemorrhage Progression-Yes | 50/90 (55.6%)             | 27/84 (32.1%)    | 4/14 (28.6%)             | 0.004           |

© 2021 Jha RM et al. JAMA Network Open.
### eTable 7. ABCC8 and TRPM4 Single-Nucleotide Polymorphisms Associated With Quantitative Change in Intraparenchymal Hemorrhage Volumes in Severe TBI

| SNP       | Model             | Hemorrhage Progression β (95% CI, p-value) | 6-hour p-value | 24-hour p-value | 5-day p-value |
|-----------|-------------------|--------------------------------------------|----------------|----------------|---------------|
| **ABCC8** |                   |                                            |                |                |               |
| rs8192695 | Additive (Reference GG) |                                            |                |                |               |
| rs8192695 | GA                | 0.19 (-0.29 – 0.67)                       | 0.44           | 0.75 (0.25 – 1.24) | 0.003*        | 0.57 (0.08 – 1.06) | 0.02     |
|           | AA                | 1.82 (0.22-3.20)                          | 0.02           | 1.49 (-0.28-3.27) | 0.10          | 1.43 (-0.33-3.20) | 0.11     |
|          | Dominant          |                                            |                |                |               |
| rs8192695 | GA, AA            | 0.34 (-0.13 – 0.81)                       | 0.15           | 0.80 (0.32 – 1.28) | 0.001*        | 0.63 (0.15 – 1.11) | 0.01     |
| rs1799859 | Additive (Reference CC) |                                            |                |                |               |
| rs1799859 | CT                | 0.02 (-0.23 – 0.27)                       | 0.87           | 0.34 (0.04 – 0.60) | 0.03          | 0.33 (0.04 – 0.62) | 0.02     |
|           | TT                | 0.12 (-0.60 – 0.83)                       | 0.75           | 0.74 (0.15 – 1.34) | 0.02          | 1.03 (0.31 – 1.77) | 0.006*   |
|          | Dominant          |                                            |                |                |               |
| rs1799859 | CT, TT            | 0.03 (-0.22 – 0.27)                       | 0.82           | 0.39 (0.11 – 0.67) | 0.007*        | 0.40 (0.12 – 0.69) | 0.006*   |
| rs4148640 | Additive (Reference GG) |                                            |                |                |               |
| rs4148640 | GT                | 0.04 (-0.21 – 0.30)                       | 0.73           | 0.34 (0.04 – 0.63) | 0.03          | 0.35 (0.06 – 0.64) | 0.02     |
|           | TT                | 0.12 (-0.59 – 0.84)                       | 0.73           | 0.74 (0.15 – 1.34) | 0.02          | 1.05 (0.31 – 1.78) | 0.005*   |
|          | Dominant          |                                            |                |                |               |
| rs4148640 | GT, TT            | 0.05 (-0.20 – 0.30)                       | 0.69           | 0.39 (0.11 – 0.67) | 0.007*        | 0.42 (0.14 – 0.70) | 0.004*   |
| **TRPM4** |                   |                                            |                |                |               |
| rs3760666 | Additive (Reference TT) |                                            |                |                |               |
| rs3760666 | TC                | -0.28 (-0.53 --0.03)                      | 0.03           | -0.18 (-0.47 – 0.12) | 0.24         | -0.18 (-0.48 – 0.12) | 0.24     |
|           | CC                | -0.40 (-0.92 – 0.12)                      | 0.14           | -0.49 (-1.16 – 0.19) | 0.16         | -0.29 (-0.92 – 0.35) | 0.37     |
|          | Dominant          |                                            |                |                |               |
| rs3760666 | TC, CC            | -0.29 (-0.54 --0.05)                      | 0.02           | -0.21 (-0.50 – 0.08) | 0.16         | -0.19 (-0.48 – 0.10) | 0.19     |
| rs1477363 | Additive (Reference CC) |                                            |                |                |               |
| rs1477363 | CA                | -0.26 (-0.52 - -0.01)                     | 0.05           | -0.24 (-0.53 – 0.06) | 0.11         | -0.27 (-0.57 – 0.02) | 0.07     |
|           | AA                | -0.31 (-0.89 – 0.26)                      | 0.28           | -0.37 (-1.21 – 0.46) | 0.38         | -0.28 (-0.94 – 0.37) | 0.39     |
|          | Dominant          |                                            |                |                |               |
| rs1477363 | CA, AA            | -0.27 (-0.52 - -0.02)                     | 0.03           | -0.25 (-0.53 – 0.04) | 0.09         | -0.28 (-0.56 – 0.01) | 0.06     |
| rs1041085 | Additive (Reference GG) |                                            |                |                |               |
| rs1041085 | GA                | -0.29 (-0.55 - -0.04)                     | 0.02           | -0.35 (-0.64 – 0.06) | 0.02         | -0.42 (-0.71 – 0.13) | 0.005*   |
|           | AA                | -0.44 (-0.91 - -0.03)                     | 0.07           | 0.61 (-1.24 – 0.02) | 0.06         | -0.50 (-1.09 – 0.08) | 0.09     |
|          | Dominant          |                                            |                |                |               |
| rs1041085 | GA, AA            | -0.32 (-0.56 - -0.08)                     | 0.01           | -0.38 (-0.66 – 0.09) | 0.009*       | -0.43 (-0.71 – 0.15) | 0.003*   |
| rs909010  | Additive (Reference TT) |                                            |                |                |               |
| rs909010  | TC                | -0.41 (-0.66 - -0.16)                     | 0.002*         | -0.42 (-0.71 – 0.12) | 0.006*       | -0.49 (-0.78 – 0.19) | 0.002*   |
|           | CC                | -0.37 (-0.79 - -0.05)                     | 0.09           | -0.47 (-1.00 – 0.06) | 0.08         | -0.47 (-0.97 – 0.03) | 0.07     |
|          | Dominant          |                                            |                |                |               |
| rs909010  | TC, CC            | -0.40 (-0.64 - -0.16)                     | 0.001*         | -0.43 (-0.71 – 0.14) | 0.003*       | -0.48 (-0.77 – 0.20) | 0.001*   |
| rs8104571 | Additive (Reference CC) |                                            |                |                |               |
| rs8104571 | CT                | -0.26 (-1.01 – 0.50)                      | 0.51           | 1.15 (-2.23 – 2.06) | 0.01         | 1.09 (-2.0 – 1.99)  | 0.02     |
|           | TT                | -                                         | -               | -               | -             | -               | -        |

*p* < 0.00931 meeting significance after Benjamin-Yekutieli correction for multiple comparisons

SNPs are significant predictors of hemorrhage expansion (binary, Table 1).

SNPs are significant (p<0.05) in all-comers regardless of craniectomy status (Supplemental Table 9).

SNPs previously reported to be predictive of intracranial pressure and/or acute CT edema after TBI.

© 2021 Jha RM et al. *JAMA Network Open.*
| SNP             | Model            | Intraparenchymal Hemorrhage Progression OR (95% CI) | 6-hour p-value | 24-hour p-value | 5-day p-value | p-value |
|-----------------|------------------|---------------------------------------------------|----------------|-----------------|---------------|--------|
| **ABCC8**       |                  |                                                   |                |                 |               |        |
| rs8192695<sup>b,c</sup> | Additive (Reference GG) | GA AA                                             | 2.98 (1.11-8.03) | 0.03            | 2.38 (0.97-5.84) | 0.06   | 2.56 (1.01-6.49) | 0.05   |
|                 | Dominant         | GA, AA                                            | 3.29 (1.24-8.71) | 0.02            | 2.63 (1.09-6.38) | 0.03   | 2.82 (1.12-7.10) | 0.03   |
| rs1799857<sup>d</sup> | Additive (Reference CC) | CT TT                                             | 1.12 (0.61-2.37) | 0.60            | 0.90 (0.50-1.62) | 0.73   | 0.81 (0.45-1.46) | 0.48   |
|                 | Recessive        | TT                                                | 2.79 (1.33-5.85) | 0.007<sup>a</sup> | 2.38 (1.22-4.64) | 0.01   | 2.10 (1.07-4.15) | 0.03   |
| **TRPM4**       |                  |                                                   |                |                 |               |        |
| rs10410857<sup>b,c</sup> | Additive (Reference GG) | GA AA                                             | 0.50 (0.27-0.94) | 0.03            | 0.44 (0.26-0.76) | 0.003<sup>a</sup> | 0.42 (0.25-0.73) | 0.002<sup>a</sup> | 0.07   |
|                 | Dominant         | GA, AA                                            | 0.54 (0.30-0.97) | 0.04            | 0.45 (0.26-0.76) | 0.003<sup>a</sup> | 0.42 (0.25-0.71) | 0.001<sup>a</sup> |       |
| rs909010<sup>b,c</sup> | Additive (Reference TT) | TC CC                                             | 0.48 (0.26-0.90) | 0.02            | 0.42 (0.24-0.73) | 0.002<sup>a</sup> | 0.38 (0.22-0.66) | 0.001<sup>a</sup> | 0.13   |
|                 | Dominant         | TC, CC                                            | 0.52 (0.29-0.95) | 0.04            | 0.44 (0.26-0.75) | 0.002<sup>a</sup> | 0.40 (0.24-0.67) | 0.001<sup>a</sup> |       |

<sup>a</sup>p < 0.00931 meeting significance after Benjamin-Yekutieli correction for multiple comparisons,
<sup>b</sup>SNPs that are significant predictors of hemorrhage expansion (binary, Table 1).
<sup>c</sup>SNPs that are significant (p < 0.05) predictors of quantitative contusion expansion volumes (continuous variable, Supplemental Table 7).
<sup>d</sup>SNPs previously reported to be predictive of intracranial pressure and/or acute CT edema after TBI.
**Table 9. ABCC8 and TRPM4 Haplotypes Associated With Contusion Expansion in Severe TBI**

| Haplotype | Model | Hemorrhage Progression OR (95% CI, p-value) | 6-hour p-value | 24-hour p-value | 5-day p-value |
|-----------|-------|---------------------------------------------|----------------|----------------|---------------|
| ABCC8 rs2237982 (C/T) - rs2283261 (A/C) - rs8192695 (G/A) - rs3819521 (C/T) |       |                                             |                |                |               |
| TCAC      | Additive | 3.78 (1.25, 11.48) | 0.02            | 3.37 (1.24-9.14) | 0.02          | 2.80 (1.04-7.59) | 0.04 |
|           | Dominant | 3.02 (1.00-9.11)   | 0.05            | 2.72 (1.02-7.29) | 0.05          | 2.24 (0.94-6.00) | 0.11 |
| TCA-C     | Additive | 3.88 (1.27-11.86)  | 0.02            | 3.34 (1.12-10.4) | 0.02          | 2.78 (1.10-7.53) | 0.04 |
|           | Dominant | 3.05 (1.00-9.8)    | 0.05            | 2.73 (1.01-7.2)  | 0.05          | 2.37 (0.94-6.7)  | 0.03 |
| TRPM4 rs3760666 (T/C) - rs1477363 (C/A) - rs10410857 (G/A) - rs909010 (T/C) |       |                                             |                |                |               |
| CAAC      | Additive | 0.58 (0.32-1.03)   | 0.06            | 0.52 (0.31-1.0)  | 0.06          | 0.54 (0.33-0.9)  | 0.02 |
|           | Dominant | 0.46 (0.24-0.94)   | 0.03            | 0.46 (0.26-0.84) | 0.03          | 0.48 (0.27-0.84) | 0.01 |
| -AAC      | Additive | 0.57 (0.32-1.02)   | 0.06            | 0.51 (0.30-0.84) | 0.06          | 0.53 (0.33-0.86) | 0.01 |
|           | Dominant | 0.47 (0.24-0.93)   | 0.03            | 0.45 (0.25-0.82) | 0.03          | 0.46 (0.26-0.81) | 0.007a |
| CA-C      | Additive | 0.57 (0.33-0.99)   | 0.04            | 0.49 (0.30-0.79) | 0.04          | 0.52 (0.33-0.82) | 0.005a |
|           | Dominant | 0.50 (0.26-0.96)   | 0.04            | 0.46 (0.26-0.80) | 0.04          | 0.48 (0.28-0.83) | 0.008a |
| - - AC    | Additive | 0.54 (0.31-0.92)   | 0.02            | 0.46 (0.28-0.73) | 0.02          | 0.48 (0.30-0.75) | 0.001a |
|           | Dominant | 0.48 (0.25-0.91)   | 0.02            | 0.43 (0.25-0.75) | 0.02          | 0.45 (0.26-0.77) | 0.003a |
| ABCC8 rs2283261 (A/C) - rs8192695 (G/A) and TRPM4 rs10410857 (G/A) - rs909010 (T/C) |       |                                             |                |                |               |
| AGAC      | Additive | 0.43 (0.18-1.0)    | 0.05            | 0.28 (0.13-0.62) | 0.002          | 0.28 (0.13-0.60) | 0.001a |
| CAGT      | Additive | 3.73 (1.24-11.1)   | 0.02            | 2.67 (0.99-7.26) | 0.05          | 2.17 (0.8-5.89)  | 0.12 |

CI= confidence interval; NS= not significant; OR= odds ratio

*p< 0.00931 meeting significance after Benjamin-Yekutieli correction for multiple comparisons
Table 10. Haplotype Distribution in the Cohort of Severe TBI

SNP order (0 = wild type, 1 = variant):
ABCC8: rs2237982-rs8192695-rs2283261-rs3819521
TRPM4: rs909010-rs10410857-rs1477363-rs3760666

| Haplotype | Frequency (proportion) |
|-----------|------------------------|
| 00000000  | 0.364198               |
| 00000001  | 0.012795               |
| 00001000  | 0.024099               |
| 00001100  | 0.0184                 |
| 00001111  | 0.177303               |
| 00010000  | 0.008384               |
| 10100000  | 0.012072               |
| 10101111  | 0.010521               |
| 10110000  | 0.195595               |
| 10110000  | 0.009866               |
| 10111000  | 0.010973               |
| 10111011  | 0.033903               |
| 10111111  | 0.079035               |
| 11100000  | 0.017407               |
| 11101111  | 0.009391               |
### eTable 11. Intraparenchymal Hemorrhage Progression Risk Polymorphisms Associated With 6-mo Glasgow Outcome Scale (GOS) Score

| SNP and model | Common OR<sup>a,b</sup> (95% CI) | p-value (model) | p-value (LR-test vs clinical only) |
|---------------|----------------------------------|----------------|----------------------------------|
| **ABCC8**     |                                  |                |                                  |
| rs2237982     | Ref (GG or GA)                   |                |                                  |
| Recessive     | AA                               | 0.45 (0.23-0.91) | 0.03<sup>b</sup> | 0.02<sup>b</sup> |
| rs2283261     | Ref (CC or CT)                   |                |                                  |
| Recessive     | TT                               | 0.45 (0.22-0.90) | 0.02<sup>b</sup> | 0.02<sup>b</sup> |
| rs8192695     | Ref (CC)                         |                |                                  |
| Dominant      | CT or TT                         | 0.38 (0.02-9.29) | 0.55                            | 0.55                |
| rs3819521     | Ref (GG or GT)                   |                |                                  |
| Recessive     | TT                               | 0.44 (0.20-0.97) | 0.04<sup>b</sup> | 0.04<sup>b</sup> |

LR-test: likelihood ratio test of model including SNP vs model with only clinical covariates

<sup>a</sup>Odds of moving one point higher on the GOS scale

<sup>b</sup>Statistically significant at p<0.05
**eTable 12. Sample Size Calculations for Genotype-Based Patient Selection in Clinical Trial**

| Group | Probability of Progression | Percent of All Comers | Sample size Required * | Number Screened |
|-------|----------------------------|-----------------------|------------------------|-----------------|
| All comers | 43.3% | 100% | 576 | 576 |
| At least one ABCC8 variant (risk) SNP | 62.5% | 26.9% | 296 | 1100 |
| At least one TRPM4 wild-type (risk) SNP | 52.9% | 58.2% | 408 | 701 |
| At least one ABCC8 variant (risk) SNP AND at least one TRPM4 wild-type (risk) SNP | 83.3% | 11.5% | 136 | 1183 |
| At least one ABCC8 variant (risk) SNP OR at least one TRPM4 wild-type (risk) SNP | 51.7% | 73.6% | 428 | 582 |
| Clinical Model Alone* | 72.2% | 17.3% | 208 | 1202 |
| Clinical Model + Genotypes* | 61.9% | 50.4% | 300 | 594 |

*Sample size for relative risk reduction of 30% in hemorrhage progression, power of 0.9

*Subgroup of patients where the model predicts >50% risk of progression. Clinical models contain the covariates from the backward elimination multivariable regression models (age, age, sex, initial GCS-score, injury severity score, coagulation factors, thrombocytopenia, and initial hemorrhage volume).
**eTable 13. Regulatory Annotations of ABCC8 and TRPM4 Single-Nucleotide Polymorphisms Associated With Hemorrhage Progression in Severe TBI**

(Includes data for high-probability causal SNPs for proxy-SNPs in LD, \( r^2 > 0.8 \))

| Genotyped SNP | High Probability Causal SNP in LD \((r^2)\) | RegulomeDB Score | Promoter Histone Marks | Enhancer Histone Marks | DNAse | Proteins Bound* | Transcription Factors with Regulatory Motifs Altered * | Transcripts with Regulatory Motifs Altered |
|---------------|---------------------------------|----------------|------------------------|------------------------|-------|----------------|------------------------------------------------|----------------------------------|
| **ABCC8 SNPs** | | | | | | | | |
| rs8192695 | | | | | | | | |
| rs77462644 (1.0) | 0.609 | Y | Y | Y | - | ATF7, CTBP1, DP2, EGR2, FOXJ2, GATA2, HDAC1, MAX, MAZ, MYC, PATZ, POL2, POLR2A, TCF7L2, USF2, VEZF1, ZNF629 | HIC1 (-2.8), LBP1 (1.6), NANOG (9.4), TCF12 (-2.6), ZFP410 (6.9) |
| rs3819521 | 0.135 | Y | Y | Y | - | FOXA (11.9), NF-kB (-3.6), STAT (12.0) |
| rs4148610* (0.96) | 0.719 | Y | Y | Y | - | HOXA13 (10.6), PAX3 (3.2), PAX5 (1.6) |
| rs4148609* (0.92) | 0.144 | Y | Y | Y | - | ETS1 |
| rs2301703* (0.81) | 0.609 | Y | Y | Y | - | CACD (-2.6), MYC (-1.8), MYF (-4.0), NR5F2 (-3.2), SMC3 (-3.9), SIN3Ak (-8.2), TCF12 (11.3) |
| rs2283254* (0.95) | 0.609 | Y | Y | Y | - | EVI (12.0) |
| rs3815066 (0.96) | 0.609 | Y | Y | Y | - | EBF (-0.8), ER-\( \alpha \) (-0.6), PLAG1 (-10.8) |
| rs2237982* and rs2283261* | 0.599 | Y | Y | Y | - | MEF2D, MYEF2, MYEF2-B |
| rs2237980 (1.0) | 0.568 | Y | Y | Y | - | ARNT2 (-12), BHLHE40 (-11.9), E2A (11.9), MXI1 (-0.1), MYC (11.4), SIN3Ak (-4.2), ZEB1 (12) |
| rs2301703* (0.92) | 0.609 | Y | Y | Y | - | see entry for rs2301703 above |
| **TRPM4 SNPs** | | | | | | | | |
| rs3760666 | - | 0.609 | N | Y | Y | ZNF512, ZNF394, ZNF596 | - |
| rs1477363 | - | 0.5896 | N | Y | N | SP1, ZNF121 | NR2F2 (-11.7), TATA (11.7) |
| rs10410857 | - | 0.329 | N | N | N | - | ARHGEF12, BCL6 (-1.7) |
| rs909010 | - | 0.609 | N | Y | N | NR2F2 | GR (-0.6), HNF4 (2.9), IRF (-1.6), RXRA (-0.1) |

* Data includes high-probability causal SNPs for proxy-SNPs in LD, \( r^2 > 0.8 \).
| rs34639121 (0.82) | 0.83 | Y | Y | Y | >250 reports of proteins bound including ATF1, CTCF, MYC, PPAR-γ | P53 (-12) |

LD= linkage disequilibrium; PWM= position weight matrix log odds scores from HaploReg V4.1; SNP= single nucleotide polymorphism

*SNPs are previously reported as significant predictors of post traumatic ICP and/or acute CT edema.

Obtained from RegulomeDB 2.0 and HaploReg V4.1.

Obtained from RegulomeDB 2.0 and HaploReg V4.1. PWM log-odds score obtained from HaploReg V4.1 annotating SNP effects on regulatory motifs and transcription factor binding affinity: Log Odds Variant− Log Odds Reference allele. An increase in log-odds scores suggest increased transcription factor binding strength based on PWMs collected by HaploReg from TRANSFAC, JASPAR and Protein Binding Microarray experimental data. Absolute values for log-odds scores for the reference and variant alleles are also available from HaploReg.

Proxy SNP in LD with more than 1 genotyped SNP
| SNP          | Model                  | Hemorrhage Progression | OR (95% CI) | Hemorrhage Progression | OR (95% CI) | Hemorrhage Progression | OR (95% CI) | Hemorrhage Progression | OR (95% CI) |
|--------------|------------------------|------------------------|-------------|------------------------|-------------|------------------------|-------------|------------------------|-------------|
|              |                        | 6-hour IPH Progression | p-value     | 24-hour IPH Progression | p-value     | 120-hour IPH Progression | p-value     |
| **ABCC8**    |                        |                        |             |                        |             |                        |             |
| rs2237982    | Additive (Reference CC)| CT                     | 0.91 (0.46-1.78) | 0.78                   | 0.97 (0.53-1.76) | 0.91                   | 0.89 (0.49-1.59) | 0.68 |
|              |                        | TT                     | 2.07 (0.91-4.73) | 0.08                   | 2.61 (1.21-5.64) | 0.02                   | 2.08 (0.97-4.46) | 0.06 |
|              |                        | Variant recessive      | TT          | 2.20 (1.07-4.52) | 0.03                    | 2.67 (1.36-5.23) | 0.004                   | 2.24 (1.15-4.39) | 0.02 |
| rs2283261    | Additive (Reference AA)| AC                     | 1.30 (0.62-2.73) | 0.48                   | 1.20 (0.63-2.72) | 0.58                   | 1.24 (0.66-2.33) | 0.96 |
|              |                        | CC                     | 2.81 (1.00-7.89) | 0.05                   | 2.85 (1.16-7.02) | 0.02                   | 2.55 (1.04-6.25) | 0.04 |
|              |                        | Variant recessive      | CC          | 2.36 (0.95-5.87) | 0.06                    | 2.66 (1.19-5.95) | 0.02                   | 2.22 (1.00-4.94) | 0.05 |
| rs8192695    | Additive (Reference GG)| GA                     | 2.71 (0.92-8.00) | 0.07                   | 2.25 (0.83-6.16) | 0.11                   | 1.98 (0.72-5.38) | 0.18 |
|              |                        | AA                     | -           | -                     | -                      | -                      | -                      | -       |
|              |                        | Variant dominant       | GT or TT    | 3.24 (1.12-9.33) | 0.03                    | 2.55 (0.95-6.85) | 0.06                   | 2.23 (0.83-5.99) | 0.11 |
| rs3819521    | Additive (Reference CC)| CC                     | 0.80 (0.40-1.59) | 0.52                   | 0.87 (0.48-1.58) | 0.64                   | 0.97 (0.54-1.76) | 0.92 |
|              |                        | CT                     | 1.67 (0.56-4.95) | 0.36                   | 2.46 (0.95-6.36) | 0.06                   | 2.15 (0.83-5.54) | 0.11 |
|              |                        | Variant recessive      | TT          | 1.87 (0.67-5.26) | 0.23                    | 2.65 (1.08-6.51) | 0.03                   | 2.18 (0.89-5.36) | 0.09 |
| **TRPM4**    |                        |                        |             |                        |             |                        |             |
| rs3760666    | Additive (Reference TT)| TC                     | 0.72 (0.37-1.43) | 0.35                   | 0.61 (0.34-1.10) | 0.10                   | 0.66 (0.37-1.18) | 0.16 |
|              |                        | CC                     | 0.54 (0.12-2.41) | 0.42                   | 0.34 (0.09-1.25) | 0.10                   | 0.32 (0.09-1.17) | 0.14 |
|              |                        | Variant dominant       | TC or CC    | 0.70 (0.36-1.35) | 0.29                    | 0.57 (0.33-1.01) | 0.06                   | 0.61 (0.35-1.07) | 0.09 |
| rs1477363    | Additive (Reference CC)| CA                     | 0.51 (0.31-1.22) | 0.16                   | 0.51 (0.28-0.93) | 0.03                   | 0.57 (0.32-1.03) | 0.06 |
|              |                        | AA                     | 0.59 (0.11-3.05) | 0.53                   | 0.45 (0.11-1.84) | 0.27                   | 0.42 (0.10-1.71) | 0.23 |
|              |                        | Variant dominant       | CA or AA    | 0.51 (0.31-1.20) | 0.15                    | 0.51 (0.28-0.90) | 0.02                   | 0.56 (0.32-0.98) | 0.04 |
| rs10410857   | Additive (Reference GG)| GA                     | 0.67 (0.34-1.32) | 0.25                   | 0.46 (0.25-0.82) | 0.009                  | 0.50 (0.28-0.89) | 0.02 |
|              |                        | AA                     | 0.41 (0.09-1.84) | 0.25                   | 0.26 (0.07-0.94) | 0.04                   | 0.24 (0.07-0.87) | 0.03 |
|              |                        | Variant dominant       | GA or AA    | 0.63 (0.33-1.23) | 0.18                    | 0.43 (0.24-0.75) | 0.003                  | 0.46 (0.26-0.81) | 0.007 |
| rs909010     | Additive (Reference TT)| TC                     | 0.59 (0.30-1.18) | 0.14                   | 0.51 (0.28-0.93) | 0.03                   | 0.55 (0.31-0.99) | 0.05 |
|              |                        | CC                     | 0.75 (0.22-2.54) | 0.65                   | 0.49 (0.17-1.39) | 0.18                   | 0.46 (0.16-1.31) | 0.15 |
|              |                        | Variant dominant       | TC or CC    | 0.62 (0.32-1.19) | 0.15                    | 0.51 (0.29-0.90) | 0.02                   | 0.54 (0.31-0.94) | 0.03 |
**Figure 1.** Opposite Associations of *ABCC8* vs *TRPM4* variant SNPs with Intraparenchymal Hemorrhage Progression in TBI

Panel of bar charts graphically depicting examples of the opposite directions of associations of *ABCC8* (A and B) vs *TRPM4* (C and D) variant SNPs with odds of hemorrhage progression in TBI at 24 hours and 5 days. Probability of progression is on the y-axis, and genotypes are on the x-axis. A dominant model for *ABCC8* SNP rs8192695 (A-i) shows increased probability of 24h hemorrhage progression with presence of the variant SNP (red) vs homozygous wild-type (blue, $P = .004$). A recessive model for rs3812695 (A-ii) shows increased probability of 24-hour progression in homozygous variants (red, $P = .027$). Additive models for rs2237982 ($P = .006$) and rs2283261 ($P = .003$) show increased probability of 5-day hemorrhage progression in homozygous variants (red) compared with both heterozygotes (green) and homozygous wild-type (blue). Conversely, dominant models at 24h for *TRPM4* SNPs rs1477363 (C-i, $P = .008$) and rs3760666 (C-ii, $P = .009$) show decreased probability of hemorrhage progression with presence of the variant SNP (red) vs homozygous wild-type (blue) at 24h. Additive models for rs10410857 (D-i, $P = .002$) and rs909010 (D-ii, $P = 3E-4$) demonstrate decreased probability of hemorrhage progression in homozygous variants (red) vs both heterozygotes (green) and homozygous wild-type (blue).
**eFigure 2.** Spatial Distribution of TRPM4 SNPs in Hemorrhage Progression After TBI

Chromosomal Location and Identification of TRPM4 SNPs as Brain eQTLs. This graph shows the -log10 \( (P\) value) of significant cis-expression quantitative trait loci (eQTLs, red dots) within the TRPM4 gene (y-axis) and their location on the gene (x-axis). Subgraphs demonstrate the single nucleotide polymorphism eQTL \( P\) values and chromosomal locations based on different tissue isolates from the genotype-tissue expression (GTEx) project with brain-specific eQTLs in the cerebellum, cortex, frontal cortex, and hippocampus. No trans-eQTLs are present. TRPM4 is encoded on the forward strand with exons delineated by vertical gray bars; exon 1 is therefore leftmost and exon 25 is located on the far right of the x-axis. Evident from the graph, majority of brain-specific cis-eQTLs are located upstream of exon 12 including the four significant TRPM4 SNPs associated with hemorrhage progression, rs3760666, rs1477363, rs10410857, rs909010 – these are highlighted by the vertical red lines.
eFigure 3. Variant TRPM4 SNPs Associated With Decreased Hemorrhage Progression in TBI are eQTL With Decreased TRPM4 Expression in Cerebellum
Panel demonstrating violin plots of normalized mRNA expression levels (y-axis) associated with the genotypes (x-axis) of four TRPM4 SNPs significantly associated with hemorrhage progression after severe traumatic brain injury (TBI). Each subpanel shows the normalized TRPM4 mRNA expression level in the cerebellum, cortex, frontal cortex, hippocampus and putamen by SNP genotype: rs3760666 (A), rs1477363 (B), rs10410857 (C) and rs909010 (D). Shaded regions in teal or blue (alternating between brain region) of the individual violin plots indicate the density distribution of mRNA expression in the samples in each respective genotype, with the white line showing the median value. The $P$ value provided for each SNP at each location indicates the $P$ value for different expression levels across genotypes for that SNP in the respective tissue location. Unlike ABCC8, all four TRPM4 SNPs are brain-specific eQTLs only in the cerebellum, with rs3760666 also having significantly different TRPM4 mRNA expression levels in the brain cortex and putamen. In all cases, mRNA expression is lower with variant TRPM4 SNPs, with a dose-dependent effect noted between homozygous wild-type, heterozygotes, and homozygous variants.
eFigure 4. Sequence Logos Demonstrating Impact of ABCC8 and TRPM4 SNPs on Transcription Factor Motifs

Schematics of sequence logos of different transcription factor binding site motifs obtained from RegulomeDB annotations for ABCC8 SNP rs2237982 (A) and TRPM4 SNP rs10410857. The y-axis in each subgraph represents a binary information tool (bits) containing two pieces of information: the height of the base pair alphabet is non linearly proportional to the frequency with which it is found at that position, and the total height of each column denotes the importance/strength of that location for transcription factor recognition and binding. Each base pair is shown in a specific color. The location altered by the SNP is highlighted in a red box. For transcription factors MEF2d, MYEF2, and MYEF2-b, ABCC8 SNP rs2237982 changes a highly conserved base-pair, and this site is highly important for determining strength of transcription factor recognition and binding. TRPM4 SNP rs10410857, only moderately affects transcription factor binding for ARHGEF12 but strongly affects BCL6.
eFigure 5. Brain Chromatin States of ABCC8 and TRPM4 Genomic Loci

| Locus     | Ratio (R) of Transcription, Enhancers | Brain | All Tissue |
|-----------|--------------------------------------|-------|------------|
| ABCC8     |                                      |       |            |
| a: rs8192695 | R=0.5 | 7 | 15 |
|           | R=0.13 | 1 | 83 |
|           | R=0.154 | 16 | 65 |
|           | R=0.233 | 9 | 68 |
|           | R=0.104 | 12 | 70 |
|           | R=0.82 | 7 | 59 |
|           | R=1.08 | 6 | 36 |
|           | R=1.32 | 0 | 25 |
|           | R=1.33 | 0 | 4 |
|           | R=1.76 | 1 | 39 |
|           | R=1.34 | 1 | 34 |
|           | R=1.77 | 0 | 80 |
|           | R=1.78 | 1 | 43 |

Annotiations from RegulomeDB showing chromatin states in regions of DNA containing significant ABCC8 and TRPM4 SNPs (200 bp bin region in the genome). The vertical red line separates chromatin states in brain tissue (left) from all-tissue samples (right). The horizontal black line separates ABCC8 SNPs (A: rs8192695, B: rs3819521, C: rs2237982, D: rs2283261) from TRPM4 SNPs (E: rs3760666, F: rs909010, G: rs1477363, H: rs10410857). The number of samples with strong transcription (green),
enhancers (yellow), heterochromatin (lavendar), repressed polycomb (dark gray) and quiescent/low transcription (light gray) is shown for each SNP in brain vs all-tissue. For each SNP, the ratio (R) of strong transcription and enhancers to repressed polycomb, quiescent states, and heterochromatin is shown. For example, for all ABCC8 SNPs, R is markedly higher in brain tissue vs other tissue, with most regional samples demonstrating strong transcriptional activity. This difference was not as pronounced with TRPM4 which was annotated to have strong transcription (green) in all tissues.
eFigure 6. Linkage Disequilibrium Maps for ABCC8 and TRPM4 SNPs Regional Loci

Linkage disequilibrium (LD) plots from the Ensembl genome browser of significant ABCC8 (A: rs2237982, B: rs2283261, C: rs3819521, D: rs8192695) and TRPM4 (E: rs3760666, F: rs1477363, and G: rs10410857) SNPs. Zoomed in chromosomal coordinates are shown for each SNP at the base of each image (x-axis), with corresponding locations of exons marked by an orange box on the top of each image. The y-axis is the $r^2$ value quantifying the extent (correlation coefficient) of LD. The threshold for perfect LD ($r^2 = 1.0$) is marked by a blue dotted horizontal line and LD ($r^2 = 0.8$) is marked by a red dotted horizontal line on each subplot. Proxy SNPs based on location and LD are shown by dots. The variant key is provided: 3 prime UTR variants (teal), missense variants (yellow), splice region variants (red), synonymous variants (green), intron variants (dark blue).
1. Jha RM, Puccio AM, Okonkwo DO, et al. (2017) ABCC8 Single Nucleotide Polymorphisms are Associated with Cerebral Edema in Severe TBI. Neurocrit Care 26:213–224. doi: 10.1007/s12028-016-0309-z

2. Jha RM, Koleck TA, Puccio AM, et al. (2018) Regionally clustered ABCC8 polymorphisms in a prospective cohort predict cerebral oedema and outcome in severe traumatic brain injury. J Neurol Neurosurg Psychiatry 89:1152–1162. doi: 10.1136/jnnp-2017-317741

3. Jha RM, Desai SM, Zusman BE, et al. (2019) Downstream TRPM4 Polymorphisms Are Associated with Intracranial Hypertension and Statistically Interact with ABCC8 Polymorphisms in a Prospective Cohort of Severe Traumatic Brain Injury. J Neurotrauma 36:1804–1817. doi: 10.1089/neu.2018.6124

4. Jha RM, Elmer J, Zusman BE, et al. (2018) Intracranial pressure trajectories: A novel approach to informing severe traumatic brain injury phenotypes. Crit Care Med 46:1792–1802. doi: 10.1097/CCM.0000000000003361

5. Kothari RU, Brott T, Broderick JP, et al. (1996) The ABCs of measuring intracerebral hemorrhage volumes. Stroke 27:1304–1305. doi: 10.1161/01.STR.27.8.1304

6. Adatia K, Newcombe VFJ, Menon DK (2020) Contusion progression following traumatic brain injury: A review of clinical and radiological predictors, and influence on outcome. Neurocrit Care. doi: 10.1007/s12028-020-00994-4

7. GTEx Consortium, Laboratory, Data Analysis & Coordinating Center (LDACC)—Analysis Working Group, Statistical Methods groups—Analysis Working Group, et al. (2017) Genetic effects on gene expression across human tissues. Nature 550:204–213. doi: 10.1038/nature24277

8. Ward LD, Kellis M (2016) HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. Nucleic Acids Res 44:D877-81. doi: 10.1093/nar/gkx1340

9. Ward LD, Kellis M (2012) HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res 40:D930-4. doi: 10.1093/nar/gkr917

10. Boyle AP, Hong EL, Hariharan M, et al. (2012) Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 22:1790–1797. doi: 10.1101/gr.137323.112

11. Yates AD, Achuthan P, Akanni W, et al. (2020) Ensembl 2020. Nucleic Acids Res 48:D682–D688. doi: 10.1093/nar/gkz966

12. Autzen HE, Myasnikov AG, Campbell MG, et al. (2018) Structure of the human TRPM4 ion channel in a lipid nanodisc. Science 359:228–232. doi: 10.1126/science.aar4510

13. Berman HM, Westbrook J, Feng Z, et al. (2000) The protein data bank. Nucleic Acids Res 28:235–242. doi: 10.1093/nar/28.1.235

© 2021 Jha RM et al. JAMA Network Open.
14. Li N, Wu J-X, Ding D, et al. (2017) Structure of a Pancreatic ATP-Sensitive Potassium Channel. Cell 168:101–110.e10. doi: 10.1016/j.cell.2016.12.028

15. Martin GM, Yoshioka C, Rex EA, et al. (2017) Cryo-EM structure of the ATP-sensitive potassium channel illuminates mechanisms of assembly and gating. Elife. doi: 10.7554/eLife.24149

16. Pettersen EF, Goddard TD, Huang CC, et al. (2004) UCSF Chimera—a visualization system for exploratory research and analysis. J Comput Chem 25:1605–1612. doi: 10.1002/jcc.20084