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Influence of ATP-Binding Cassette Polymorphisms on Neurological Outcome After Traumatic Brain Injury

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Abstract

Background—As important mediators of solute transport at the blood–brain and blood–cerebrospinal fluid barriers, ATP-binding cassette (ABC) transporters (including ABCB1, ABCC1, and ABCC2), impact the bioavailability of drugs and endogenous substrates in the brain. While several ABCB1, ABCC1, and ABCC2 single nucleotide polymorphisms (SNPs) have been identified, their impact on outcome after traumatic brain injury (TBI) is unknown.

Hypothesis—ABCB1, ABCC1, and ABCC2 SNPs are associated with Glasgow Outcome Scale (GOS) score after TBI.

Methods—DNA samples from 305 adult patients with severe TBI (Glasgow Coma Scale, GCS score ≤ 8) were genotyped for tagging SNPs of ABCB1 (rs1045642; rs1128503), ABCC1 (rs212093; rs35621; rs4148382), and ABCC2 (rs2273697). For each SNP, patients were dichotomized based on presence of variant allele for multivariate analysis to determine associations with GOS assigned at 6 months adjusting for GCS, Injury Severity score, age, and patient sex.

Results—For ABCB1 rs1045642, patients homozygous for the T allele were less likely to be assigned poor outcome versus those possessing the C allele [CT/CC; odds of unfavorable GOS = 0.71(0.55−0.92)]. For ABCC1 rs4148382, patients homozygous for the G allele were less likely to be assigned poor outcome versus those possessing the A allele [AG/AA; odds of unfavorable GOS = 0.73(0.55−0.98)].

Conclusions—In this single-center study, patients homozygous for the T allele of ABCB1 rs1045642 or the G allele of ABCC1 rs4148382 were found to have better outcome after severe TBI. Further study is necessary to replicate these very preliminary findings and to determine whether these associations are due to central nervous system bioavailability of ABC transporter drug substrates commonly used in the management of TBI, brain efflux of endogenous solutes, or both.

Keywords

Blood-brain barrier; Head trauma; Membrane transporter; Multidrug resistance protein; Multidrug resistance-associated protein; P-Glycoprotein

Introduction

Traumatic brain injury (TBI) remains a significant public health concern with ∼ 1.7 million cases occurring annually in the United States and causing a third of all injury related deaths [1]. Individuals, who experience TBI with comparable pre-hospital function and demographics, often display variable clinical outcomes. While these differences can be explained by the heterogeneous nature of TBI in terms of primary injury and treatment, host specific factors, such as genotype, may also play a role. A growing body of literature suggests that genetic factors can modulate the pathophysiology of secondary brain damage [2-4].

ATP-binding cassette (ABC) transporters are a family of proteins whose role includes regulating drug substrate absorption, distribution, and excretion [5]. During periods of brain injury, ABC transporters participate in the transport of target substrates across the blood–brain barrier (BBB) [6]. Of clinical importance, ABC-mediated transport can enhance the active efflux of therapeutic substances in a range of central nervous system (CNS) disease states, such as malignancy, psychiatric disorders, and epilepsy [7, 8]. Accordingly, genetic differences in ABC transporters at the BBB and blood–cerebrospinal fluid (CSF) barriers (BCSFB) may influence brain bioavailability of, and consequently individual responses to, CNS-targeted medications, including those commonly used in the management of TBI.

Genetic variation, in the form of single nucleotide polymorphism (SNP), is implicated in the functional activity of ABCB1 (*aka* multidrug resistance protein 1 [MDR1] or P-glycoprotein), ABCC1, and ABCC2 [*aka* multidrug resistance-associated proteins (MRP) 1 and 2] transporters [9-11]. Association studies in Caucasian, Egyptian, and Croatian populations demonstrate that the C allele of the rs1045642 ABCB1 polymorphism is related to increased protein expression and anti-epileptic drug (AED) resistance [12-14]. A study conducted in the Netherlands found several SNPs in the ABCC1 gene to be associated with lung function in chronic obstructive pulmonary disease patients [10]. Korean and German population-based studies linked polymorphisms in the ABCC2 gene to adverse AED reaction and non-response to AED therapy, respectively, [11, 15]. Given the importance of ABC transporters at the BBB and BCSFB, the relevance of the functional BBB after TBI, and pharmacological (narcotics, AEDs) and endogenous (glutathione) ABC transporter substrates potentially relevant to TBI patients, we sought to determine whether there was an association between tagging SNP-related haplotype blocks of ABCB1, ABCC1, and ABCC2 genes, and neurological outcome after severe TBI in humans.

Methods

Study Design and Subjects

With the approval of the University of Pittsburgh Institutional Review Board, we conducted a retrospective study on prospectively collected DNA samples from 305 consecutive participants from the University of Pittsburgh Brain Trauma Research Center (BTRC) database. This database included patients admitted to the University of Pittsburgh Medical Center Neurotrauma unit between May 2000 and November 2009 with associated clinical outcome data and DNA samples. Subjects aged 18–74 years having experienced severe TBI, defined as initial or deterioration to a Glasgow Coma Scale (GCS) score ≤8, with external ventricular drain (EVD) placement were included in this study. Individuals with pre-existing neurologic deficit, penetrating TBI, and cardiac or respiratory arrest were excluded. A legal authorized representative provided initial informed consent and continued assent was obtained if possible. DNA extracted from blood or CSF was cataloged and stored at −80 °C for batch analysis. Demographic, injury, and treatment data were obtained from medical records.

Tagging SNP Selection

HapMap Genome Browser Build 35 [16] was utilized to select tagging SNPs accounting for the variability in the ABCB1, ABCC1, and ABCC2 genes, including regions spanning 1 kb of the 5′ and 3′ flanking regions of these genes. Tagging SNPs rs1045642 and rs1128503 were selected for ABCB1; rs212093, rs35621, and rs4148382 for ABCC1; and rs2273697 for ABCC2. Tagging SNPs with a minor allele frequency ≥20 % and $r^2 \geq 0.80$ were utilized [17] and selection based on western European ancestry (CEU) represented the majority of the TBI subjects in our study.

Genotyping

A commercially available kit (Qiaamp kit, Qiagen, Chatsworth, CA, USA) was utilized to extract DNA samples from CSF obtained from the EVD as part of standard of care or from blood samples using a simple salting out procedure [18]. TaqMan allele discrimination technology and commercially available assays (Applied Biosystems, Foster City, CA, USA) were employed for genotyping. Genotyping assignments and amplification were conducted using the ABI7000 and SDS 2.0 software (Applied Bio-systems, Foster City, CA, USA). Genotypes were independently assigned by two individuals who were blinded to phenotype data. Data were then compared and any discrepancies between results were resolved with additional genotyping. Only genotypes achieving consensus were assigned; as such, some genotyping data are missing. Genotypes were dichotomized into variant present and variant absent (homozygous wild type) combinations for analyses.

Outcome Measure

Primary outcome was measured by Glasgow Outcome Scale (GOS) score [19]. As part of the BTRC database, GOS was assigned at 3, 6, 12, and 24 months following injury if possible. This score was obtained by direct interview if available or, if not, by telephone. Outcome assignments were completed by a neuropsychological technician under the supervision of an attending neurosurgeon or a neuropsychologist. For analysis, GOS score assigned at 6 months was utilized as these data were most complete. As a measure of presenting injury severity and level of consciousness, initial GCS score was defined as the first score assigned by the admitting neurosurgeon upon arrival to our Level 1 Trauma facility. The Injury Severity score (ISS) was assigned by the Trauma surgery team.

Statistical Analysis

Associations between clinical variables (age, sex, initial GCS, ISS, and Marshall computerized tomography classification [20]) and genotype for each tagging SNP were determined using the Kruskal–Wallis test. For assessment of independent associations between genotype and the primary outcome variable GOS assigned at 6 months, analysis was conducted using multivariable logistic regression, according to the criteria recommended by Bagley and White [21]. The clinical variables initial GCS score, age, patient sex, and ISS were included in the statistical model due to established influence on outcome after TBI in humans. Dichotomized genotypes were utilized in multivariable logistic regression analysis to determine independent associations. Associations with outcome are presented as odds ratio (OR) and 5th to 95th confidence limits (CL). Statistical

analysis was conducted using Stata 10.0 (StataCorp LP, College Station, TX, USA) or SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA) statistical software.

Results

Demographics and Genotype Frequencies

Study demographic data are shown in Table 1. Sample sizes and genotype frequencies were calculated for each SNP (Tables 2, 3; Supplementary Tables 1–4), and were similar to published data available through the International HapMap Project, Public release #27, 2009-02 [16]. No statistically significant associations between ABCB1, ABCC1, and ABCC2 polymorphisms and the clinical variables age, patient sex, initial GCS, or ISS were detected.

Logistic Regression Analysis

Bivariate analysis examining associations between genotype and GOS assigned at 6 months yielded two SNPs for inclusion in multivariable logistic regression models ($P < 0.3$): rs1045642 of ABCB1 ($P = 0.13$) and rs4148382 of ABCC1 (P = 0.26). The absence of the C allele (TT vs. CT/CC genotype) for the rs1045642 SNP of ABCB1 was found to be independently associated with GOS assigned at 6 months when controlling for initial GCS, ISS, age, and patient sex (Table 4). Patients with the TT genotype were less likely to be assigned poor neurological outcome (lower GOS) than those possessing the C allele [OR 0.71 (0.55–0.92) TT vs. CT/CC genotype, $P = 0.01$]. The absence of the A allele (GG vs. GA/AA genotype) for the rs4148382 SNP of ABCC1 was found to be independently associated with GOS assigned at 6 months when controlling for initial GCS, ISS, age, and patient sex (Table 5). Patients with the GG genotype were less likely to be assigned poor neurological outcome than those possessing the A allele [OR 0.73 (0.55–0.98) GG vs. GA/AA genotype, $P = 0.04$].

Although, our patient population was relatively homogenous in terms of ethnicity, to ensure the results were not due to ethnic stratification, race was added as a variable in logistic analysis. The addition of race into the statistical model did not change outcomes (data not shown).

Discussion

In this preliminary single-center study, we have shown that the SNP for ABCB1 (also referred to as MDR1 and P-glycoprotein) rs1045642 and the SNP for ABCC1 (also referred to as MRP1) rs4148382 are associated with neurological outcome after severe TBI. More favorable neurological outcome was associated with those individuals in our cohort having the TT genotype of rs1045642 or the GG genotype of rs4148382.

For ABCB1, this relationship could be explained by the functional consequences of having a C allele. The rs1045642 polymorphism, found within exon 26, tags a haploblock of ∼4.7 kb based on HapMap Phase III data [16], and results in a synonymous change at residue 1145 from an ATT codon to an ATC codon, both which code for isoleucine. This “silent” change, which is located within the intracellular domain of the bound protein between the second Q-

loop and Signature motifs, has been shown to have functional consequences [22]. Studies have suggested that nucleotide variation at rs1045642 within ABCB1 (also called C3435T) structurally alters substrate interaction sites and influences the level of protein expression potentially by introducing a translation pause due to the introduction of a rare codon [9, 22, 23]. We speculate that this difference could explain our findings as it modifies one or more of the ABC transporters primary functions by: alteration of pharmacologic substrate carrying capacity, derangement of transport of unknown endogenous substrates, or disruption of BBB function as a whole.

Alteration of membrane bound transporters via “silent” mutations and possible changes in protein folding could affect neurologic outcome by disturbing the transporters ability to bind and move substrate. Both known and unknown exogenous and endogenous substrate transport could be modified based on genotype change. Multiple studies have concluded that the variant allele of rs1045642 ABCB1 decreases the efficacy of antiepileptic medications due to increased ABCB1 transporter function [12, 14, 24, 25]. This includes the drug phenytoin, which is exported across the BBB via ABCB1 (MDR1, P-glycoprotein) and was commonly used in this cohort of TBI patients. Furthermore, during ischemic brain injury in animal models, genetic knockout of ABCB1 promotes the accumulation of neuroprotective pharmacologic substances [26]. These findings support the notion that genetic modulation of the ABCB1 gene could impact outcome following brain injury by altered substrate transport, and are consistent with the association between the loss-of-function ABCB1 polymorphism (TT of rs1045642) and better neurological outcome observed in our study. A prospective study examining genotype–phenotype (blood:brain drug substrate levels) relationships in this regard appears warranted.

In contrast, other reports refute the claim that ABCB1 polymorphisms are associated with functional activity or related to neurologic disease states. Studies involving the TT genotype of rs1045642 have shown increased, decreased, and no genotypic influence on the expression of the ABCB1 transporter protein [27–29], and two recent meta-analyses looking at ABCB1 haplotypes in AED resistance also suggested a lack of influence in epilepsy [30, 31]. This body of data would propose that substrate transport is not affected and therefore could not be the cause of improved neurologic outcome in patients with the loss-of-function ABCB1 polymorphism TT of rs1045642.

Antiepileptic drugs, however, are not the only substrates for ABCB1. Several other therapeutics used in the management of TBI patients including barbiturates, narcotics, sedatives, and antibiotics are ABCB1 transporter substrates [32]. Additionally, there is a host of endogenous substrates that have been identified [33], including a possible role for ABCB1 in amyloid-β clearance in patients with Alzheimer's disease [34]. It is possible to speculate that brain bioavailability of many or all of these substrates could be important in both the management and ultimate clinical outcome of patients after TBI. It follows that these sub-stances, whether endogenous or exogenous, known or unknown, could be impacted by transporter changes leading to different clinical outcomes as suggested in this study.

While the essential role for ABC transporters in normal BBB function is established [35], and the potential role for ABC transporters in neurodegenerative diseases is emerging [36], the role for ABCC1 after TBI is underexplored. Given the importance of functional ABCC1 in drug resistance and glutathione homeostasis [37], it is tempting to speculate that altered ABCC1 function could influence brain bioavailability of glutathione and glutathione-conjugates [38] after TBI. In patients with chronic obstructive pulmonary disease, individuals homozygous for the G allele (GG genotype) had reduced ABCC1 protein levels, reduced inflammatory cells in bronchial biopsies, and better pulmonary function compared with heterozygous (GA) individuals [39]. While biologically plausible, our data are far too preliminary to speculate that a similar pattern of reduced inflammation and improved function is occurring in patients with GG versus GA/AA genotypes after TBI.

This preliminary single-center study has limitations. Association studies do not prove causality. It is likely that additional complex epigenetic and/or post-translational interactions ultimately determine clinical phenotype. In this regard, future studies examining functional genotype–phenotype relationships (e.g., ABCB1 SNPs and cross brain phenytoin concentrations) are needed to complement outcome studies. In addition, TBI is a heterogeneous disease and uncontrollable factors may have influenced the ability to detect statistical significance. We attempted to control for this heterogeneity by including measures of injury severity (initial GCS and ISS) and Marshall computerized tomography classification in the univariate and/or multivariate statistical models. Furthermore, these findings should be replicated prospectively in larger independent samples including subjects from different ethnicities for validation. Nonetheless, this work represents a first attempt to understand the relationship between ABC transporter polymorphisms and outcome in humans after TBI, and is the largest study to date on genotype of ABC transporters and effect on neurological outcome. Additional study is needed to see if effective concentrations of commonly prescribed ABCB1 substrates and/or endogenous ABCC1 substrates vary with the patient genotype. Treatment approaches using modulators of ABCB1 (e.g., cyclosporine A, tacrolimus) and/or ABCC1 (e.g., probenecid, furosemide) could present a novel way to overcome genotype variation.

In conclusion, we found that the ABCB1 SNP rs1045642 and ABCC1 SNP rs4148382 are associated with 6-month GOS in patients with severe TBI. Further investigation is required to determine functional consequences of each SNP, and larger prospective studies are required to validate the present single-center findings. Nonetheless, these data showing associations between neurological outcome and ABCB1/ABCC1 genotype represent a logical first step for future management of TBI patients in the era of personalized medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Patient demographics (*N* = 305)

Age (years)	36.0 ± 15.6
Male (%)	240/305 (79)
Caucasian (%)	284/305 (93)
African-American (%)	21/305 (7)
Initial Glasgow Coma Scale score	6 (4–7)
Six months Glasgow Outcome Scale score	3 (1–4)
Injury Severity Score	34 (26–43)
Mortality (%)	90/305 (30)

Data are median (interquartile range) or mean ± standard deviation

Table 2
ABCB1 rs1045642 frequencies and clinical characteristics by genotype

	TT	CT	CC	*P*†
n (%)	66 (22)	162 (53)	77 (25)	–
Age (years)	38 ± 16	35 ± 15.0	38 ± 16	0.14
Patient sex (% male)	71	82	79	0.25
Initial Glasgow Coma Scale score	6 (4–7)	6 (4–7)	6 (4–7)	0.97
6 mo Glasgow Outcome Scale score	3 (1–4)	3 (1–4)	3 (1–3)	0.13
Injury Severity Score	34 (28–43)	34 (26–43)	34 (26–43)	0.73
Marshall CT classification (%)‡				0.95
Diffuse injury I	5 (8)	7 (4)	5 (6)	
Diffuse injury II	31 (47)	75 (47)	31 (40)	
Diffuse injury III	7 (11)	29 (18)	20 (26)	
Diffuse injury IV	12 (18)	23 (14)	10 (13)	
Evacuated mass lesion	3 (5)	4 (3)	1 (1)	
Non-evacuated mass lesion	8 (12)	21 (13)	10 (13)	
Mortality (%)	21.2	30.9	32.5	0.19

Data are median (interquartile range) or mean ± standard deviation

†Kruskal–Wallis test

‡Missing data points

Table 3
ABCC1 rs4148382 frequencies and clinical characteristics by genotype

	GG	GA	AA	*P*[†]
n (%)	246 (83)	48 (16)	2 (1)	–
Age (years)	36 ± 16	35 ± 15	42 ± 6	0.55
Patient sex (% male)	80	77	50	0.53
Initial Glasgow Coma Scale score	6 (5–7)	6 (5–7)	7 (6, 7)	0.80
6 mo Glasgow Outcome Scale score	3 (1–4)	3 (1–3)	3 (3, 4)	0.26
Injury Severity Score	34 (27–43)	30 (26–43)	38 (33–43)	0.77
Marshall CT classification (%)[‡]				0.49
Diffuse injury I	10 (4)	5 (10)	0 (0)	
Diffuse injury II	115 (47)	20 (42)	1 (50)	
Diffuse injury III	39 (16)	14 (29)	0 (0)	
Diffuse injury IV	38 (16)	4 (8)	1 (50)	
Evacuated mass lesion	7 (3)	1 (2)	0 (0)	
Non-evacuated mass lesion	34 (14)	4 (8)	0 (0)	
Mortality (%)	28.0	33.3	0	0.58

Data are median (interquartile range) or mean ± standard deviation

[†]Kruskal–Wallis test

[‡]Missing data points

Table 4

Dichotomized ABCB1 rs1045642 variant allele multivariate logistic regression analysis

	TT	CT/CC	OR[†]	*P*
Age	38.0 ± 15.5	35.2 ± 15.6	0.98 (0.96–1.0)	0.07
Patient sex (% male)	71	80	1.62 (0.83–3.1)	0.16
Initial Glasgow Coma Scale score	6 (4–7)	6 (4–7)	1.13 (0.91–1.39)	0.26
Injury Severity Score	34 (28–43)	34 (26–43)	1.00 (0.97–1.03)	0.95
6 mo Glasgow Outcome Scale score[‡]	3 (1–4)	3 (1–4)	0.71 (0.55–0.92)	0.01
1 Dead (%)	14 (23.0)	75 (34.3)		
2 Vegetative (%)	4 (6.6)	8 (3.7)		
3 Severe disability (%)	19 (31.2)	67 (30.6)		
4 Moderate disability (%)	13 (21.3)	46 (21.0)		
5 Normal (%)	11 (18.0)	23 (10.5)		

Data are median (interquartile range) or mean ± standard deviation

[†]Odds ratio (5–95 % confidence interval)

[‡]Missing data points

Table 5
Dichotomized ABCC1 rs4148382 variant allele multivariate logistic regression analysis

	GG	GA/AA	OR[†]	*P*
Age	36.4 ± 15.9	34.8 ± 14.4	0.99 (0.96–1.01)	0.21
Patient sex (% male)	80	76	0.98 (0.44–2.16)	0.96
Initial Glasgow Coma Scale score	6 (5–7)	6 (5–7)	1.20 (0.93–1.54)	0.17
Injury Severity score	34 (26–43)	30 (26–43)	0.99 (0.95–1.02)	0.37
6 mo Glasgow Outcome Scale score[‡]	3 (1–4)	3 (1–3)	0.73 (0.55–0.98)	0.04
1 Dead (%)	69 (30.3)	16 (36.4)		
2 Vegetative (%)	11 (4.8)	2 (4.6)		
3 Severe disability (%)	67 (29.4)	15 (34.1)		
4 Moderate disability (%)	49 (21.5)	8 (18.2)		
5 Normal (%)	32 (14.0)	3 (6.8)		

Data are median (interquartile range) or mean ± standard deviation

[†]Odds ratio (5–95 % confidence interval)

[‡]Missing data points