ABCG2 c.421C>A Is Associated with Outcomes after Severe Traumatic Brain Injury

Solomon M. Adams,¹ Yvette P. Conley,^{2,3} Dianxu Ren,⁴ David O. Okonkwo,⁵ Ava M. Puccio,⁵ C. Edward Dixon,^{5,6} Robert S.B. Clark,⁶ Patrick M. Kochanek,⁶ and Philip E. Empey^{6,7}

Abstract

Traumatic brain injury (TBI) is a leading cause of death with no pharmacological treatments that improve outcomes. Transporter proteins participate in TBI recovery by maintaining the central nervous system (CNS) biochemical milieu. Genetic variations in transporters that alter expression and/or function have been associated with TBI outcomes. The ATP-binding cassette transporter, *ABCG2*, is a uric acid (UA) transporter that effluxes UA from cells in the CNS and is responsible for systemic UA clearance. Uric acid is a CNS antioxidant and/or a biomarker that might support TBI recovery. Our objective was to investigate the impact of *ABCG2* SNP: c.421C>A on TBI outcomes. Two cohorts (discovery [$N=270$] and replication [$N=166$]) were genotyped for *ABCG2* c.421C>A. Glasgow Outcome Scale (GOS) scores were collected at 3, 6, 12, and 24 months post-injury and compared with mixed-effects multiple ordinal regression controlled for time post-injury, age, sex, time, post-injury imaging determined hemorrhage types, and Glasgow Coma Scale score. Variant alleles (genotype) were associated with better GOS scores ($p=0.01$ [discovery] and $p=0.02$ [replication]), whereas genotype*age interaction was associated with worse GOS scores ($p=0.03$ [discovery] and $p=0.01$ [replication]). Reversed coefficient directionality suggests variant allele(s) are protective up to approximately age 34 years. Overall, variant alleles at *ABCG2* c.421C>A associate with better GOS scores post-injury in two independently sampled cohorts. This finding is mitigated by increasing subject age. This suggests that *ABCG2* might have an age-dependent effect on TBI recovery and should be explored in future mechanistic studies.

Keywords: biomarkers; genetic factors; human studies; outcome measures; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) affects nearly 2 million people every year and is the leading cause of mortality for children and young adults in the United States.^{1,2} Despite its morbidity and mortality, no pharmacological treatments exist that improve TBI outcomes, and prognostication remains challenging.³ Investigations have sought deeper understanding of the cellular processes that regulate secondary injury post-TBI, focusing on oxidative stress and excitotoxicity, among others.^{3–5} Identifying mechanisms that mitigate these pathways might provide insight into novel treatment and prognostication modalities. Transporters expressed in the central nervous system (CNS) have been a recent focus, with particular interest in the effects of functional genetic polymorphisms on recovery and outcomes after severe TBI.

Genetic polymorphisms in ATP-binding cassette (ABC) transporters are associated with TBI outcomes. Jha and colleagues recently identified ABCC8 (SUR1) single-nucleotide polymorphisms (SNPs) that were predictive of cerebral edema post-TBI.⁶ Cousar and colleagues investigated neurological outcome post-TBI in association with SNPs in the transporters, ABCB1 (PGP, MDR1), ABCC1 (MRP1), and ABCC2 (MRP2), and found that variations in ABCB1 and ABCC1 were associated with poor outcomes post-injury.⁷ These provide a mechanistic framework that will drive studies into the impact of transporter genetic variations on the disposition of endo- and exogenous molecules post-TBI.

The ABC transporter, subfamily G, member 2 (*ABCG2*, BCRP) is a 72-kDa membrane transport protein expressed in a variety of tissues, including the CNS. *ABCG2* effluxes a diverse range of substrates, including xenobiotics, uric acid, and porphyrins.⁸

¹Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania.

²Health Promotion and Development, School of Nursing, University of Pittsburgh, Pittsburgh, Pennsylvania.

³Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

⁴Health and Community Systems, School of Nursing, University of Pittsburgh, Pittsburgh, Pennsylvania.

⁵Neurological Surgery, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

⁶Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, Pennsylvania.

⁷Pharmacy and Therapeutics, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania.

Within the brain, ABCG2 is expressed in endothelial/epithelial cells of the blood–brain barrier (BBB), blood–cerebrospinal fluid barrier, microglia, and neurons. It is expressed on the apical membrane and faces blood, removing its substrates from the brain compartment, thus playing a primary role in both limiting CNS exposure and removing molecules generated within the CNS.⁹ ABCG2 is one of the primary CNS efflux transporters with respect to expression and substrate removal, which generates interest in the impact of ABCG2 functional variation on TBI outcomes.¹⁰

Genetic variations impacting the function of ABCG2 have been characterized. The SNP: rs2231142 (*ABCG2* c.421C>A), is a common missense polymorphism coding for a lysine in place of glutamine at amino acid position 141, resulting in decreased protein expression.¹¹ This variation has been investigated in several CNS pathologies and has shown relationships with the onset of Parkinson’s disease, Alzheimer’s disease, and others.^{12,13} Our objective was to determine the association of *ABCG2* c.421C>A with outcomes after severe TBI in multiple cohorts. We hypothesized that subjects with at least one variant allele would have improved outcomes post-TBI.

Methods

Population

Subjects were identified from the University of Pittsburgh Brain Trauma Research Center (Pittsburgh, PA) following approval by the local institutional review board. Informed consent was obtained from the subjects’ surrogates before data collection. Criteria for inclusion were: age 16 and older; Glasgow Coma Scale (GCS) score of 8 or less in the absence of medications known to affect GCS; and external ventricular drain (EVD) placement. To diminish the risk for population stratification skewing results, only white subjects were included in the final analysis. Patients with penetrating trauma and those with previous neurological impairment were excluded. Two independent cohorts were identified to provide a cohort for discovery and replication. Cohorts were built by date of injury (separated by approximately 4 years) from the same clinical site. Glasgow Outcome Scale (GOS) scores were collected by qualified neuropsychologists 3, 6, 12, and 24 months post-injury. The GOS ranges from 1 to 5, where a score of 1 corresponds to deceased, 2 corresponds to a patient in a persistent vegetative state, 3 corresponds to a patient with severe disability, 4 corresponds to a patient with minor disability, and 5 corresponds to little or no disability.¹⁴ To control for injury characteristics based on pathoanatomical data, computed tomography imaging data were reviewed for hemorrhage types associated with the injury. Patients were coded based on the presence or absence of one or more of the following: epidural hematoma; subdural hematoma; subarachnoid hemorrhage; intraparenchymal hemorrhage; intraventricular hemorrhage; and/or evidence of diffuse axonal injury.

Genotyping

DNA was isolated from cerebrospinal fluid (CSF) samples taken from the EVD or from blood samples using a commercially available kit (Qiaamp kit; Qiagen, Chatsworth, CA). Genotyping for *ABCG2* c.421C>A was performed with a TaqMan™ allelic discrimination assay for the discovery cohort (Applied Biosystems, Foster City, CA) and a mix of TaqMan + Illumina Human Core Exome v1.2 (Illumina, San Diego, CA) for the replication cohort. Genotype data quality were addressed by using technical replicates, blinded double calls of raw data, assessment for significant deviation from Hardy-Weinberg equilibrium, and compared with published genotype frequencies for the non-Finnish European population from the Broad Institute’s gnomAD Browser.¹⁵

Statistical analysis

Demographic data for the discovery and replication cohorts were compared using the *t*-test, chi-square test, or Fisher’s exact test. All genetic association studies were carried out using a dominant variant allele model (i.e., phenotype magnitude in CC < CA/AA). Subjects with missing hemorrhage-type data were coded with a dummy variable to account for the missing data while avoiding list-wise exclusion. To determine the association of *ABCG2* c.421C>A with outcomes, GOS score was treated as an ordinal variable¹⁶ and compared with mixed-effect multiple ordinal regression using the package Ordinal for R (R Foundation for Statistical Computing, Vienna, Austria).¹⁷ The assumption of proportionality of odds was tested by first dichotomizing GOS along four possible cut points [1|2-5, 1-2|3-5, 1-3|4-5, and 1-4|5], then controlled for age, sex, time, post-injury imaging determined hemorrhage types, and GCS with random effects of subjects with mixed-effects binomial logistic regression using the LME4 package for R (R Foundation for Statistical Computing).¹⁸ Finally, estimated 95% confidence intervals were compared for all coefficient estimates for each break point. The model was controlled for time post-injury, age, sex, time post-injury, imaging determined hemorrhage types, and GCS. Skewed continuous variables were log-transformed to improve model fit. Significance for random effects were determined with analysis of variance of a mixed model and a standard model excluding the random-effect variable. Results are presented as model parameters and odds ratios, where appropriate, which correspond to the odds of being in any higher GOS level. All statistical analyses were carried out using R software (v3.3.1; R Development Core Team, Vienna, Austria).

Results

The discovery and replication cohorts contained 270 and 166 subjects, respectively. Table 1 shows the demographic information for each cohort. Notably, subjects in the replication cohort were significantly older than the discovery cohort. The frequency of the “A” (variant) allele was higher in the replication cohort than the discovery cohort, though this did not reach statistical significance ($p=0.09$). *ABCG2* c.421C>A adhered to Hardy-Weinberg equilibrium in both cohorts separately and combined ($p>0.05$).

TABLE 1. DEMOGRAPHIC INFORMATION FOR DISCOVERY AND REPLICATION COHORTS

	Discovery cohort (N=270)	Replication cohort (N=166)	p value
Age Median (IQR)	33 (23–47)	36.5 (24–54)	0.03
Female (%)	21.1	22.3	0.86
GCS Median (IQR)	6 (4–7)	7 (5–7)	0.22
Hemorrhage type			
None	2 (0.7)	2 (1.2)	1
Epidural hematoma	29 (10.7)	17 (10.2)	0.99
Subdural hematoma	134 (49.6)	49 (29.5)	<0.001
Subarachnoid hemorrhage	132 (48.9)	43 (25.9)	<0.001
Intraparenchymal hemorrhage	65 (24.1)	39 (23.5)	0.98
Intraventricular hemorrhage	54 (20.0)	7 (4.2)	<0.001
Diffuse axonal injury	58 (21.5)	21 (12.7)	0.03
No imaging data	46 (17.0)	24 (14.5)	0.56
<i>ABCG2</i> c.421C>A (CC) N (%)	227 (84.1)	128 (77.1)	0.09
<i>ABCG2</i> c.421C>A (CA/AA) N (%)	43 (15.9)	38 (22.9)	

IQR, interquartile range (25–75%); GCS, Glasgow Coma Scale.

TABLE 2. DISCOVERY AND REPLICATION MIXED-EFFECTS ORDINAL REGRESSION

Factor	Discovery cohort (N=270)		Replication cohort (N=166)	
	Coefficient (95% CI)	p value	Coefficient (95% CI)	p value
<i>ABCG2</i> c.421C>A	22.03 (4.53, 39.53)	0.01	25.94 (4.47, 47.42)	0.02
ln(age)	−6.69 (−9.01, −4.37)	<0.001	−4.37 (−7.21, −1.53)	0.003
<i>ABCG2</i> c.421C>A * ln(age)	−5.58 (−10.56, −0.60)	0.03	−8.03 (−14.18, −1.88)	0.01
GCS score	2.18 (1.55, 2.82)	<0.001	1.47 (0.68, 2.27)	<0.001
Hemorrhage type				
None	10.14 (0.95, 19.34)	0.03	2.19 (−7.05, 11.43)	0.64
Epidural hematoma	−0.37 (−2.99, 2.24)	0.78	5.40 (1.54, 9.26)	0.01
Subdural hematoma	−0.56 (−2.46, 1.35)	0.57	−1.99 (−4.78, 0.79)	0.16
Subarachnoid hemorrhage	−1.59 (−3.45, 0.28)	0.10	0.85 (−1.76, 3.47)	0.52
Intraparenchymal hemorrhage	1.90 (−0.18, 3.98)	0.07	1.25 (−1.72, 4.22)	0.41
Intraventricular hemorrhage	0.52 (−1.69, 2.72)	0.65	0.19 (−5.11, 5.50)	0.94
Diffuse axonal injury	1.48 (−0.79, 3.75)	0.20	1.04 (−2.29, 4.37)	0.54
No imaging data	−2.55 (−5.57, 0.48)	0.10	2.32 (−1.42, 6.05)	0.22
Sex (M vs. F)	1.01 (−0.99, 3.01)	0.32	−0.52 (−3.07, 2.02)	0.68
Months post-injury	0.12 (0.09, 0.14)	<0.001	0.09 (0.05, 0.13)	<0.001
Subject (random effect)	N/A	<0.001	N/A	<0.001

GCS, Glasgow Coma Scale; M, male; F, female; N/A, not applicable.

Calculated minor allele frequencies (MAFs) were 7.96%, 12.35%, and 9.63% in the discovery, replication, and combined cohorts, respectively. These did not significantly deviate from MAF for *ABCG2* c.421C>A in the non-Finnish European population based on the gnomAD Browser (discovery $p=0.08$, replication $p=0.27$, combined $p=0.52$).¹⁵

The mixed-effects ordinal regression model was built with covariates age, sex, time post-injury, imaging determined hemorrhage types, and GCS forced in the model to control for injury severity and patient characteristics that might impact recovery independently. Covariates were individually tested with genotype for two-way interactions. The assumption of proportionality of odds was met in all models. The final model for the discovery cohort, and replication cohorts are summarized in Table 2. We found a significant association with the “A” allele in both cohorts ($p=0.01$ [discovery] and $p=0.02$ [replication]). Additionally, we found a significant interaction between the presence of the “A” allele and age ($p=0.03$ [discovery] and $p=0.01$ [replication]). Finally, both cohorts were combined into a final group and assessed with the same model covariates. The coefficient for the conditional (genotype) effects were positive (23.56; $p<0.001$), and the coefficient of the interaction was negative (−6.69; $p<0.001$), suggesting that the effect of genotype is mitigated by increasing age. The final full model is summarized in Table 3.

The odds of a favorable outcome were much higher in younger subjects with at least one variant allele at *ABCG2* c.421C>A, and this effect is adjusted with age. The age at which the effect of age completely mitigated the genotype effect was calculated by solving for age in: $1 = e^{\beta_{\text{genotype+age}} \ln(\text{age})} * e^{\beta_{\text{genotype}}}$ where β refers to coefficients from the combined cohort model defined by the subscript. This simplifies to: $\text{age} = e^{\left(\frac{\beta_{\text{genotype}}}{\beta_{\text{genotype+age}}}\right)}$, which is approximately ~34 years, after which having at least one variant allele at *ABCG2* c.421C>A is predictive of a poor outcome compared to those with nonvariant alleles. Figure 1 shows the proportion of subjects with good (GOS, ≥ 4) outcomes broken up by age >34 and genotype. To determine the odds ratio associated with genotype conditional upon subject age, odds ratios for heterozygote and homozygote versus wild type were plotted with increasing age bound by interquartile range (Fig. 2).

Discussion

In this study, we sought to determine the association of *ABCG2* c.421C>A with outcomes after severe TBI by utilizing a robust discovery-replication method. This allowed for replication in an independent cohort and strengthens the findings. We found that subjects with at least one variant (A) allele at *ABCG2* c.421C>A had improved odds of having a higher GOS score, and that the beneficial effect was mitigated by increasing age to a point of inflection at ~34 years old. Based on the observed data versus the model, it appears more likely that the genotype effect is present in younger individuals, and is not an important factor in the older population, rather than a reversal of association in subjects >34 years old.

TABLE 3. MODEL FOR COMBINED DISCOVERY AND REPLICATION COHORTS

Factor	Coefficient	SE (95% CI)	p value
<i>ABCG2</i> c.421C>A	23.56	6.68 (10.46, 36.66)	<0.001
ln(age)	−5.93	0.92 (−7.73, −4.12)	<0.001
<i>ABCG2</i> c.421C>A * age	−6.69	1.89 (−10.39, 2.99)	<0.001
GCS Score	1.89	0.25 (1.40, 2.38)	<0.001
Hemorrhage type			
None	5.54	3.35 (−1.03, 12.12)	0.10
Epidural hematoma	2.12	1.09 (−0.01, 4.25)	0.05
Subdural hematoma	−0.90	0.76 (−2.39, 0.59)	0.24
Subarachnoid hemorrhage	−0.45	0.75 (−1.92, 1.02)	0.55
Intraparenchymal hemorrhage	1.45	0.85 (−0.22, 3.12)	0.09
Intraventricular hemorrhage	0.47	1.02 (−1.52, 2.46)	0.64
Diffuse axonal injury	1.16	0.97 (−0.74, 3.05)	0.23
No imaging data	−0.31	1.15 (−2.56, 1.95)	0.79
Sex (M vs. F)	0.29	0.81 (−1.29, 1.87)	0.72
Months post-injury	0.11	0.01 (0.09, 0.13)	<0.001
Subject (random effect)	N/A	N/A	<0.001

GCS=Glasgow Coma Scale; M, male; F, female; N/A, not applicable.

FIG. 1. Percent of individuals with a favorable (GOS, 4–5) outcome at each time point based on the presence of one or two variant alleles. Plot is broken at the age of inflection (34 years) to demonstrate the mitigation of the association with advancing age. Data based on combination of discovery and replication cohorts analyzed in a dominant variant allele model to ease interpretation. * $p < 0.05$; ** $p < 0.01$. GOS, Glasgow Outcome Scale.

The molecular phenotype of *ABCG2* c.421C>A is decreased *ABCG2* protein expression attributed to improper folding, ubiquitination, and proteasome mediated degradation.¹⁹ The consequence of carrying a variant allele at *ABCG2* c.421C>A is decreased expression to half that of wild-type *ABCG2*.²⁰ Variant alleles are associated with higher risk for gout attributed to decreased renal efflux of the *ABCG2* substrate, uric acid. This leads to hyperuricemia and potentially higher brain levels of uric acid. Converse to its pathological roles, uric acid is a potent neural antioxidant. Matsuo and colleagues investigated the role of *ABCG2* c.421C>A in the onset of Parkinson's disease under the hypothesis that increased uric acid in the brain would provide neuroprotection. They found that patients with at least one variant (A) allele developed Parkinson's disease 1.6 years later than those without a variant allele ($p = 0.025$).²¹ This finding suggests that dysfunction of *ABCG2* on the BBB might confer neuroprotection, which might direct new treatment options for TBI.

A potential explanation for our findings might be that people who have at least one variant allele at *ABCG2* c.421C>A have decreased efflux of uric acid from the brain and thus higher brain concentrations of uric acid. Increased concentrations may promote brain recovery post-TBI.^{22,23} Uric acid elevation therapy has been proposed as a potential treatment option for multiple neurological pathologies because of its neuroprotective effects.²⁴ Clinical trials have already investigated the use of exogenous uric acid as a treatment for ischemic stroke and have provided evidence that uric

acid therapy might prevent early ischemic worsening compared to placebo ($p = 0.01$).²⁵ This is supported by uric acid's endogenous antioxidant activity in the brain and its role in mitigating synaptic glutamate levels through upregulation of the glial glutamate transporters, SLC1A3 (EAAT-1) and SLC1A2 (EAAT-2). Du and colleagues also found that inhibition of these transporters in an *in vitro* model for spinal cord injury promoted apoptosis. Thus, combining the antioxidant roles of uric acid with its antiglutamate effects strongly promotes the ability of uric acid and thus *ABCG2* in protecting the brain following injury.^{26,27} Counter to this theory, Maetzler and colleagues evaluated *ABCG2* c.421C>A along with variations in other uric acid transporters (SLC17A3 and SLC2A9) in patients with Lewy body disorders (LBD). They did not find an association with *ABCG2* c.421C>A, but rather found that the "T" allele at the SLC17A3 SNP, rs1165205, was associated with higher CSF uric acid levels, and that subjects with LBD had lower uric acid levels in serum and CSF than controls.²⁸ However, uric acid baseline serum levels are age dependent, and the population with LBD is generally older than study populations with TBI.²⁹ It is also plausible that the role of *ABCG2* in regulation of amyloid-beta could explain some of our findings. A study by Jullienne and colleagues found amyloid-beta accumulation in their model for juvenile TBI.³⁰ The polymorphism, *ABCG2* c.421C>A, is thought to impact the regulation of amyloid-beta and may confer protection against Alzheimer's disease.^{12,31,32} Indeed, genetic risk factors for Alzheimer's disease (e.g., *APOE* $\epsilon 4$) are also risk factors for poor

FIG. 2. Odds ratios for any increase in GOS score compared to reference for having one (CA) or two (AA) variant alleles plotted with increasing age. Data based on combination of discovery and replication cohorts analyzed in a dominant variant allele model to ease interpretation. Error lines represent 95% confidence intervals. GOS, Glasgow Outcome Scale.

clinical outcomes from TBI, potentially related to similar amyloid- β plaque pathology.³³

Hukkelhoven and colleagues recently showed that advancing age independently predicts poor outcome. They found that an age threshold of 39 years old provided the best prediction for poor outcomes and mortality.³⁴ Our findings provide supportive data that the recovery trajectory post-TBI changes in the fourth decade of life. Future study will seek to explain the mitigation of genotype effect that happens in patients >34 years old. Given that this explanation is speculative, it does provide insight into the potential changes in uric acid generation and metabolism with age. This might relate to changes in CNS plasticity and response to injury that occur at this age. It is also possible that this is related to a change in microglia polarization (i.e., M1 vs. M2 phenotype), though this area remains controversial.^{35,36} The response to brain levels of uric acid may be age related, wherein younger individuals might exhibit increased sensitivity to its role in the CNS antioxidant reserve. Finally, age-related differences in expression of compensatory mechanisms (i.e., other transport systems that compensate for ABCG2) might limit the benefit of having variant alleles. Full understanding of this finding requires further mechanistic study into the biochemical disposition of patients suffering from TBI in the context of genotype at *ABCG2* c.421C>A.

A limitation of our study is the single-center study design. However, both groups were independently sampled at different time points, allowing for a classical experimental replication. Variation in fixed effects, while showing the same association with similar magnitude across two heterogeneous cohorts, is a strength of the findings, though it might challenge generalizability. Similarly, the interaction between genotype and age is interesting, but requires further study given that it could result from population stratification. Finally, although replication of results in a separate cohort provides strong support of the finding, it is not possible to establish causation from a retrospective evaluation. Further investigation into the molecular mechanism associated with the genetic association might better explain the results of this preliminary study.

Conclusion

An association of *ABCG2* c.421C>A with outcomes following severe TBI was identified in a discovery and replication cohort. Mixed-effects multiple ordinal regression with GOS scores as the dependent variable showed an age*genotype interaction, suggesting that having at least one *ABCG2* variant allele was predictive of improved GOS score in subjects <34 years old, and of similar or lower GOS scores in subjects >34 years old. *ABCG2* function may impact brain recovery pathways after injury in an age-dependent manner.

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Author Disclosure Statement

No competing financial interests to disclose.

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Address correspondence to:
 Philip E. Empey, PharmD, PhD
 Pharmacy and Therapeutics, School of Pharmacy
 University of Pittsburgh
 205 Salk Pavilion
 335 Sutherland Drive
 Pittsburgh, PA 15261
 E-mail: pempey@pitt.edu