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- Dr. Kochanek is a co-author of several US patents or provisional patents (below):

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    a. Title: Method to improve neurologic outcomes in temperature managed patients
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ABCC8 Single Nucleotide Polymorphisms are associated with Cerebral Edema in Severe TBI

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Abstract

Objective—Cerebral Edema (CE) in TBI is the consequence of multiple underlying mechanisms, and is associated with unfavorable outcomes. Genetic variability in these pathways likely explains some of the clinical heterogeneity observed in edema development. A role for Sulfonylurea-receptor-1 (Sur1) in CE is supported. However, there are no prior studies examining the effect of genetic variability in the Sur1 gene (*ABCC8*) on the development of CE. We hypothesize that *ABCC8* single nucleotide polymorphisms (SNP) are predictive of CE.

Methods—DNA was extracted from 385 patients. SNPs in *ABCC8* were genotyped using the Human Core Exome v1.2 (Illumina). CE measurements included acute CT edema, mean and peak intracranial pressure (ICP), and need for decompressive craniotomy.

Results—14 SNPs with minor-allele frequency>0.2 were identified. 4 SNPS rs2283261, rs3819521, rs2283258 and rs1799857 were associated with CE measures. In multiple regression

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models, homozygote-variant genotypes in rs2283261, rs3819521, and rs2283258 had increased odds of CT edema (OR=2.45, p=0.007; OR=2.95, p=0.025; OR=3.00, p=0.013), had higher mean (β=3.13,p=0.000; β=2.95,p=0.005; β=3.20,p=0.008) and peak (β=8.00,p=0.001; β=7.64,p=0.007; β=6.89,p=0.034) ICP. The homozygote wild-type genotype of rs1799857 had decreased odds of decompressive craniotomy (OR=0.47, p=0.004).

Conclusions—This is the first report assessing the impact of *ABCC8* genetic variability on CE development in TBI. Minor allele *ABCC8 SNP* genotypes had increased risk of CE, while major SNP alleles were protective—potentially suggesting an evolutionary advantage. These findings could guide risk stratification, treatment responders, and the development of novel targeted or gene-based therapies against CE in TBI and other neurological disorders.

Keywords

ABCC8; cerebral edema; traumatic brain injury; single nucleotide polymorphism (SNP); sulfonylurea receptor-1 (Sur1)

INTRODUCTION

Traumatic brain injury (TBI) is a heterogeneous disease. Clinical variability develops immediately from initial impact through recovery, making this population challenging to study. Non-modifiable differences include demographic factors (age, sex, race), initial impact mechanism (blunt vs penetrating, velocity, force), and type of primary injury (diffuse axonal injury, subdural vs epidural vs intraparenchymal hemorrhages). However, these factors only partially explain ensuing clinical heterogeneity with respect to extent of primary injury, secondary injury development (e.g. cerebral edema(CE), tissue hypoxia, seizures), treatment response, and outcomes. Genetic variability has been increasingly implicated in contributing to observed clinical variability after TBI[1,2].

Genetic differences in TBI have primarily been examined with regards to outcome associations including genes like *ApoE, p53, IL*[2]. However, there has been limited evaluation of pathways contributing to CE /intracranial hypertension-both pivotal pathophysiologic factors associated with mortality and unfavorable prognosis in TBI. CE occurs in > 60% of severe TBI (sTBI) patients with mass lesions and 15% of those with normal computed tomography(CT) scans on presentation[3]. The bulk of critical care in sTBI is currently dedicated towards management of intracranial hypertension and CE.

Multiple underlying pathways have been implicated in CE formation after sTBI and are potential candidates for exploration of genetic variability. These include aquaporin-4 (AQP-4), high-mobility-group-box protein-1 with toll-like-receptor 4, Na+-K(+)-2Cl(-) cotransporter and sulfonylurea receptor (Sur1)-transient receptor potential cation channel member-4 (Trpm4) [4–11]. Single nucleotide polymorphisms (SNP) in the *AQP-4* gene are associated with functional outcome, however have not been evaluated with regards to CE generation[1]. Although current therapies against CE are reactionary and non-specific (hyperosmolar treatment, neuromuscular blockade, cerebrospinal fluid (CSF) drainage, decompressive craniotomy), ongoing identification of causative pathways involved in CE has the potential to lead to the development of targeted treatment[7,10,12,13]. Genetic

polymorphisms in these pathways could alter gene expression and regulation, as well as modify protein structure and function. This may affect degree and timing of CE development as well as individual responses to specific therapies. A priori identification of a patient's risk for CE, dominance of a certain pathway, and likelihood of response to targeted therapy could have practical and important theranostic implications.

Of the known mechanisms involved in CE development, the Sur1 pathway is particularly unique. Sur1 is a sulfonylurea-receptor and transmembrane protein, encoded by *ABCC8.* Other members of the ABC-transporter family have been recognized as important mediators of solute transport at blood-brain and blood-CSF barriers, and genetic variations have been associated with outcomes after TBI[14]. Sur1 is an ideal target for investigation in central nervous system (CNS) diseases because it is not normally expressed in the brain, but is upregulated in injury[7,15]. Additionally, an existing FDA approved anti-diabetes medication (glyburide) inhibits Sur1 at non-hypoglycemic doses and has shown promise against cerebral edema in Phase 1 and 2 clinical trials[16–18]. Upregulated Sur1 by itself performs no recognized function; rather, it is a regulatory protein. It undergoes obligate association with a non-selective cation channel (Trpm4) allowing for channel opening, depolarization, water influx and oncotic edema, eventually followed by cell death.

Given the combination of Sur1's regulatory function and specific upregulation during CNS injury, genetic polymorphisms in *ABCC8* have the potential to significantly influence CNS-specific regulation and expression, CE development, and response to targeted therapy. To our knowledge, this association remains currently undefined. We undertook an exploratory candidate gene approach to determine potential associations between *ABCC8* SNPs and measures of CE. We hypothesized that coding and non-coding SNP variations in *ABCC8* would be associated with measures of CE in sTBI.

METHODS

Study Design

Subjects were prospectively enrolled through the Brain-Trauma Research Center at the University of Pittsburgh. Eligibility was determined by the presence of sTBI defined as Glasgow Coma Scale (GCS) score <9, placement of an external ventricular drain (EVD) for therapeutic CSF drainage per standard care, and age 16–80 years. Exclusion criteria were penetrating brain injury, brain death and pregnancy. 385 consecutively enrolled patients who consented to the study primarily of North-American ancestry (CEU population) between 2006–2013 were included. All subjects/health-care proxies provided informed consent including the collection of genetic material. The University of Pittsburgh Institutional Review Board approved the study.

Genotyping and SNP identification

DNA samples were obtained on admission-while the timing of DNA collection is not relevant to a research study involving SNPs (since these polymorphisms are constant per individual)[19], obtaining SNP analyses prior to the development of cerebral edema may eventually be important in clinical practice in order to serve as a useful genetic biomarker.

Blood was centrifuged and DNA extracted from white blood cells by the simple salting out method[20]. If blood was unavailable, DNA was extracted from excess CSF in the ventriculostomy bag that would have otherwise been discarded[21]. All extracted DNA was stored at 4°C in 1XTE buffer.

Genotype data was generated using Human Core Exome v1.2 from Illumina in the University of Pittsburgh Genomics Core laboratory. For quality control, blind technical duplicates were included and any discrepancies were rectified using raw data or re-genotyped. For additional quality control, samples or SNPs that did not have a minimum 95% call rate were excluded. Data for the *ABCC8* gene was abstracted using its position in genome build 37/hg19, to correspond with the build for Human Core Exome v1.2. Research assistants involved in genotyping SNPs were blinded to CE development outcomes. Pairwise linkage disequilibrium (LD) between SNPs (r^2 and D') was determined using JLIN software[22].

Data Collection

Demographic and outcome data were collected by a research assistant masked to *ABCC8* SNPs. Three outcome measures assessed development of CE.

1. Edema on initial head CT (size of ventricles, basilar cisterns; transfalcine herniation; loss of gray/white matter differentiation) is associated with raised ICP after TBI [23,24], and was included as an outcome. Determinations were made by official radiologist reports.
2. Hourly ICP - subcategorized into mean and peak ICP during the course of neuromonitoring (at our institution this is typically for 5 days).
3. Decompressive craniotomy for intracranial hypertension. These surgeries were not performed for hematoma evacuations, but were operations involving skull removal or lobectomies due to intraoperative concerns for CE. Based on our institutional protocol, decompressive craniotomy is considered when the patient has elevated ICP despite adequate sedation/analgesia, continuous CSF drainage by EVD, hyperosmolar therapy, propofol infusion to a maximum of 60 mcg/kg/minute, and neuromuscular blockade. The need for decompressive craniotomy was determined by the attending neurosurgeon responsible for the patient due to concerns for swelling. This information was gained objectively from the medical charts in the operative report: concern for cerebral edema as an indication for surgery, the decision to leave the skull flap off due to the subjective intraoperative edematous nature of the brain, or craniotomies where a lobectomy was performed due to swelling concerns.

Statistical Analysis

Descriptive statistics of baseline variables were reported as means±standard deviations. Linear regression models evaluated the independent relationship between *ABCC8* SNPs and continuous outcomes (ICP). Logistic regression models evaluated the independent relationship between *ABCC8* SNPs and categorical outcomes (CT edema, decompressive craniotomy). Odds ratios, β-coefficients, and 95% confidence intervals (CI) were calculated

based on modes of inheritance: dominant (AA vs Ab+bb), recessive (bb vs AA+Ab), heterogzygous (Ab vs AA+bb), as well as by an allele difference model (A vs b)[1,25]. Multiple variable logistic and linear regression models were developed with clinically relevant variables (age, gender, initial GCS score) to control for confounders in evaluating the independent relationship between *ABCC8* SNPs and CE development. Tests were two-tailed. Although exploratory, multiple comparisons were adjusted using the established Benjamin-Yekutieli (B-Y) method-a ***more*** conservative modification of the standard False Discovery Rate (FDR) correction used in genetics studies, since the Bonferroni method has been previously cited as inappropriately conservative for SNP evaluation[26,27]. In the B-Y method, the critical significance level is determined by: $\alpha/\sum_{i=1}^{k} 1/i$ where α = pre-correction significance level (0.05), k= number of hypotheses, and i= i_{th} comparison. Analyses were performed using Stata 14.0(StataCorp, TX).

RESULTS

Demographic and clinical characteristics are summarized in Table 1. 44% of patients had edema noted on acute CT, and 33% underwent decompressive craniotomy. Of those who underwent this surgery, 68% were performed on the day of admission, 14% occurred on day 1, and 11% occurred between days 2–5. Fourteen *ABCC8* SNPs with MAF>0.2 were identified. The *p*-value after adjusting for multiple hypotheses testing (14 SNPS, 4 outcomes) was 0.0108. Four SNPs (rs2283261, rs3819521, rs2283258 and rs1799857) were associated with measures of CE in both univariate and multivariate single locus analyses. The four SNPs were in close physical proximity (Figure 1A), and there was likely some, but not complete LD with each other (all $r^2 < 0.8$, Figure 1B–C).

rs2283261 is associated with acute CT edema, mean and peak ICP

There were 50.4% heterozygotes (AC) for rs2283261; 14% of patients were homozygous for the minor-allele (CC, Table 2). Homozygous-minor patients had higher mean and peak ICPs, and frequency of acute CT edema (Table 2). In univariate analyses (Table 3), rs2283261 homozygote-variant genotype increased odds of acute CT edema (OR 2.46, p=0.006), mean ICP (β=3.60, p <0.001) and peak ICP (β=8.29, p=0.001). Heterozygotes were protected against CT edema (OR 0.54, p=0.006), had lower peak ICP (β=−5.87, p=0.002) and tended to have lower mean ICP (β= −1.57, p=0.029-however this did not survive the B-Y correction). Concordantly, in the allele difference model, the wild-type allele(A) was protective for CT edema (OR 0.41, p=0.006), and these patients had lower mean (β= −3.70, p=0.000) and peak (β= −8.29, p=0.001) ICPs. These results retained significance in multivariate analyses (Table 4) where homozygote-variants had increased odds of CT edema (OR=2.45, p=0.007), higher mean ICP by 3.13 mmHg (p=0.000) and higher peak ICP by 8 mmHg (p=0.001). The wild-type allele remained protective against acute CT edema (p=0.007) and ICP (mean p=0.000; peak p=0.001).

rs3819521 is associated with acute CT edema, mean and peak ICP

There were 47 % heterozygotes (CT) for rs3819521. 10.4% of patients were homozygous for the minor-allele (TT, Table 2). Homozygous-minor patients had higher mean and peak

ICPs, and frequency of acute CT edema (Table 2). Similar to rs2283261, wild-type rs3819521 allele (C) was protective against ICP and acute CT edema in both univariate (Table 3) and multivariate (Table 4) analyses. Homozygote-variant genotypes had higher mean ICP (β=3.42, p=0.002), higher peak ICP (β=8.23, p=0.004), and tended to have higher odds of CT edema (OR=2.43, p=0.019). Maintaining internal consistency, the wild-type allele protected against higher mean (p=0.002) and peak ICP (p=0.004). Heterozygotes were protected against the measures of CE including acute CT edema (OR=0.53, p=0.005) and peak ICP (β=−5.67, p=0.002). These findings remained robust in multivariate analyses where the homozygous-variant genotype remained associated with increased risk of edema, and the wild-type allele was protective (Table 4).

rs2283258 is associated with acute CT edema, mean and peak ICP

There were 44.4 % heterozygotes (AG) for rs2283258, and 7.8% of patients were homozygous for the minor allele (AA, Table 2). Homozygous minor patients had higher mean and peak ICPs, and frequency of acute CT edema (Table 2). In univariate analyses, patients with homozygous-variant genotype had increased odds of acute CT edema (OR=3.13, p=0.01), and had higher mean ICP (β=3.84, p =0.002). Peak ICP was also higher by 6.89 mmHg in patients with the homozygous-variant genotype (p=0.034) but this did not maintain significance after correcting for multiple comparisons. The presence of the wild-type allele (G) was protective against acute CT edema (OR=0.32, p=0.01) and mean ICP (β= −3.84, p =0.002). Heterozygotes had a trend towards decreased CT edema and lower peak ICP like the other SNPs but this was not statistically significant. These findings were confirmed in multivariate analyses (Table 4) where the homozygous-variant genotype was associated with increased odds of acute CT edema (OR=3.0, p=0.013) and mean ICP(β=3.2, p=0.008), and the wild-type allele was protective.

rs1799857 is associated with Decompressive Craniotomy

There were 49.1% heterozygotes (GA) for rs1799857, and 18.7% of patients were homozygous for the minor-allele (AA, Table 2). This SNP was associated with odds of decompressive craniotomy. In univariate analyses (Table 3), the homozygous wild-type genotype (GG) decreased odds of decompressive craniotomy (OR=0.47, p=0.002), and presence of the variant-allele (A) increased odds of the surgery (OR=2.13, p=0.002). This retained robustness in the multivariate model (Table 4).

DISCUSSION

Much of neurocritical care in sTBI focuses on monitoring and treating CE and intracranial hypertension. Sur1 is a key regulatory protein involved in CE generation in multiple CNS disorders including TBI and ischemic stroke[7–9,15]. Sur1 expression has been demonstrated in human contusional tissue[28]. We have exciting preliminary data suggesting human CSF Sur1 levels are undetectable in non-injured controls, elevated in patients with sTBI, and trajectories may correlate with CE and outcomes (*In Press*, Critical Care Medicine, abstract presented at American Academy of Neurology, 2016[29]). The efficacy of inhibiting this pathway with Glyburide has shown promise, and is being evaluated by ongoing clinical trials[16,17]. Particularly in light of its regulated expression and regulatory

function, genetic variability in the Sur1 gene, *ABCC8*, could provide important information regarding patient risk-stratification, monitoring, efficacy of targeted therapies, and prognosis - not only in TBI but also in other neurologic diseases affected by CE like ischemic stroke.

To our knowledge, this is the first analysis evaluating the relationship between genetic variability in *ABCC8* and development of CE in sTBI. This study identifies 3 *ABCC8* SNPs (rs2283261, rs3819521, and rs2283258) significantly associated with multiple measures of CE including radiographic edema on initial CT, and clinical measurements of ICP. A fourth SNP (rs1799857) was associated with odds of having a decompressive craniotomy.

CE associations with rs2283261, rs3819521, rs2283258 and rs1799857

For rs2283261, rs3819521, and rs2283258, homozygous-variant genotypes appeared to confer increased risk of CE and presence of wild-type alleles was protective-potentially reflecting an evolutionary advantage. In rs2283261 and rs3819521, the increased risk of CE measures was eliminated in heterozygotes with 1 SNP/minor-allele. Interestingly, heterozygotes may potentially be more protected than homozygous wild-type-one potential explanation is that the Sur1-Trpm4 cation channel is a hetero-octameric structure comprised of four Sur1 subunits, and four Trpm4 subunits[15]. Any heterogeneity in Sur1 splicing/protein structure (vs. a homogeneous structure in homozygotes) may possibly reduce optimal protein-protein interactions and therefore channel efficiency and subsequent edema development.

Unlike the 3 intronic SNPs where homozygous-variant genotypes conferred increased risk of CE measures, in rs1799857 (exon 12), the homozygous wild-type genotype was protective against odds of decompressive craniotomy. Presence of the variant-allele eliminated the protective effect and increased odds of surgery. Although in our population rs1799857 was not significantly associated with acute CT edema, the same trend as decompressive craniotomy was observed; the homozygous wild-type genotype decreased odds of acute radiographic edema and the minor allele increased those odds (p=0.096). It is not unexpected that rs1799857 was associated with decompressive craniotomy, but not ICP. As evident from Table 1, >80% of the surgeries were performed on D0-D1 after injury, thereby likely obviating risks of subsequent intracranial hypertension.

Potential significance of rs2283261, rs3819521, rs2283258 and rs1799857

Much of the existing research on genetic variation in *ABCC8* is reported in the diabetes literature. The role of Sur1 was initially appreciated in pancreatic β cells, where Sur1 associates with the potassium channel (Kir6.2) [15,30–32]. In this literature, *ABCC8* SNPs are associated with disorders of glucose metabolism like congenital hyperinsulinism and neonatal diabetes[30,32,33]. Reported SNPs for these diseases are located throughout coding (exons 1–16, 18–39) and non-coding (promoter, introns 3, 8, 10–11, 14–16, 18–19, 22, 24–25, 29, 32, 36, 38) regions of the *ABCC8* gene[30].

Our study identifies 3 novel SNPs (rs2283261, rs3819521, and rs2283258) associated with acute CT edema, mean and peak ICP in a sTBI population. These SNPs are in different non-coding portions of the *ABCC8* gene (Figure 1); rs3819521 is in intron 3, rs2283258 is in intron 7, and rs2283261 is in intron 10. As with most human genomic SNPs, these non-

coding portions may be significant in protein regulation, expression and modifications (only 60,000 of the originally reported 1.42 million SNPs in the human genome were within exons)[19]. Previously reported polymorphisms in introns 7 and 10 have been associated with aberrant splicing and hyperinsulinism[30,34–38]. Aberrant splicing due to an intron 10 SNP has also been shown to influence a transmembrane domain of Sur1 protein[36]. Although SNPs in intron 3 have been reported in connection with insulin disorders, these remain unclassified and of unclear functional significance[30,36].

The fourth significant SNP identified in our population, rs1799857, is located in exon 12 that encodes a Sur1 transmembrane domain. Substitution of variant-allele A in place of G, results in a synonymous variant at position 562 (of 1581); although there is no change in the amino acid Histidine, even in synonymous variants the effect of the polymorphism may not be silent, could impact mRNA stability, and can be further evaluated at the mRNA level[39].

While there is a strong pathophysiologic basis for genetic variability in *ABCC8* influencing the presence and degree of brain edema development, the precise mechanisms remain to be elucidated. The specific impact of rs2283261, rs3819521, rs2283258 and rs1799857 on Sur1 protein structure, function, expression, splicing or regulation requires further investigation beyond the scope of this study. Although any potential causality of the relationships is presently unknown, the identified SNPs could nonetheless provide valuable insights as risk-stratification biomarkers. This study lays the groundwork for additional research to assess the functional impact of these SNPs on the Sur1 pathway and CE.

Limitations

This was a single-center population thereby introducing the possibility of selection bias. SNP identification was limited based on the existing coverage of the *ABCC8* gene. Since treatment with hyperosmolar therapy could affect measures used to assess the development of cerebral edema such as ICP or CT findings, incorporating this information using a validated measure such as ‘Therapeutic Intensity Level’ would have been useful, but was unavailable. Fortunately, since these maneuvers reduce measures of cerebral edema, this limitation while important reduces the likelihood of a falsely positive relationship. Incorporation of the use of hyperosmolar therapy using measures such as the TIL is nonetheless warranted in further confirmatory studies. Additionally, our sample size was relatively small for genetic studies and requires a validation cohort. However, notwithstanding the limited sample, our findings demonstrated relatively large effect sizes, were robust to adjusting for potential confounders in multivariate regression models, and survived statistical correction for multiple comparisons. Given our limited sample size, we used a candidate gene approach and did not evaluate the impact of genetic variability on other related proteins in the pathway, such as the Sur1 regulated channel Trpm4. Moreover, although we are limited in terms of sample size due to the incidence of severe TBI, this is one of the largest polymorphism studies reported in this disease[1,14,21,25,40]. We eagerly anticipate validation in future cohorts facilitated by emerging multi-center studies such as Transforming Research and Clinical Knowledge in TBI (TRACK-TBI). We did not include outcome measures in our study–this was intentional; given the underlying pathophysiology of the Sur1 pathway, the focus of our study was limited to genetic variability related to CE

development which is a unique target relevant to neurointensivists. Finally, as with most genetic association studies, our study is limited in that the relationships between identified SNPs and CE measures are currently associative in nature.

CONCLUSIONS

As the molecular understanding of underlying pathways contributing to CE becomes increasingly sophisticated and preclinical findings are translated to humans, recognition of potential genetic influences becomes progressively more relevant. The Sur1 pathway is distinctive. Since Sur1 is normally absent in the CNS, not only is it a potentially specific therapeutic target against CE, but the degree of upregulation and expression of this regulatory protein (and resultant CE) may vary based on genetic differences. This study demonstrates the involvement of genetic variations in *ABCC8* (rs2283261, rs3819521, rs2283258 and rs1799857) in CE development in sTBI. Our data provide the foundation for further research into understanding the functional implications of these and other *ABCC8* polymorphisms on CE. Our findings need to be validated in additional populations. If validated, this could also inform the use of certain *ABCC8* polymorphisms as biomarkers to a priori identify sTBI patients with altered Sur1 expression, structure or function and an increased risk of CE. In the rapidly evolving world of individualized medicine, understanding the impact of genetic variability in the Sur1 protein and pathway could eventually guide CE evaluation including categorization of treatment responders, and advance the development of targeted or gene-based therapies.

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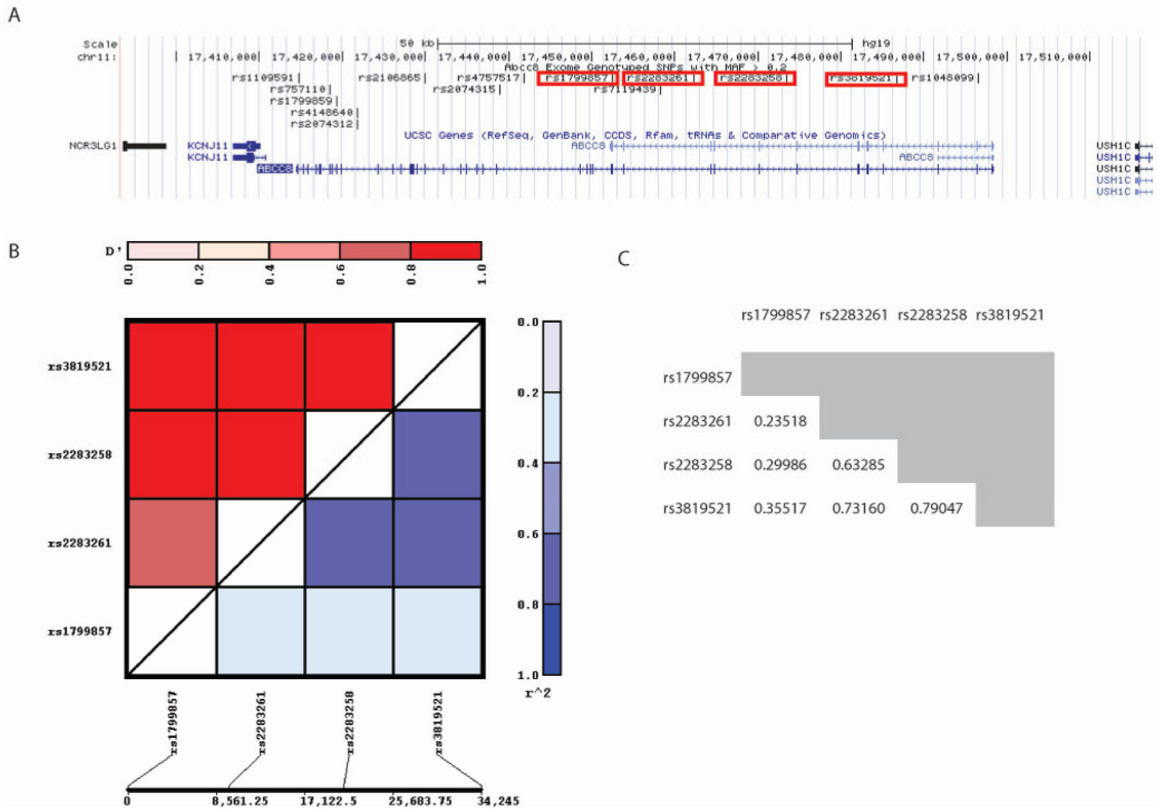

Figure 1.

(A) The chromosomal locations of all 14 SNPs identified in ABCC8 with MAF > 0.2 based on the UCSC genome browser (GRCh38/hg38). The SNPS significantly associated with CE measures (rs2283261, rs3819521, rs2283258 and rs1799857) are boxed in red. LD plot (B) and r2 values (C) between rs2283261, rs3819521, rs2283258 and rs1799857 were generated using the JLIN program. The results show that none of the r2 values between any of the 4 SNPs are > 0.8.

TABLE 1

PATIENT CHARACTERISTICS

Variable (n=385)	Mean (SD, Range)
Age	37.9 (16.8, 16–77)
Initial GCS	5.83 (1.51, 3–8)
	Frequency n (%)
Gender (M)	304 (79.0%)
Race	
• White	380 (98.7%)
• Asian/Pacific Islander	2 (0.52%)
• Other	3 (0.78%)
Edema noted on acute CT (Y)	146 (0.44)
Decompressive Craniotomy (Y)	128/385 (0.33)
• Day 0	87/128 (0.68)
• Day 1	18/128 (0.14)
• Days 2, 3, 4 or 5	14/128 (0.11)

Table 1 summarizes the patient characteristics of the severe TBI population overall (n=385). All patients had severe TBI (GCS 3–8. Mean 5.83), and were predominantly male (79%).

TABLE 2

SNP ALLELE AND GENOTYPE FREQUENCIES IN THE SEVERE TBI POPULATION

SNP		Homozygous Major	Heterozygous	Homozygous Minor	Major Allele	Minor Allele
rs2283261	Total Frequency (%)	137 (35.6%)	194 (50.4%)	54 (14%)	331 (86%)	248 (64.4%)
A=major C= minor	• Average ICP, Mean (SD)	10.0 (3.1)	9.6 (3.6)	***13.5 (9.2)***	9.8 (3.4)	24.5 (11.0)
	• Peak ICP, Mean (SD)	27.0 (13.0)	23.1 (9.4)	***32.8 (18.8)***	10.5 (5.7)	25.4 (12.9)
	• Acute CT Edema * Frequency Y (%)	53 (47.3%)	64 (36.8%)	***29 (63%)***	117 (40.9)	93 (42.3)
	• Decompressive Surgery Frequency Y (%)	45 (32.9%)	65 (33.5)	18 (33.5%)	110 (33.2)	83 (33.5)
rs3819521	Total Frequency (%)	164 (42.6)	181 (47.0)	40 (10.4)	345 (89.6)	221 (57.4)
C= major T= minor	• Average ICP, Mean (SD)	10.3 (3.2)	9.7 (5.1)	***13.4 (7.8)***	9.9 (4.32)	10.5 (5.9)
	• Peak ICP, Mean (SD)	27.2 (12.4)	22.9 (10.7)	***33.1 (18.5)***	24.9 (11.7)	25.0 (13.3)
	• Acute CT Edema * Frequency Y (%)	67 (48.6)	58 (36.2)	***21 (63.6)***	125 (41.8)	79 (40.7)
	• Decompressive Surgery Frequency Y (%)	58 (35.4)	58 (35.4)	12 (30.0)	116 (33.6)	70 (31.7)
rs2283258	Total Frequency (%)	184 (47.8)	171 (44.4)	20 (7.8)	355 (92.2)	201 (52.2)
G=major A=minor	• Average ICP, Mean (SD)	10.2 (3.3)	9.9 (5.5)	***13.9 (7.5)***	10.0 (4.5)	10.6
	• Peak ICP, Mean (SD)	26.6 (12.3)	23.7 (12.2)	***33.0 (16.9)***	25.2 (12.3)	25.3 (13.4)
	• Acute CT Edema *v Frequency Y (%)	72 (46.8)	56 (36.8)	***18 (69.2)***	128 (41.8)	74 (41.6)
	• Decompressive Surgery Frequency Y (%)	61 (33.2)	57 (33.3)	10 (33.3)	118 (33.2)	67 (33.3)
rs1799857	Total Frequency (%)	124 (32.2)	189 (49.1)	72 (18.7)	313 (81.3)	261 (67.8)
G=major A=minor	• Average ICP, Mean (SD)	10.9 (5.6)	10.2 (5.0)	9.7 (3.2)	10.5 (5.3)	10.1 (4.6)
	• Peak ICP, Mean (SD)	27.2 (14.0)	25.1 (13.1)	25.4 (10.0)	26.0 (10)	25.1 (12.3)
	• Acute CT Edema * Frequency Y (%)	40 (37.4)	76 (46.9)	30 (47.6)	116 (43.1)	106 (47.1)
	• Decompressive Surgery Frequency Y (%)	***28 (22.6)***	72 (38.1)	28 (38.9)	100 (32.0)	100 (38.3)

Table 2 summarizes the relative frequencies of the SNP genotypes and alleles in the TBI population, as well as for the various measures of cerebral edema. For example, row 1 evaluates rs2283261. 35.6% of the TBI population were wild-type (homozygous-major), 50.4% were heterozygotes, and 14% were homozygous for the variant SNP. Focusing on the homozygous major genotype (AA) for rs2283261, in patients with this genotype, acute CT edema occured in 47.3%, the mean ICP was 10.0 ± 3.1 mmHg, peak ICP was 27.0 ±13.0, and decompressive craniotomies were performed 32.9% of the patients. In contrast, the TBI sample contained 14% of patients with homozygous minor genotype of the rs2283261 SNP (CC) in. In these patients (CC), acute CT edema occured in a higher percentage (63%) of patients, mean and peak ICP was also higher 13.5 ±9.2 and 32.8 ±g18.8.

* CT edema data were available for 332 patients of the total 385.

TABLE 3

UNIVARIATE ANALYSIS OF *ABCC8* SNP ASSOCIATION WITH CEREBRAL EDEMA IN TBI

SNP		Acute CT Edema		Average ICP		Peak ICP		Decompressive Craniotomy	
		Odds Ratio (95% CI)	***p***	***β-coefficient (95% CI)***	***p***	***β-coefficient (95% CI)***	***p***	***Odds ratio (95% CI)***	***P***
rs2283261 Intron 10	Homozygous major (AA)	1.23 (0.78–1.94)	0.38	−0.51 (−2.04–1.01)	0.51	1.56 (−2.4–5.5)	0.44	0.97 (0.62–1.51)	0.91
	Homozygous minor (CC)	***2.46 (1.29–4.69)***	***0.006***	***3.70 (1.85–5.56)***	***0.000***	***8.29 (3.42–13.17)***	***0.001***	1.005 (0.55–1.85)	0.99
	Heterozygous (AC)	***0.54 (0.35–0.84)***	***0.006***	*−1.57 (−2.98–−0.16)*	*0.029*	***−5.87 (−9.48–−2.27)***	***0.002***	1.023 (0.67–1.56)	0.91
	Allele A	***0.41 (0.21–0.77)***	***0.006***	***−3.70 (−5.56–−1.84)***	***0.000***	***−8.29 (−13.2–−3.42)***	***0.001***	1.00 (0.54–1.83)	0.99
	Allele C	0.82 (0.52–1.29)	0.38	0.52 (−1.01–2.04)	0.51	−1.56 (−5.52–2.41)	0.44	1.03 (0.66–1.60)	0.90
rs3819521 Intron 3	Homozygous major (CC)	1.37 (0.88–2.13)	0.16	−0.17 (−1.62–1.28)	0.82	2.24 (−1.51–6.00)	0.24	1.18 (0.77–1.81)	0.45
	Homozygous minor (TT)	*2.43 (1.16–5.13)*	*0.019*	***3.42 (1.29–5.55)***	***0.002***	***8.23 (2.67–13.80)***	***0.004***	0.85 (0.42–1.72)	0.65
	Heterozygous (CT)	***0.53 (0.34–0.82)***	***0.005***	−1.29 (−2.7–0.12)	0.07	***−5.67 (−9.29–−2.06)***	***0.002***	0.90 (0.59–1.38)	0.64
	Allele C	*0.41 (0.19–0.87)*	*0.019*	***−3.42 (−5.55–−1.29)***	***0.002***	***−8.23 (−13.8–−2.67)***	***0.004***	1.18 (0.58–2.41)	0.65
	Allele T	0.73 (0.47–1.13)	0.16	0.17 (−1.28–1.62)	0.82	−2.24 (−6.00–1.51)	0.24	0.85 (0.55–1.30)	0.45
rs2283258 Intron 7	Homozygous major (GG)	1.23 (0.80–1.91)	0.72	−0.41 (−1.84–1.01)	0.57	1.36 (−2.35–5.07)	0.47	0.99 (0.65–1.52)	0.97
	Homozygous minor (AA)	***3.13 (1.32–7.42)***	***0.01***	***3.84 (1.49–6.28)***	***0.002***	7.80 (1.40–14.21)	*0.017*	1.00 (0.46–2.21)	0.99
	Heterozygous (GA)	*0.58 (0.38–0.91)*	*0.016*	−0.84 (−2.27–0.58)	0.25	−3.92 (−7.60–−0.25)	*0.037*	1.01 (0.66–1.54)	0.97
	Allele G	***0.32 (0.18–0.76)***	***0.010***	***−3.84 (−6.28–−1.40)***	***0.002***	−7.80 (−14.21–−1.4)	*0.017*	1.00 (0.45–2.20)	0.99
	Allele A	0.81 (0.52–1.25)	0.34	-.41 (−1.01–1.84)	0.57	−1.36 (−5.07–2.35)	0.47	1.01 (0.66–1.54)	0.97
rs1799857 Exon 12	Homozygous major (GG)	0.67 (0.42–1.07)	0.096	0.85 (−0.64–2.32)	0.26	2.02 (−1.83–5.87)	0.3	***0.47 (0.29–0.77)***	***0.002***
	Homozygous minor (AA)	1.20 (0.69–2.08)	0.52	−0.83 (−2.71–1.05)	0.39	−0.60 (−5.50–4.30)	0.81	1.36 (0.80–2.30)	0.26
	Heterozygous (GA)	1.26 (0.82–1.95)	0.29	−0.303 (−1.73–1.12)	0.68	−1.52 (−5.22–2.19)	0.42	1.54 (1.004–2.36)	0.048
	Allele G	0.83 (0.48–1.45)	0.52	0.83 (−1.05–2.71)	0.39	0.60 (−4.30–5.50)	0.81	0.74 (0.43–1.25)	0.26
	Allele A	1.49 (0.93–2.39)	0.096	−0.85 (−2.32–0.64)	0.26	−2.02 (−5.88–1.84)	0.30	***2.13 (1.31–3.47)***	***0.002***

Table 3: Univariate analyses demonstrating the association of each of the 4 SNPs with measures of cerebral edema including mean and peak ICP, acute CT edema, and need for decompressive craniotomy. Odds ratios (OR) are provided for binary outcomes (CT edema, decompressive craniotomy), and βcoefficients for continuous outcomes (mean and peak ICP).

TABLE 4

MULTIVARIATE ANALYSIS OF *ABCC8* SNP ASSOCIATION WITH MEASURES OF CEREBRAL EDEMA IN TBI

SNP		Acute CT Edema		Average ICP		Peak ICP		Decompressive Craniotomy	
		Odds Ratio (95% CI)	***p***	***β-coefficient (95% CI)***	***p***	***β-coefficient (95% CI)***	***p***	***Odds ratio (95% CI)***	***P***
rs2283261	Homozygous minor (CC)	***2.45 (1.28–4.70)***	***0.007***	***3.13 (1.50–5.13)***	***0.000***	***8.00 (3.13–12.86)***	***0.001***	0.99 (0.53–1.87)	0.99
	Heterozygous (AC)	***0.53 (0.34–0.83)***	***0.005***	*−1.20 (−2.56–−0.16)*	*0.084*	***−5.17 (−8.74–−1.60)***	***0.005***	0.96 (0.61–1.48)	0.84
	Allele A	***0.41 (0.21–0.78)***	***0.007***	***−3.31 (−5.13–−1.50)***	***0.000***	***−8.00 (−12.86–−3.13)***	***0.001***	1.01 (0.53–1.89)	0.99
rs3819521	Homozygous minor (TT)	2.36 (1.11–5.01)	0.025	***2.95 (0.88–5.02)***	***0.005***	***7.64 (2.11–13.16)***	***0.007***	0.83 (0.40–1.74)	0.63
	Heterozygous (CT)	***0.52 (0.33–0.82)***	***0.004***	−0.70 (−2.08–0.69)	0.32	***−4.61 (−8.24–−0.96)***	***0.013***	0.80 (0.51–1.25)	0.33
	Allele C	0.42 (0.20–0.90)	0.025	***−2.95 (−5.02–−0.88)***	***0.005***	***−7.64 (−13.2–−2.11)***	***0.007***	1.20 (0.58–2.50)	0.63
rs2283258	Homozygous minor (AA)	***3.00 (1.26–7.18)***	***0.013***	***3.20 (0.83–5.58)***	***0.008***	6.89 (0.52–13.25)	*0.034*	1.03 (0.46–2.34)	0.94
	Allele G	***0.33 (0.14–0.80)***	***0.013***	***−3.20 (−5.58–−0.83)***	***0.008***	−6.89 (−13.25–−0.52)	*0.034*	0.97 (0.43–2.19)	0.94
rs1799857	Homozygous major (GG)	0.67 (0.42–1.08)	0.102	0.82 (−0.61–2.23)	0.26	1.90 (−1.88–5.68)	0.32	***0.47 (0.28–0.78)***	***0.004***
	Allele A	1.49 (0.92–2.39)	0.102	−0.81 (−2.23–0.61)	0.26	−90 (−5.68–1.88)	0.32	***2.12 (1.28–3.51)***	***0.004***

Table 4: Multivariate analyses demonstrating the association of each of the 4 SNPs with measures of cerebral edema including mean and peak ICP, acute CT edema, and need for decompressive craniotomy after controlling for age, gender, and initial GCS score. Adjusted Odds ratios (OR) are provided for binary outcomes (CT edema, decompressive craniotomy), and β-coefficients for continuous outcomes (mean and peak ICP).