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Decreased DNA Methylation of *RGMA* is Associated with Intracranial Hypertension After Severe Traumatic Brain Injury: An Exploratory Epigenome-Wide Association Study

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Author contributions

RMJ and YPC are the principal investigators of the cerebral edema and epigenome-wide association studies of TBI, respectively. RMJ, YPC, DEW, JRS, and DL conceived and designed the study. BEZ, SMD, AMP, and DOO collected the phenotype data. DL, YL, AIA, and SL performed the statistical analysis and contributed to the initial writing of the manuscript. All authors reviewed, edited, and approved the final manuscript.

Conflicts of Interest

RMJ is a paid consultant and is on advisory boards for Biogen and ASTRAL (Antagonizing SUR1-TRPM4 to Reduce the Progression of Intracerebral Hematoma and Edema Surrounding Lesions clinical trial). The other authors declare no conflicts of interest.

Ethical approval/informed consent

The University of Pittsburgh Institutional Review Board approved this study.

Supplementary Information

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Abstract

Background: Cerebral edema and intracranial hypertension are major contributors to unfavorable prognosis in traumatic brain injury (TBI). Local epigenetic changes, particularly in DNA methylation, may influence gene expression and thus host response/secondary injury after TBI. It remains unknown whether DNA methylation in the central nervous system is associated with cerebral edema severity or intracranial hypertension post TBI. We sought to identify epigenome-wide DNA methylation patterns associated with these forms of secondary injury after TBI.

Methods: We obtained genome-wide DNA methylation profiles of DNA extracted from ventricular cerebrospinal fluid samples at three different postinjury time points from a prospective cohort of patients with severe TBI (n = 89 patients, 254 samples). Cerebral edema and intracranial pressure (ICP) measures were clustered to generate composite end points of cerebral edema and ICP severity. We performed an unbiased epigenome-wide association study (EWAS) to test associations between DNA methylation at 419,895 cytosine–phosphate–guanine (CpG) sites and cerebral edema/ICP severity categories. Given inflated p values, we conducted permutation tests for top CpG sites to filter out potential false discoveries.

Results: Our data-driven hierarchical clustering across six cerebral edema and ICP measures identified two groups differing significantly in ICP based on the EWAS-identified CpG site cg22111818 in *RGMA* (Repulsive guidance molecule A, permutation $p = 4.20 \times 10^{-8}$). At 3–4 days post TBI, patients with severe intracranial hypertension had significantly lower levels of methylation at cg22111818.

Conclusions: We report a novel potential relationship between intracranial hypertension after TBI and an acute, nonsustained reduction in DNA methylation at cg22111818 in the *RGMA* gene. To our knowledge, this is the largest EWAS in severe TBI. Our findings are further strengthened by previous findings that *RGMA* modulates axonal repair in other central nervous system disorders, but a role in intracranial hypertension or TBI has not been previously identified. Additional work is warranted to validate and extend these findings, including assessment of its possible role in risk stratification, identification of novel druggable targets, and ultimately our ability to personalize therapy in TBI.

Keywords

Traumatic brain injury; Intracranial hypertension; Epigenome-wide association study (EWAS); DNA methylation; Repulsive guidance molecule A (*RGMA*)

Introduction

Traumatic brain injury (TBI) affects 1.7 million people annually in the United States and causes 30% of all injury-related deaths [1]. Two distinct but related secondary complications, cerebral edema and intracranial hypertension, contribute strongly to unfavorable prognosis after TBI and remain important causes of morbidity and mortality in intensive care units worldwide [2, 3]. Current therapies, such as hyperosmolar therapy, neuromuscular blockade, hypothermia, cerebrospinal fluid (CSF) drainage, deep sedation with general anesthesia, and decompressive craniectomy, are reactive, invasive, and associated with significant side effects. Understanding the molecular underpinnings of cerebral edema and increases in intracranial pressure (ICP) could inform the subsequent development of molecularly targeted therapies; this is an unmet need. Studies have shown that genetic variation in relevant molecular pathways are associated with cerebral edema [4–8]. However, gene expression is not only determined by genetic variability but may also be influenced by DNA methylation levels.

DNA methylation is a dynamic epigenetic mechanism of regulating gene expression responsive to both internal or external stimuli [9]. Unlike fixed polymorphisms in DNA sequences, methylation status may change with time and local microenvironments. Brain injury may disturb otherwise stable DNA methylation status in specific and relevant genes, potentially affecting their transcriptional status and subsequently modifying the patient's downstream response to injury. Few studies have examined DNA methylation patterns in TBI; most are in animal models [10–12], two evaluated human peripheral serum (N = 12 and 15, respectively) [13, 14], and one evaluated human brain tissue methylation (N = 17 patients with TBI vs. 19 controls) [15]. Overall, these studies support a role for DNA methylation in pathogenesis, response to therapy, and neurodegeneration in TBI [16–18]. However, these studies are limited beyond just sample size: given the importance of regional differences in methylation patterns, studies of serum samples may not reflect the locally injured microenvironment, and tissue samples, although highly specific to regional differences, are not routinely available to guide clinical management because these are typically obtained after a biopsy or during an autopsy.

We performed an epigenome-wide study evaluating the association between longitudinal methylation patterns in ventricular CSF and intracranial hypertension or cerebral edema after severe TBI in 120 patients. To our knowledge, changes in methylation status associated with the development of post-TBI cerebral edema or ICP have not been reported in humans. Identifying differentially methylated cytosine–phosphate–guanine (CpG) sites may not only provide mechanistic insight into these secondary injuries but also identify biomarkers for risk stratification in patients with TBI, facilitating personalized clinical care.

Methods

Patient Cohort

In this study, we enrolled patients aged 16–80 with severe TBI [Glasgow Coma Scale (GCS) score of 3–8] with closed head injuries, who were admitted to a single level 1 trauma center and had an external ventricular drain (EVD) placed as standard of care. Pregnant and

incarcerated patients were excluded. Proxy informed consent was obtained from patients' healthcare proxies, and patients were reconsented following recovery when possible. The University of Pittsburgh Institutional Review Board approved this study.

DNA Methylation

Daily CSF samples were collected over the first 5–6 days post injury via EVD. The CSF samples were processed and the cellular components were used for DNA extraction. DNA was extracted by using the Qiamp extraction kit and protocol (Qiagen Inc., Frederick, MD). For this study, CSF samples from days 1, 3, and 5 post admission were used for DNA methylation data collection. Whenever CSF samples could not be collected on the target day, samples collected on the subsequent day (days 2, 4, and 6) were substituted. We obtained a total of 368 samples (3 samples per patient collected at days 1–2, days 3–4, and days 5–6 plus 8 technical replicates). Methylation data were obtained by Illumina HumanMethylation450k bead chips at 485,512 CpG sites. We implemented a series of quality control procedures on the methylation data. At the probe level, the data were first subjected to functional normalization to remove batch effects and technical artifacts by using the funtooNorm R package [19]. Next, cross-reactive, single-nucleotide polymorphism overlapping, sex-chromosomal, and low-quality probes were detected and removed by using the minfi [20] and ENmix [21] R packages. The number of probes removed at each step can be found in Table S1. At the sample level, 17 (4.6%) of 368 samples were identified as low-quality or outlier samples and were removed. After quality control, a total of 419,895 CpG sites and 351 samples were retained, and we obtained methylation M values based on the formula $M = \log_2\left(\frac{\text{Beta}}{1 - \text{Beta}}\right)$. As described in a later section, samples from patients who did not have complete cerebral edema and ICP measurements were further removed, leaving 254 samples used for statistical analysis.

Cerebral Edema and ICP Measurement

The first noncontrast computed tomography (CT) scan of the head obtained at our institution was assessed by trained research staff for signs of cerebral edema, including (1) midline shift (mm), (2) basal cistern compression, (3) loss of gray–white differentiation, (4) ventricular effacement, and (5) herniation. A patient with any of these signs was recorded as having evidence of cerebral edema on the admission head CT scan, and an ordinal scale (0–5) in which patients received one point for the presence of each characteristic was created as a metric of edema severity. Hourly ICP measurements were recorded via closed EVD over the first 120 h from admission or until invasive monitoring was removed.

ICP Level Clustering

We sought to combine cerebral edema and ICP measures into a single composite end point for each patient. This reduction of dimensionality conferred two major advantages: increased power by combing multiple measurements and a reduced likelihood of type I error with fewer comparisons. Six measurements were used for this purpose, including (1) the degree of midline shift (mm), (2) the number of signs of cerebral edema (presence of midline shift, basal cistern effacement, sulcal effacement, ventricular effacement, and herniation), (3) the mean ICP, (4) the peak ICP, (5) the proportion of ICP spikes greater than 20 mm Hg, and

(6) the proportion of ICP spikes greater than 25 mm Hg. The last two measurements were included simultaneously to capture different intracranial hypertension phenotypes—patients with ICP levels frequently in the range of 20–25 mm Hg may have an ICP burden similar to patients with intermittent spikes > 25 mm Hg but may have a distinct pathophysiology.

Without a priori knowledge of which cerebral edema/ICP measurement(s) are most informative in classifying patients, we implemented an unbiased data-driven approach to create a composite end point, i.e., we assigned patients into clusters of different degrees of cerebral edema/ICP severity. After we removed patients with missing data ($n = 31$), the above six measurements were standardized and hierarchical clustering was performed by using R version 3.5.2 (R Project for Statistical Computing, Vienna, Austria) [22]. The optimal number of clusters was determined on the basis of the average silhouette width, a measure of how similar an object is to its own cluster compared with other clusters. Silhouette width was maximal when patients were divided into two clusters (Fig. S1). The two clusters that differed significantly in ICP, but not cerebral edema indices, are hereafter referred to as high vs. low ICP severity clusters.

Statistical Analysis

Descriptive statistics of patient characteristics were reported as means ± standard deviations or counts and percentages. We conducted a cross-sectional epigenome-wide association study (EWAS) of the composite ICP severity cluster, evaluating three time points separately in linear regression models with empirical Bayes moderation using the limma R package [23]. Covariates included age, sex, initial GCS score, and surrogate variables computed from the whole-genome methylation data by the sva R package [24]. One common confounder in EWAS is cell type heterogeneity, which stems from the distinct epigenetic profiles of different cell types and differential cell type compositions of different samples. We accounted for this potential confounding by adjusting for surrogate variables, which account for any uncontrolled sources of variation, including cell type heterogeneity and batch effects; this also decreases the risk of confounding-related type 1 error, which was particularly important given our small sample size [24]. The widely used epigenome-wide significance threshold for the Illumina HumanMethylation450k chip is 2.4×10^{-7} [25], and even though the three time points are not completely independent, we used a more stringent study-wide threshold of $p < 2.4 \times 10^{-7}/3 = 8 \times 10^{-8}$ to account for these three time points (using the Bonferroni method); this conservative approach was used to further minimize risk of a type 1 error. Top CpG sites were further tested for association with each of the six individual measurements while adjusting for age, sex, initial GCS score, and surrogate variables. Because quantile–quantile (Q–Q) plots of the epigenome-wide p values revealed global inflation (Results), we obtained empirical p values for the CpG sites, which surpassed our EWAS threshold by randomly permuting the cluster membership. This permutation step is important and effective for reducing the type I error rate [26]. Given the large number of iterations needed (maximally 1×10^9 times) and the need to recompute surrogate variables for each iteration, which was computationally prohibitive, we used an alternative approach to account for cell type heterogeneity in the permutation analysis. Specifically, we used a standard reference-free approach implemented in the RefFreeEWAS R package [27] to estimate the cell type proportions for each sample and included the cell type proportions as

a set of fixed covariates in all iterations of the permutation analysis, in addition to age, sex, and initial GCS score.

Results

Demographic and clinical characteristics are summarized in Table 1. The patient population had a mean age of 40.3 ± 17.3 years, and women accounted for 20% of the cohort. Most patients (89%) were White, with 9% Black and 2% Asian. Approximately two thirds of the patients had a GCS score of 6–8. The plurality of patients had either subdural hemorrhage (36%) or intraparenchymal hemorrhage/contusion (31.5%) as their primary injury pattern. Injuries were predominantly either frontal (33.7%) or diffuse (27%), with < 5% involving the brainstem or cerebellum.

ICP Severity Clustering

Among the 120 patients with TBI, 31 were missing at least one ICP or cerebral edema measure necessary for clustering. The hierarchical clustering of the remaining 89 patients identified two clusters categorizing ICP severity: high ($n = 10$) vs. low ($n = 79$) (Table 1, Fig. 1). Patients in the high ICP group had significantly higher average values for all ICP measurements. For example, the group means of mean ICP and peak ICP in the high vs. low group were 21.0 vs. 10.9 mm Hg and 37.2 vs. 25.8 mm Hg, and the high group had on average 30% of the hourly ICP being greater than 25 mm Hg, whereas that percentage for the low group was only 1.7%. Distributions of age, sex, race, and initial GCS score were similar between the two groups; however, the limited sample size (particularly of the high group) precluded meaningful comparisons of these important characteristics. Patients in the high ICP group were more likely to have an unfavorable functional recovery and higher disability score at 3 and 12 months post injury, although the differences were not statistically significant.

Lower *RGMA* Methylation is Associated with Intracranial Hypertension

For the analysis of day 1–2, day 3–4, and day 5–6 data, the numbers of patients with complete data in the low vs. high group were 62 vs. 6, 64 vs. 8, and 58 vs. 7, respectively. Accounting for cell type heterogeneity, we still observed p value inflation for the EWAS of days 3–4 (genomic inflation factor $\lambda = 1.14$), but not for days 1–2 ($\lambda = 1.03$) and days 5–6 ($\lambda = 0.89$), as shown in the Q–Q plots (Figs. 2, S2).

Four CpG sites passed the study-wide significance threshold (Table 2), all of which were from the analysis of day 3–4 data. The top signal, cg22111818 *RGMA*, had an association p value of 1.78×10^{-11} and a Bayes factor of 12.7, and its methylation level was on average 50% lower in the high ICP group compared with the low ICP group. We recalculated the association p value of these CpG sites using permutation to address and minimize the impact of global inflation on estimates and likelihood of type 1 error. This CpG remained significant in the permutation test (empirical $p = 4.20 \times 10^{-8}$). Among the six measurements used for clustering, cg22111818 showed the strongest association with the proportion of ICP spikes greater than 25 mm Hg ($p = 5.31 \times 10^{-7}$; Table 3), although this did not meet the stringent genome-wide significance threshold. Patients in the high ICP group had a

large decrease in cg22111818 methylation levels at day 2 and a partial recovery at day 5, resulting in a significant difference in methylation status only on days 3–4 (Fig. 3). The other three CpG sites were not significantly associated with intracranial hypertension in the permutation. As a sensitivity analysis, we repeated the EWAS using the RefFreeEWAS approach [27], which is a more direct method to control for cell type heterogeneity than surrogate variable analysis. The inflation and the EWAS results remained largely unaffected (Fig. S4, Table S2).

Sensitivity Analysis Confirms *RGMA* Association with Intracranial Hypertension

In the data-driven clustering analysis, the individual variables important in differentiating patient subgroups were not known a priori. As shown in Table 1, the two identified clusters did not differ in the two cerebral edema measures. We therefore performed a post hoc sensitivity analysis using three of the original six variables (mean ICP, peak ICP, and proportion of ICP spikes greater than 25 mm Hg) to recluster the patient cohort and examine the *RGMA* association using the same approach as in the primary analysis. The patient composition of the new clusters was similar to that of the original cluster: all 89 patients stayed in their original clusters. The only change was that 2 and 10 additional patients were assigned to the new high and low clusters, respectively, whereas they had been excluded from the original analysis because of missing CT data necessary for clustering. The detailed association results are shown in Figs. S5, S6 and Table S3. The *RGMA* association at days 3–4 was robust to different versions of the composite end point tested (original permutation $p = 4.2 \times 10^{-8}$, sensitivity analysis permutation $p = 5.0 \times 10_{-9}$).

cg22111818 Methylation and Gene Expression

We queried a public data set containing matched methylation and messenger RNA data and found that there was an inverse correlation between cg22111818 and *RGMA* expression in several tissues (TCGA Wanderer data set, http://maplab.imppc.org/wanderer/) (Fig. S3), although no samples of brain tissue were available.

Discussion

In this EWAS, we report a novel association between lower mean CSF *RGMA* methylation levels and intracranial hypertension after severe TBI. To our knowledge, this is the largest EWAS in human TBI and is further strengthened by using ventricular CSF rather than serum samples. The role of *RGMA* methylation has not previously been reported in TBI and could potentially inform the development of biomarkers for risk stratification. Furthermore, it may ultimately represent a target/pathway for therapeutic intervention: future multicenter and larger clinical studies should be performed to validate these findings, and preclinical studies in TBI models are warranted to explore the potential molecular link between *RGMA* and intracranial hypertension.

RGMA encodes a glycosylphosphatidylinositolanchored glycoprotein expressed predominantly in the developing and adult central nervous system (CNS) [28]. Existing evidence supports a role of *RGMA* in various biological processes in the CNS. The protein acts as a repulsive guidance cue directing axonal growth in developing neurons [29]. It

also has important roles in neural tube closure, neuronal differentiation, and axon survival and regeneration [30–32]. It has recently been reported to have increased expression in immune-cell-related neuroinflammation and neurodegeneration in experimental autoimmune encephalomyelitis (specifically in pathogenic T helper 17 cells) [33]. In vitro and in vivo experiments have demonstrated a role for RGMA in traumatic CNS injuries [34] and other neurological disorders [35–39]. *RGMA* was reported to be markedly upregulated in multiple cell types in the traumatically injured rat and human spinal cords [40], and in perilesional tissue after TBI in humans [34]. Anti-RGMA antibodies were shown to promote repair of the damaged spinal cord and relieve neuropathic pain after spinal cord injury [40, 41]. These results are consistent with our findings that a decreased *RGMA* methylation level was associated with more severe ICP based on the general negative relationship between DNA methylation and gene expression; however, currently the biological systems implicated by RGMA in severe TBI (and intracranial hypertension) and the molecular mechanisms by which *RGMA* expression influences ICP remain unknown and important to explore in future research. Speculatively, enhancing neuroinflammation is one possible mechanism by which RGMA is related to cerebral edema and intracranial hypertension. Future studies investigating how the methylation status of cg22111818 could regulate *RGMA* expression during ICP elevation may yield valuable insights into molecular mechanisms of injury and pathophysiological responses to it.

It is also important to note that *RGMA* may not be the only site relevant to methylation changes in cg22111818. CHD2, a chromatin remodeler in the chromodomain helicase DNA binding protein family, is adjacent in the same topological domain. This gene has been shown to be relevant, indeed occasionally causative, in several CNS disease pathologies, such as early onset encephalopathy and intellectual disability [42]. Currently, this possibility remains speculative and requires validation in future studies and biological models.

Interestingly, in our study, CT findings did not match the ICP measures in the high (10 patients) vs. low (79 patients) ICP clusters. This CT–ICP dissociation is not surprising given the complex and nonlinear relationship between cerebral edema and ICP, particularly in TBI [43]. Although related via the Monro–Kellie doctrine, autoregulation, intracranial compliance, and elastance, ICP levels do not reflect the same degree of edema generation across patients—another important reason for personalized medicine. In our study, this dissociation was likely further confounded by the low number of high ICP patient samples as well as the specific CT measures used (midline shift and number of signs of edema). As seen in Table 1, the high ICP group did have higher values for both CT measures vs. the ‘low’ group; however, we were limited in power to declare statistical significance.

This study has several strengths. Ventricular CSF may be a more relevant source than blood for understanding the methylomic changes that occur within the CNS post TBI because it directly bathes the brain parenchyma and circulates proximally to the injury location. Although our study contained only 89 patients, it is currently the largest human EWAS reported in TBI. Additionally, by reducing the dimensionality of the outcomes of interest from six quantitative measurements to a single binary categorical variable, we gained statistical power in the EWAS.

However, our results should also be interpreted in light of several limitations. First, no causality has been established. The changes in *RGMA* methylation may simply be an epiphenomenon related to intracranial hypertension, with no role in either pathophysiology or protective response. Second, relatively infrequent sampling hindered a precise characterization of temporality in the relationship between *RGMA* methylation and ICP changes. Similarly, grouping samples collected within a 2-day period might have introduced some level of heterogeneity. Further research is needed to identify the utility of *RGMA* as a therapeutic target, particularly because DNA methylation is dynamic and encodes longer-term change. A focus on identifying specific cell types involved will also be valuable. Thus, future multicenter studies with larger carefully characterized patient cohorts will enable deeper interrogations of the trajectories of methylation changes and how they may predict the progression of intracranial hypertension and other complications in patients with TBI. Furthermore, mechanistic bench research manipulating this site (removing/artificially activating) would valuably inform its utility as a biomarker and/or biological target as well as elucidate underlying pathobiological mechanisms by which this gene is related to ICP.

Array-based technology is inherently limited vs. sequencing in terms of missing potentially important data; future studies sequencing specific RGMA loci in independent samples would be informative. Use of the Bonferroni procedure to account for the three cross-sectional EWASs possibly led to a conservative analysis because methylation levels across time points are not expected to be independent. We also observed inflation in the EWAS *p* values. Typical reasons for inflation include small sample sizes, an imbalanced case/control ratio, and uncontrolled confounding. The use of surrogate variable analysis protected our analysis from being confounded by unmeasured sources of variation. The effectiveness of this approach in controlling for cell type heterogeneity was supported by a sensitivity analysis in which we compared current results with the results from the RefFreeEWAS approach [27], which is specifically for cell proportion estimation. The two methods gave the same level of inflation and similar *p* values for the top CpG sites (Fig. S4, Table S2). Furthermore, we handled the EWAS inflation using a permutation test, and therefore the *RGMA* association was less likely to be affected by this *p* value inflation.

Therapeutic intensity levels were not available for our cohort. At our institution, there is a standardized/protocolized approach to stepwise ICP management in severe TBI that limits interpatient variability. This includes details about patient positioning, euthermia, sedation, and hyperosmolar therapy, with third tier approaches including paralysis and decompressive craniectomy. Antiepileptic regimens are also standardized. Despite this protocolized approach, differences in ICP-lowering therapies and antiepileptic agents/incidence of seizures can impact the measured ICP and therefore confound our results. As noted above, to control for unknown (or unmeasured) sources of variation, including clinical heterogeneity, we employed the statistical technique of surrogate variable analysis, which is thought to be superior to directly adjusting individual covariates because it also accounts for unmeasured variability [24]. Furthermore, our results were robust to heterogeneity in the sensitivity analyses.

Despite being the largest TBI EWAS, the sample size was relatively small; this limited the power of our study and our ability to adjust for additional covariates, raising the possibility of residual confounding. This is particularly important in a disease such as severe TBI that is highly heterogeneous; indeed, different forms of primary injury, as well as different pathophysiological mechanisms that lead to cerebral edema and intracranial hypertension (e.g., direct compression, mass effect, cytotoxic edema, vascular injury/hyperemia), may differentially impact DNA methylation. Although it is not possible to individually adjust for all potential confounders especially in a cohort of this size, as outlined above, we accounted for these unmeasured/unknown potential confounders using surrogate variable analysis. The small size of the high group further added to the imbalanced case/control ratio, which was likely a contributing factor to the statistical inflation we observed. However, the finding of *RGMA* was robust to adjustment for cell type heterogeneity and known confounders, and it survived permutation testing. Validation in independent cohorts is needed to confirm our findings. Another effect of a small sample size is the potential impact on reduction of power and increase in risk of type 1 error; we implemented several statistical measures to minimize this risk (stringent p value threshold, Bonferroni corrections for both CpG islands and multiple time points, permutation analyses); nonetheless, the risk cannot be entirely eliminated and needs to be acknowledged. A repeated measures design evaluating the change in expression of *RGMA* as a biomarker was not performed in the current exploratory study because there was no a priori knowledge of this gene's role in severe TBI and/or ICP; however, this would be valuable to explore in future work. Additionally, an independent validation study is needed in a separate, preferably multicenter, cohort.

Finally, our study was limited to patients with severe TBI. Although this allowed us to use ventricular CSF, which is drained as part of standard care, it precluded our ability to determine whether *RGMA* plays a role in the development of more subtle cerebral edema and/or axonal swelling, which can be seen in less severe cases across the important TBI injury spectrum.

Conclusions

In an EWAS of 89 patients with severe TBI, we report a novel association between decreased *RGMA* methylation and intracranial hypertension. This association warrants further evaluation in larger cohorts. The potential role of *RGMA* in the development of intracranial hypertension also merits exploration in preclinical TBI models. If validated, this finding could play a role in patient risk stratification as well as the identification of new targeted therapies to advance precision medicine management in TBI care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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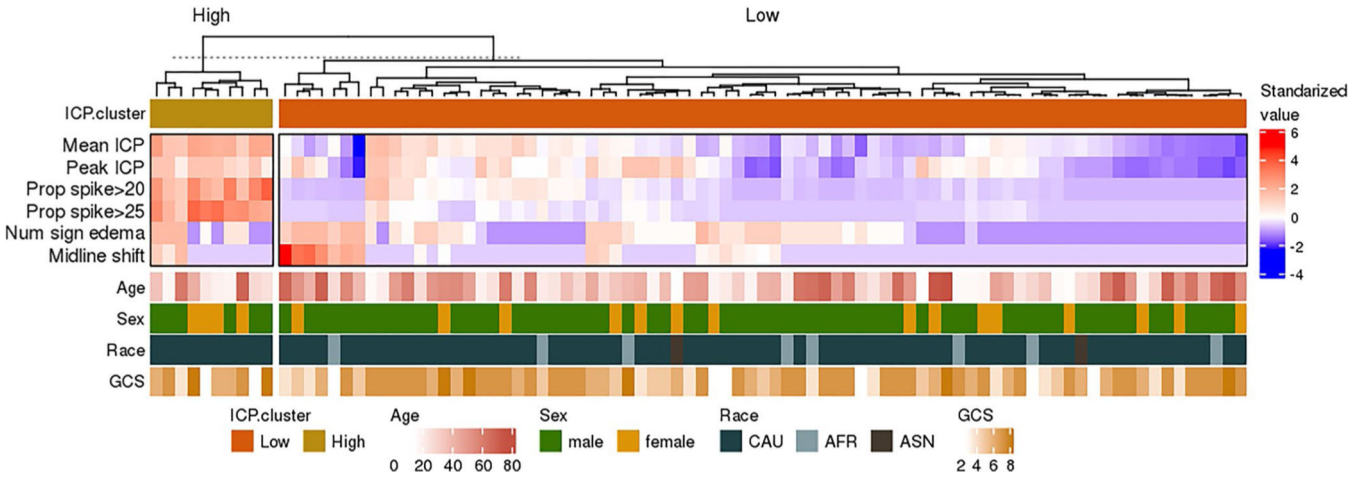

Fig. 1.

Clustering of cerebral edema and ICP measurements. The top dendrogram shows the high ICP (left) and the low ICP (rights) clusters.Eighty-nine patients are laid out horizontally, and rows represent different characteristics. Middle heat maps with a blue–red color scale show the standardized value of the six individual measurements for each patient. Age, sex, race, and initial GCS score were annotated below the heat maps. *AFR* African, *ASN* Asian, *CAU* Caucasian, *GSC* Glasgow coma scale, *ICP* Intracranial pressure

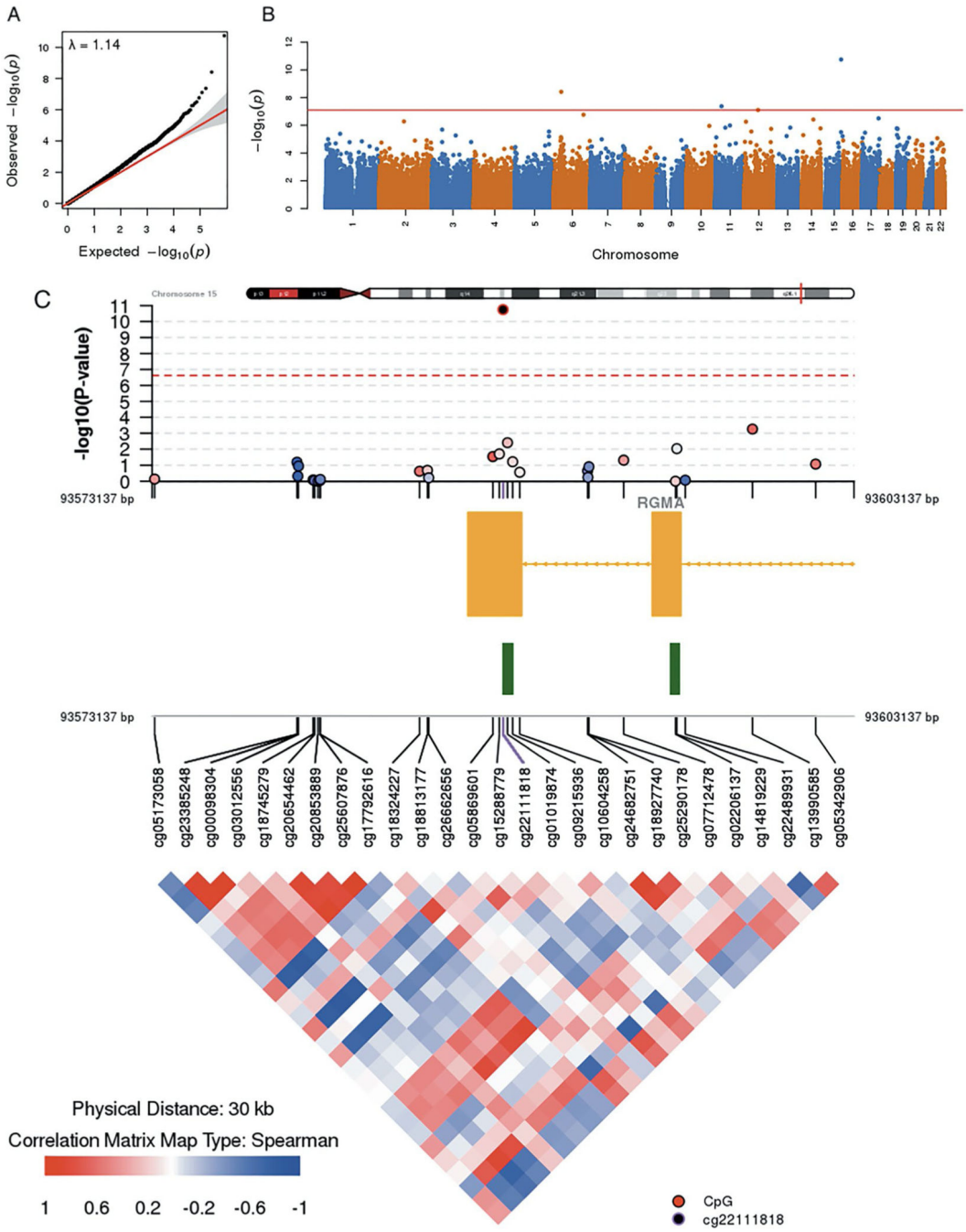

Fig. 2.

EWAS of the composite ICP level cluster at days 3–4. **a** Q–Q plot. **b** Manhattan plot. **c** CoMet plot for the *RGMA* locus. The upper panel shows the EWAS − log10(*p* value); the yellow track shows the gene information, and the thick yellow blocks represent exons; the green blocks are CpG islands; the bottom panel is a heat map of the methylation correlation pattern. *CpG* Cytosine–phosphate–guanine, *EWAS* Epigenome-wide association study, *ICP* Intracranial pressure, *Q–Q* Quantile–quantile

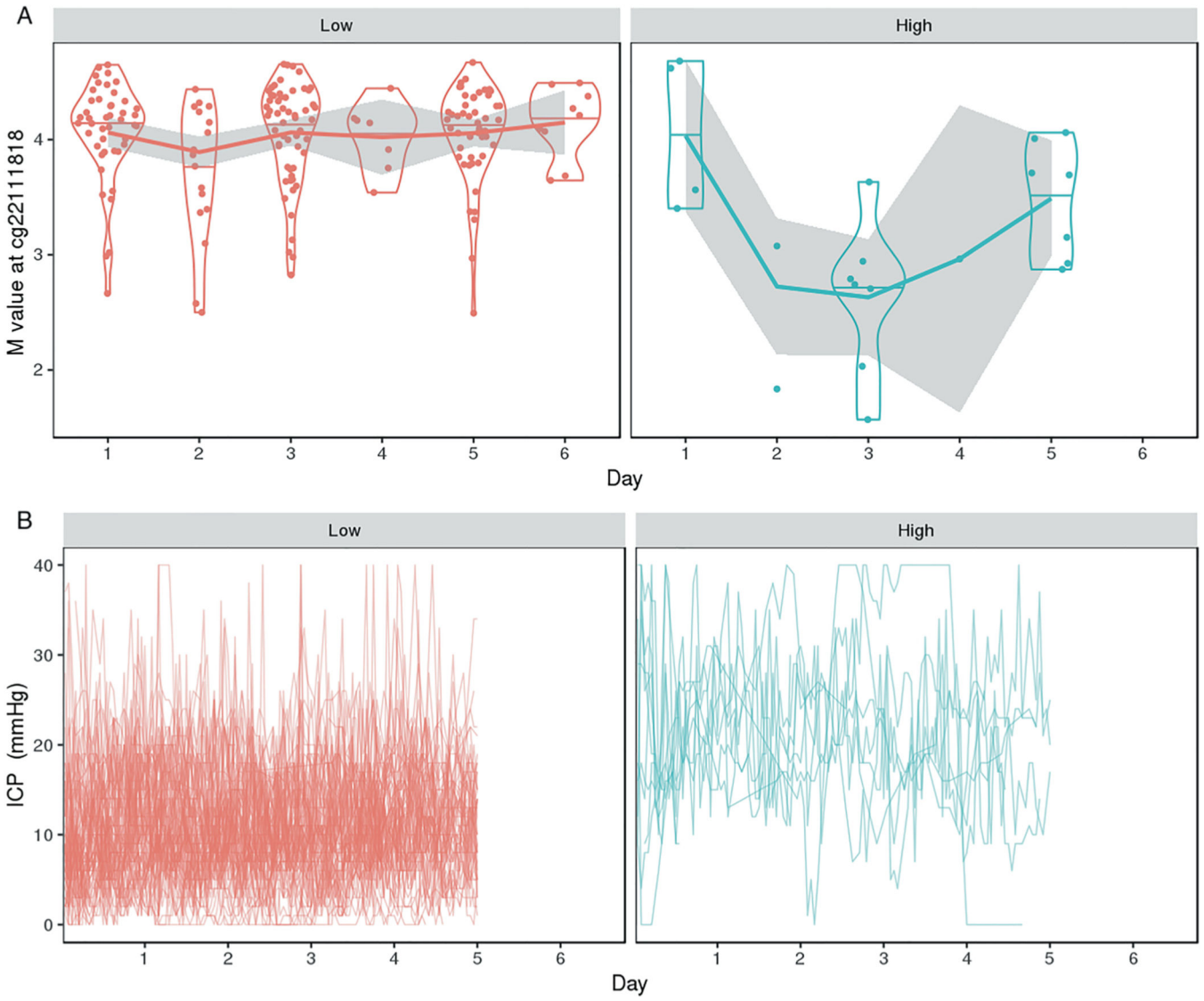

Fig. 3.

Trajectory of cg22111818 methylation level (**a**) and trajectory of ICP (**b**) in the high (left, *n* = 64) and the low (right, *n* = 8) ICP level clusters. **a** Violin plots are drawn for cg22111818 methylation with an increment of 1 day, and the horizontal lines in the violin plot denote the median. No violins are drawn at days 2, 4, and 6 in the high group because of small numbers of observations. **b** Hourly ICP trajectory lines are drawn for each patient up to 120 h (day 5) post hospitalization. *ICP* Intracranial pressure

Table 1

Characteristics of the 89 patients with TBI analyzed in EWAS, overall and split by the composite ICP group membership

	Overall (*N* = 89)	By ICP level		
		High (*n* = 10)	Low (*n* = 79)	*p* value[a]
Age (years)	40.3 ± 17.3	34.7 ± 17.5	41.0 ± 17.3	0.29
Sex (female)	19 (21.3%)	4 (40%)	15 (19.0%)	0.26
Race				0.49
White	79 (88.8%)	10 (100%)	69 (87.3%)	
Black	8 (9.0%)	0 (0%)	8 (10.1%)	
Asian	2 (2.2%)	0 (0%)	2 (2.5%)	
Initial GCS score				0.26
3	8 (9.0%)	2 (20.0%)	6 (7.6%)	
4	11 (12.3%)	1 (10.0%)	10 (12.7%)	
5	9 (10.1%)	0 (0%)	9 (11.4%)	
6	18 (20.2%)	3 (30.0%)	15 (19.0%)	
7	36 (40.4%)	2 (20.0%)	34 (43.0%)	
8	7 (7.9%)	2 (20.0%)	5 (6.3%)	
Craniotomy				1.00
Yes	12 (13.5%)	1 (10%)	11 (13.9%)	
No	77 (86.5%)	9 (90%)	68 (86.1%)	
Craniectomy				0.33
Yes	28 (31.5%)	5 (50%)	23 (29.1%)	
No	61 (68.5%)	5 (50%)	56 (70.9%)	
Primary injury pattern				0.59
DAI	10 (11.2%)	0 (0%)	10 (12.7%)	
EDH	5 (5.6%)	1 (10%)	4 (5.1%)	
IPH/contusion	28 (31.5%)	3 (30%)	25 (31.6%)	
IVH	1 (1.1%)	0 (0%)	1 (1.3%)	
SAH	13 (14.6%)	3 (30%)	10 (12.7%)	
SDH	32 (36.0%)	3 (30%)	29 (36.7%)	

	Overall (*N* = 89)	By ICP level		
		High (*n* = 10)	Low (*n* = 79)	*p* value[a]
Primary injury location				0.08
Brainstem	2 (2.2%)	0 (0%)	2 (2.5%)	
Cerebellar	1 (1.1%)	1 (10%)	0 (0%)	
Diffuse	24 (27.0%)	1 (10%)	23 (29.1%)	
Frontal	30 (33.7%)	4 (40%)	26 (32.9%)	
Intraventricular	1 (1.1%)	0 (0%)	1 (1.3%)	
Missing	10 (11.2%)	0 (0%)	10 (12.7%)	
Parietal	6 (6.7%)	0 (0%)	6 (7.6%)	
Temporal	12 (13.5%)	3 (30%)	9 (11.4%)	
Tentorium	3 (3.4%)	1 (10%)	2 (2.5%)	
GOS[b] at 3 months				0.60
Unfavorable	70 (78.7%)	9 (90%)	61 (77.2%)	
Favorable	19 (21.3%)	1 (10%)	18 (22.8%)	
GOS at 12 months				
Unfavorable	41 (46.1%)	8 (80%)	33 (41.8%)	0.14
Favorable	36 (40.4%)	2 (20%)	34 (43.0%)	
Missing	12 (13.5%)	0 (0%)	12 (15.2%)	
DRS[c] at 3 months	14.0 ± 11.1	20.4 ± 12.0	13.2 ± 10.7	0.09
DRS at 12 months	13.2 ± 13.0	19.8 ± 12.6	12.1 ± 12.9	0.11
CE measurements				
Midline shift (cm)	2.1 ± 4.7	2.3 ± 3.9	2.0 ± 4.8	0.64
Number of signs of edema	1.8 ± 1.8	2.3 ± 2.2	1.7 ± 1.8	0.44
Mean ICP (mm Hg)	12.1 ± 4.7	21.0 ± 1.9	10.9 ± 3.6	4.80E-07
Peak ICP (mm Hg)	27.1 ± 8.8	37.2 ± 3.9	25.8 ± 8.5	1.00E-04
Proportion of ICP spikes greater than 20 mm Hg	10.8% ± 16.3%	49.0% ± 13.3%	5.9% ± 8.2%	2.99E-07
Proportion of ICP spikes greater than 25 mm Hg	4.9% ± 9.6%	30.0% ± 7.3%	1.7% ± 2.7%	9.44E-08

CE cerebral edema, *DAI* Diffuse axonal injury, *DRS* Disability rating scale, *EDH* Epidural hemorrhage, *EWAS* Epigenome-wide association study, *GOS* Glasgow outcome scale, *GSC* Glasgow coma scale, *ICP* Intracranial pressure, *IPH* Intraparenchymal hemorrhage, *IVH* Intraventricular hemorrhage, *SAH* Subarachnoid hemorrhage, *SDH* Subdural hemorrhage, *TBI* Traumatic brain injury

[a] *p* Values from comparing the two groups: χ^2 test for sex, race, initial GSC, craniotomy, craniectomy, primary injury pattern, primary injury location, and GOS; Wilcoxon rank-sum test for other variables

[b] Dichotomized as either favorable (4–5) or unfavorable (1–3)

[c] Take values from 0 to 30, and a large score indicates high disability

Table 2

Significant associations from the EWAS of the composite ICP level cluster at days 3–4

CpG	CHR:POS[a]	Gene	CpG island	Log (fold change)[b]	EWAS *p* value[c]	Bayes factor	Empirical *p* value[c]
cg22111818	15:93588137	*RGMA*	Island	− 1.00	**1.78E–11**	12.7	**4.20E–08**
cg19664267	6:33245163	*B3GALT4*	Island	0.90	**3.84E–09**	8.7	9.06E–07
cg16967296	11:27450634	*LGR4*	OpenSea	− 1.10	**4.25E–08**	6.9	5.56E–05
cg15016701	12:63211683	*PPM1H*	OpenSea	0.46	**7.87E–08**	6.4	3.05E–07

EWAS Epigenome-wide association study, *ICP* Intracranial pressure

[a] Position is based on GRCh37

[b] Log (fold change) of the methylation levels comparing the high ICP cluster with the low ICP cluster

[c] Bolded *p* values were less than the genome-wide multiple-testing corrected significance threshold of 8×10^{-8}

Table 3

Association between cg22111818 at days 3–4 and individual CE and ICP measurements

Measurement	β coefficient[a]	*p* value	Bayes factor
Mean ICP	0.99	0.21	− 6.6
Degree of midline shift	1.00	0.96	− 7.6
Number of signs of edema	0.98	0.21	− 5.9
Peak ICP	0.99	0.07	− 6.7
Proportion of ICP spikes greater than 20 mm Hg	0.52	1.00E–04	1.0
Proportion of ICP spikes greater than 25 mm Hg	0.24	5.31E–07	5.0

CE cerebral edema, *ICP* Intracranial pressure

[a]Changes in the methylation level of cg22111818 per one unit increase in the corresponding CE measures