The influence of CYP2B6 variants on traumatic brain injury patient outcome after rapid sequence intubation

by

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Bachelor of Science in Nursing, University of Pittsburgh, 2022

Submitted to the Graduate Faculty of the

University Honors College in partial fulfillment

of the requirements for the degree of

Bachelor of Philosophy

University of Pittsburgh

2022

UNIVERSITY OF PITTSBURGH

UNIVERSITY HONORS COLLEGE

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November 22, 2022

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A traumatic brain injury (TBI) is a disruption in normal brain function due to any disturbance to the head. These injuries are usually devastating; leading to lifetime disability or early death. It has been found that genetic polymorphisms can impact outcomes after a TBI. A treatment that is commonly used in these patients is sedation – usually with an opioid or propofol. This study aimed to investigate the relationship of the *CYP2B6* polymorphism and the outcomes of TBI patients while also considering propofol administration. The cytochrome P450 enzyme CYP2B6 is involved in the biotransformation of many drugs, including anesthetic agents. Studies have found that variants of *CYP2B6* can lead to decreased systematic clearance of propofol. This could potentially lead to life-threatening complications. Despite this knowledge there have been no formal investigations regarding this polymorphism's effect on drug administration and TBI patient outcome, warranting a need for a study of this nature.

This study is a retrospective analysis of prospectively collected participants from the Brain Trauma Research Center (BRTC) in the Department of Neurosurgery at the University of Pittsburgh Medical Center (UPMC) Presbyterian Hospital. Inclusion criteria for this database included: a severe blunt TBI, Glasgow Coma Scale (GCS) score 3-8, and not following commands. Participants also met the additional inclusion criteria of permission to obtain a genetic sample. With this criteria, participants (n=440) were further phenotyped by collecting the drugs admitted to each patient during their hospital stay. Allelic Discrimination of *CYP2B6* was performed using two SNPs (rs2279343 and rs3745274) through Taqman Assays. Neurological outcomes were evaluated using the Glasgow Outcome Scale (GOS) and Disability Rating Scale

(DRS). Individuals with the rs3745274 TT genotype and rs2279343 GG genotype were more likely to have worse outcomes. This could be due to decreased metabolism or systematic clearance of drugs. These findings suggest that *CYP2B6* may impact patient outcome through drug metabolism and further investigation should be done regarding the role of *CYP2B6* variants on TBI patient outcome.

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PREFACE

I would like to start off by thanking everybody involved in this process for being so flexible. There were a lot of speed bumps in the road, but I'm thankful for their commitment and always believing I could reach this goal. I would like to thank my committee members Dr. Ava Puccio, Dr. Dianxu Ren, and Dr. Ruchira Jha for their time, support, and expertise. I would also like to thank Sandra Delouches for her patience and support in the lab. As well as teaching me what I needed to know to successfully complete my data collection. I would like to thank the patient's and families who enrolled in this study and continuously contribute to the development of medical knowledge. Finally, I would like to extend the greatest gratitude to my chair of my dissertation, Dr. Yvette Conley. Without Dr. Conley's support and guidance, this study would not exist.

1.0 BACKGROUND

A traumatic brain injury (TBI) is defined as a disruption in normal brain function caused by any disturbance to the head. [1-2] TBI is a leading cause of death and disability in the United States, accounting for approximately 2.87 million emergency department visits, hospitalizations, and deaths during 2014. [2] In 2021, the National Institutes of Health report approximately 51,000 deaths due to TBI related injuries. [14] The Centers for Disease Control and Prevention have stated over the span of eight years, from 2006 to 2014, emergency department visits related to TBI have increased 54%, while hospitalization rates have decreased by 8% and death rates have decreased by 6%. [2] This can suggest an increase in TBI-related survivals, meaning an increase in the knowledge of TBI treatment and the need for additional treatments for this patient population. Even after initial TBI-related hospitalization, half of TBI patients will experience a decline in their quality of daily life, or die, within 5 years. [3] Those alive 5 years after their TBI still feel the presence of their injury as 57% are moderately to severely disabled, 55% are unemployed, and 50% are rehospitalized at least once. [3]

There are substantial differences in the severity of different TBIs and the short-term and long-term effects. [2-4] TBI patients can have lifelong symptomology, such as headaches and sleep disorders, as well as memory problems, depression, and slower thinking. [4] The severity of a TBI is usually assessed through the length of loss of consciousness, the length of memory loss, how responsive the patient was after the injury occurred, and a computed tomography (CT) scan, pupil reactivity, and Glasgow Coma Score (GCS). [4] The CT scan shows the presence of brain bruising, bleeding, or swelling. [4] Most people experience a mild TBI, or concussion, but some people also experience moderate or severe TBIs. [2,4]

For the purposes of this study, the focus is on severe TBI. Studies have shown genetic polymorphisms may have a potential influence on treatment outcomes after severe TBI. [5] Studies have shown after the initial injury, patients are likely to suffer secondary injury to their brain due to hypoxia, hypercapnia, and/or hypotension. [6, 8] The current medical management for severe TBI patients includes sedation of the patient to reduce intracranial pressure (ICP), optimize ventilation, and reduce the patient's potential stress response. [6-7] Common sedatives given to patients include propofol, benzodiazepines, and opioids. [7]

Propofol has become a first-line sedative for TBI patients in the intensive care unit (ICU) due to its rapid onset and short duration of action, minimal effect on renal and hepatic systems, and its neuroprotective properties including the ability to decrease ICP. [8] Although disadvantages of this drug include the absence of amnesia at low doses, no analgesic effects, tolerance, decreased mean arterial pressure (MAP) and cerebral perfusion pressure (CPP), and propofol infusion syndrome. [8-9] Propofol infusion syndrome is a rare, fatal complication characterized by severe metabolic acidosis and circulatory collapse. [8] Risk factors for propofol infusion syndrome include use greater than 48 hours, dosage greater than 4 mg/kg/hour, neurologic pathologies, inadequate diet, and young age. [8] Propofol infusion syndrome has also been found to occur at higher rates in children than adults. [8] Therapeutic hypothermia, which is commonly used in the treatment of severe TBI patients, has also been shown to precipitate propofol infusion syndrome in patients receiving doses less than 4 mg/kg/hour. [8]

The purpose of this proposed study is to examine the relationship between *CYP2B6* variants and the outcomes of TBI patients while also taking propofol administration into account. The cytochrome P450 enzyme CYP2B6 is involved in the biotransformation of drugs, especially the biotransformation of some anesthetic agents, including propofol and ketamine. [10 - 11] The

most common functionally deficient allele is *CYP2B6*6* [Q172H, K262R], which occurs at frequencies of 15 to 60% in different populations. [11] This allele, which leads to slower metabolism of propofol, leads to lower expression in the liver as alternative splicing on mRNA leads to a reduction in the activity of the enzyme. [10-12] Slower metabolism of propofol could lead to systemic build-up, which could put patients at an increased risk of developing propofol infusion syndrome. [13- 14]

Although variants of the *CYP2B6* gene have been shown to disrupt normal functioning of anesthetic metabolism, and the multiple studies done concerning anesthetic use in TBI patients, there has been little research done combining both. During my literature search, no results were found concerning *CYP2B6* variants and the outcomes of severe TBI patients after receiving propofol during their hospital stay. Also, there were only 12 results when searching *CYP2B6* variants and propofol. This shows there is a gap in the literature. It has been acknowledged that *CYP2B6* variants could cause decreased metabolism of propofol, as well as long-term sedation of TBI patients with propofol could potentially lead to adverse outcomes, creating a reason to investigate the relationship between variants of the *CYP2B6* polymorphism and outcomes of severe TBI patients while taking propofol administration into account.

2.0 PURPOSE

The aim of this study is to explore the relationship of *CYP2B6* variants on the outcome of TBI patients after receiving propofol throughout their initial hospital stay after injury. This study helps expand the knowledge base surrounding genetic influence on TBI outcomes, as well as drug metabolism. This study examines patient outcomes 3- and 6-months post-injury, as well as acute data throughout the patient's initial hospital stay. Patient outcome measures include the disability rating scale (DRS), and the Glasgow Outcome Scale (GOS) as granular neurological outcomes. The goal of this project is to initiate this area of research so that eventually findings can influence patient care regarding the use of certain anesthetics based on precision medicine and the chance that genetics may influence patient outcomes, especially at the beginning of the patient's care. The development and use of particular practices regarding anesthetic use in TBI patients could be promising in promoting better outcomes. Also acknowledging propofol infusion syndrome as a potentially fatal complication in this population.

3.0 EXPECTED OUTCOMES

In accordance with the role the *CYP2B6* polymorphism plays on the decreased metabolism of propofol and impacted metabolism on other drugs, the expected outcome is TBI patients with *CYP2B6* variants, specially the *6*6* variant, will have significantly poorer outcome at the 6-month outcome measure compared to patients who have different variants. Another expected outcome is patients who have variants of the *CYP2B6* polymorphism and receive propofol will have significantly poorer outcomes than those who also have *CYP2B6* variants but received a different sedative. The DRS measures are anticipated to emulate the results of the GOS and contribute a better understanding of the correlation between *CYP2B6* variants and outcomes of severe TBI patients while considering propofol administration.

4.0 METHODS AND MEASUREMENTS

4.1 Participants

The University of Pittsburgh Institutional Review Board approved the parent study from which the data and biospecimens were derived. Written informed consent was given from the next of kin. If the patient recovered cognitively by the follow-up visit, they were approached for continued participation consent. This ongoing study includes patients admitted to the University of Pittsburgh Medical Center Presbyterian Hospital neuro intensive care unit. The parent study investigates genomic and epigenomic changes that occur after severe TBI through the genetic analysis and collection of cerebrospinal fluid (CSF) and blood. Inclusion criteria to be met included a closed head injury with a hospital-admission Glasgow Coma Score (GCS) ≤8 prior to the administration of paralytics or sedatives, aged 16 to 80 years old, not brain dead, and draining cerebrospinal fluid (CSF) via external ventricular drain as standard of care. Participants were followed for 24 months following injury to measure a GOS, and DRS at 3, 6, 12, and 24 months, although for this study only 3 month and 6-month outcomes were studied. We currently have data on 464 patients admitted with a severe TBI and these individuals also have DNA available for genotyping and outcome data available for analyses.

After determining eligibility and receiving informed consent, initial GCS scores, demographic information, and record of drugs received throughout the patient's hospital stay were extracted from the medical record. Hospital staff assisted in collecting blood and CSF specimens and alerted research staff when samples were ready to be picked up for processing in

the genetic labs. Samples were processed according to lab protocol and then frozen for future DNA extraction.

4.2 Patient Assessment

For participants to meet eligibility criteria for the study, they had to have a severe TBI indicated by a GCS ≤8 upon initial assessment of the patient upon arrival to the hospital. GSC measures coma severity based on eye opening, verbal response and motor response. [17] Participants were followed for two-years post-injury to record additional long-term data. Evaluations were completed by neuropsychological technicians under the direction of a neuropsychologist at the Brain Trauma Research Center (BTRC) at the University of Pittsburgh Medical Center Presbyterian Hospital at 3 and 6-months after the injury. The evaluations consisted of a neuropsychological battery including the Glasgow Outcome Scale (GOS) and Disability Rating Scale (DRS). The GOS-Extended was performed in later enrollment years; however, to obtain a larger sample set, the GOS score was used for this analysis.

The GOS investigates a participant's ability to function independently and care for themselves. The GOS is rated based on the following scale: 1 = death, 2 = persistent vegetative state, 3 = severe disability, 4 = moderate disability, 5 = good recovery. [18]

The DRS is used to evaluate participant's functional outcomes and abilities after a TBI. The participants are rated on a scale of 0-30, where a score of 0 is indictive of no disability, while a score of 30 indicates death. There are three primary categories of impairment studied including impairment, disability, and handicap. There are seven subcategories including eye-

opening, motor responses, communication, cognitive skill necessary for self-care, physical/cognitive abilities, over-all dependence, and employment. [19]

4.3 Genotype Data Collection

For this study, DNA samples were extracted from one of two sources. The preferred source was 10mL whole venous blood. The blood was centrifuged to isolate the white blood cells, then DNA was extracted using the salting out protocol, previously described. The secondary source was DNA extracted from CSF drainage into a ventriculostomy bag that would have otherwise been discarded. The DNA was extracted using the instructions from the manufacturers of the Qiamp Midi kit (Qiagen, Valencia, CA, USA). All DNA samples were stored at 4°C in 1x TE buffer.

The *CYP2B6*6* allele was identified by genotyping two single nucleotide polymorphisms (SNPs; rs3745274, 516 G>T and rs2279343, 785A>G). Both of these polymorphisms were genotyped using Taqman Allele Discrimination Assays using standard long protocols: 23µl of master mix (see Table 1 for composition) and 2µl of a working dilution of genomic DNA were combined and loaded into the PCR system for cycling with the following conditions: a hold cycle of 95.0°C for 10 minutes, 50 cycles of the PCR stage of 95°C for 15 seconds and 58°C for 90 seconds, then a post-read stage of 60°C for 30 seconds, followed by an indefinite hold at 10°C. Each allele was assigned a specific fluorescent symbol (shown in Figure 1.0). For rs3745274, homogenous GG individuals were assigned FAM on the x-axis, while homogenous TT individuals were assigned VIC on the Y-axis and heterozygotes having one FAM and one

VIC labeled allele were clustered in the middle. For rs2279242, homogenous GG individuals were assigned FAM on the x-axis, homogenous AA individuals were assigned VIC on the Y-axis and heterozygotes having one FAM and one VIC labeled allele were clustered in the middle. For quality control, data was double called by two individuals and non-concordance rectified by re-visiting the raw data or re-genotyping. Negative controls were included and significant deviation from Hardy Weinberg Equilibrium was tested.

Table 1. Master Mix Preparation for 20x Assay (rs3745274)

Sterile water	10 µl
20x Concentration Assay (rs3745274)	1.25 µl
TaqMan Universal PCR Master Mix No AmpErase	12.5 µl
Extracted DNA	2 µl
	25 µl per sample

Table 2. Master Mix Preparation for 40x Assay (rs2279242)

Sterile water	10 µl
40x Concentration Assay (rs2279242)	0.625 µl
TaqMan Universal PCR Master Mix No AmpErase	12.5 µl
Extracted DNA	2 µl
	25 µl per sample

Figure 1.0 Example of Taqman Results
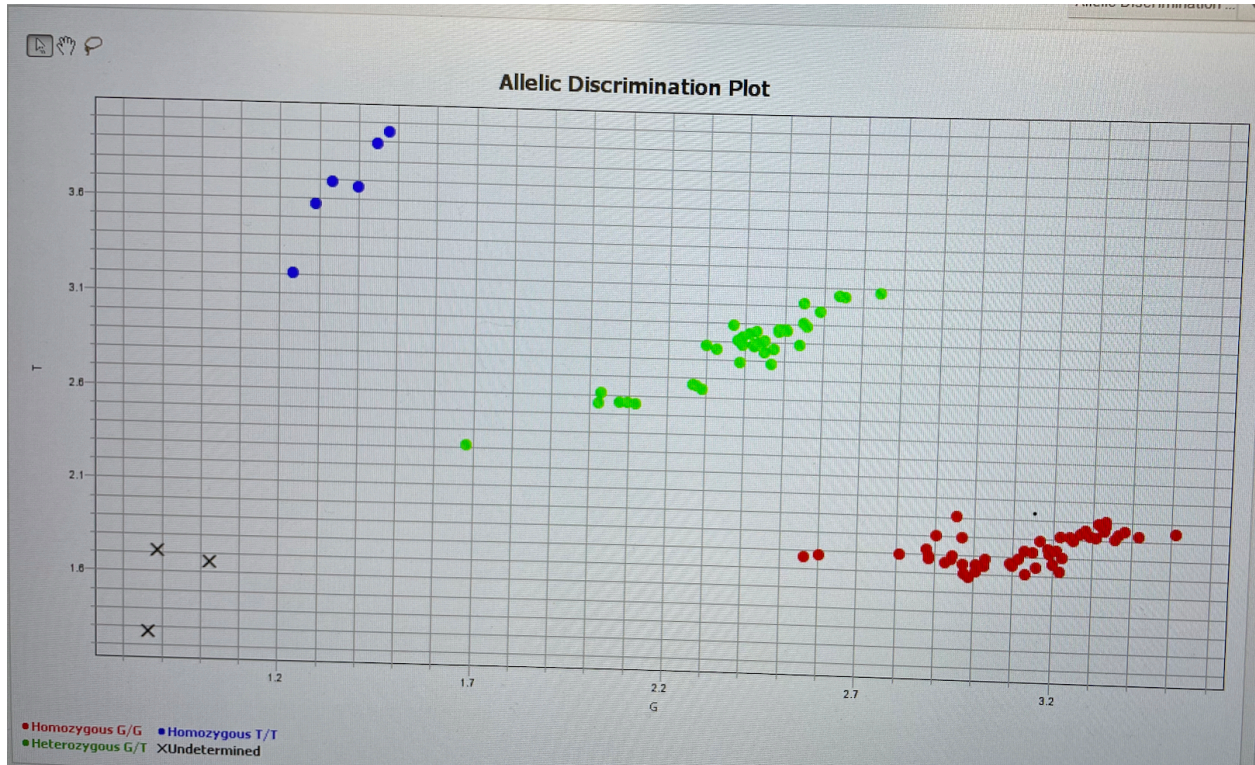
4.4 Statistical Analysis

The independent variable in this study was the *CYP2B6* polymorphism variant, as well as if propofol was given to the patient or not. The dependent variables are GOS, and DRS outcomes. Covariates include sex, age, extent of injury (GCS), and comorbidities. Because the anesthetic used was also investigated, a retrospective review of participants' anesthesia records were collected in order to analyze the data concerning the propofol use, patient outcome, and patient genetics. Since GOS can be dichotomized into poor outcomes (GOS 1,2) and favorable outcomes (GOS 3, 4, 5) logistic regression models can be used as an analytical approach. Each GOS time point will be analyzed separately. The GOS and DRS were analyzed at 3-months and 6-months using a chi-squared test, and then analyzed by both propofol use and genotype using a two-way

ANOVA test. Tests were also run to control for propofol and genotype to investigate

relationships between these variables. Findings were considered significant if the p-value was

≤0.05. 95% confidence intervals and odds ratios were also calculated.

This final sample set analyzed was comprised of 440 participants with clinical data and
genetic samples. The average age was 37.5 years (range 16-77), with the population being
mostly white (n=407) males (n=347) with 80.9% receiving propofol (n = 356). The most
common rs2279343 genotype being AA (n=188) and most common rs3745274 genotype being
GG (n=210). GCS scores were dichotomized into severity of injury, with GCS 3-4 being
compared to a GCS of 5-8. Of the participants, 24.4% had a GCS of 3-4 and 75.6% had a GCS of
5-8. Hardy Weinberg equilibrium was met by both SNPs (rs2279343 and rs3745274). Additional
demographic data for each SNP is shown in Table 3 and Table 4 below.

Table 3. Sample Demographics

Characteristics	TBI (n=440)
Age (years; mean ± SD)	37.51 ± 16.78
Sex	
Male	347 (78.86%)
Female	93 (21.14%)
Race	
White	407 (92.5%)
Asian, Indian	3 (0.68%)
Black, African American	29 (6.59%)
Other	1 (0.23%)
Glasgow Coma Scale	
3-4	107 (24.43%)
5-8	331 (75.57%)

Table 4. Sample Genotypes

Genotype	TBI (n=440)
rs3745274	n= 396
GG	210 (53.03%)
GT	146 (36.87%)
TT	40 (10.1%)
rs2279343	n= 375
AA	188 (50.13%)
AG	154 (41.07%)
GG	33 (8.8%)

Table 5 outlines the frequency of GOS by genotype. There were no significant findings related to genotype on the frequency of mortality at each timepoint (p-value ≥ 0.05). Though, it is interesting to point out there is a decrease in the amount of participants with a severe disability to death, characterized by a GOS of 1-3.

Table 5. Frequency of Mortality (GOS) by Genotype

GOS	rs3745274	rs2279343
3-month	(n=367)	(n= 347)
1-3	n=285 (77.66%)	n=271 (78.1%)
4-5	n=82 (22.34%)	n=76 (21.9%)
6-month	(n=363)	(n=343)
1-3	n=250 (68.87%)	n = 234 (68.22%)
4-5	n=113 (31.13%)	n= 109 (31.78%)

After analysis using an Analysis of Maximum Likelihood Estimates, propofol is found to be significant for GOS in the 3-month timepoint (p-value = 0.0432). It was also found that

rs3745274 genotype GT is significant for better outcomes than GG in GOS 6-month outcome (p-value = 0.0451). There is also borderline significance with rs2279343 genotype AA (p-value =0.0625) with GOS outcomes at three months. As well as borderline significant with rs3745274 genotype GG at 6-month GOS (p-value = 0.0529). Then using an Odds Ratio Estimate, it was shown that there is a less likely chance for getting worse outcomes with rs3745274 genotypes GG and GT than genotype TT and rs2279343 genotypes AA and GA versus genotype GG at all time-points.

Table 6. Analysis of Maximum Likelihood Estimates for GOS for rs3745274

GOS	Estimate	Standard Error	Wald Chi-Square	p-value
3-month GOS				
GG	-0.8310	0.5297	2.4615	0.1167
GT	-0.6052	0.5454	1.2313	0.2672
6-month GOS				
GG	-0.9401	0.4856	3.7480	0.0529
GT	-1.0003	0.4991	4.0160	0.0451

Table 7. Analysis of Maximum Likelihood Estimates for GOS for rs2279343

GOS	Estimate	Standard Error	Wald Chi-Square	p-value
3-month GOS				
AA	-1.4359	0.7708	3.4697	0.0625
GA	-1.3095	0.7764	2.8445	0.0917
6-month GOS				
AA	-0.8891	0.5582	2.5372	0.1112
GA	-1.0117	0.5640	3.2180	0.0728

Table 8. rs2279343 Odds Ratio Estimates for GOS

GOS	Point Estimate	95% Wald Confidence Limits

3- month GOS			
rs2279343 AA vs GG	0.238	0.053	1.078
rs2279343 GA vs GG	0.270	0.059	1.236
6- month GOS			
rs2279343 AA vs GG	0.411	0.138	1.227
rs2279343 GA vs GG	0.364	0.120	1.098

Table 9. rs3745274 Odds Ratio Estimates for GOS

GOS	Point Estimate	95% Wald Confidence Limits	
3- month GOS			
rs3745274 GG vs TT	0.436	0.154	1.230
rs3745274 GT vs TT	0.546	0.187	1.590
6- month GOS			
rs3745274 GG vs TT	0.391	0.151	1.012
rs3745274 GT vs TT	0.368	0.138	0.978

The same tests were also done for DRS at 3-month and 6-month timepoints. These findings also align with GOS score, with participants with rs3745274 genotypes GG and GT exhibiting better outcomes versus those with genotype TT. As well as participants with rs2279343 genotypes AA and GA exhibiting better outcomes than those with genotype GG. Propofol was also found to be significant for participants to receive better outcomes with 6-month DRS (p-value = 0.0257) and borderline significance with 3-month DRS (p-value= 0.0532).

Table 10. Analysis of Maximum Likelihood Estimates for DRS for rs3745274

DRS	Estimate	Standard Error	Wald Chi-Square	p-value
3-month DRS				
GG	-0.1041	0.4122	0.0638	0.8007
GT	-0.0750	0.4233	0.0314	0.8594
6-month DRS				
GG	0.0597	0.4052	0.0217	0.8829
GT	0.0627	0.4190	0.0224	0.8811

Table 11. Analysis of Maximum Likelihood Estimates for DRS for rs2279343

DRS	Estimate	Standard Error	Wald Chi-Square	p-value
3-month DRS				
AA	-0.4373	0.4713	0.8611	0.3534
GA	-0.4315	0.4767	0.8191	0.3654
6-month DRS				
AA	-0.1171	0.4456	0.0691	0.7927
GA	-0.2040	0.4531	0.2028	0.6524

Table 12. rs2279343 Odds Ratio Estimates for DRS

DRS	Point Estimate	95% Wald Confidence Limits	
3- month DRS			
rs2279343 AA vs GG	0.646	0.256	1.626
rs2279343 GA vs GG	0.650	0.255	1.654
6- month DRS			
rs2279343 AA vs GG	0.889	0.371	2.130
rs2279343 GA vs GG	0.815	0.336	1.982

Table 13. rs3745274 Odds Ratio Estimates for DRS

DRS	Point Estimate	95% Wald Confidence Limits	
3- month DRS			
rs3745274 GG vs TT	0.901	0.402	2.021
rs3745274 GT vs TT	0.928	0.405	2.127
6- month DRS			
rs3745274 GG vs TT	1.062	0.480	2.349
rs3745274 GT vs TT	1.065	0.468	2.420

6.0 DISCUSSION

The aim of this study was to investigate the relationship of *CYP2B6* variants on the outcome of severe TBI patients, especially regarding propofol drug metabolism. *CYP2B6* is heavily involved in drug metabolism, especially the metabolism of frequently used anesthetics. Variants of this gene can cause adverse drug reactions, though little research has been done regarding the impact this gene may have on severe TBI patients in which an adverse drug reaction could be potentially life-threatening.

Participants in this study reflect national demographic trends, as well as those commonly seen in the Pittsburgh region, with the study being mostly composed of white males. Of the 440 participants, 92.5% were white, 78.9% were male and the average age was 37.5 years old. Distribution of the rs2279343 SNP was AA 50.1%, GA 41.1%, and GG 8.8%. Distribution of the rs3745274 was GG 53%, GT 36.9%, and TT 10.1%

The variations of *CYP2B6* were collected using two SNPs rs2279343 and rs3745274. The rs374524 variant is associated with the *CYP2B6*9* allele. This allele causes decreased function to no function of the protein. [21] GG is the wildtype. The rs2279343 variant is found as the *CYP2B6*4* allele alone, as well as in combination with haplotypes. This allele is known to have normal to increased function. [22] Increased function alleles lead to greater metabolism and increased clearance of the drug, in which patients potentially need higher doses of the drugs. The AA is the wild type.

One of the most common and studied *CYP2B6* variant is the *CYP2B6*6*, which is a combination of *CYB2B6*4* and *CYP2B6*9*. The rs3745274 SNP is also commonly used to describe *CYP2B6*6*. This allele is known to have decreased function and protein expression.

[22] It is also the most common polymorphism of the gene. Phenotypically, GG homozygotes are rapid metabolizers, GT heterozygotes have intermediate activity of the enzyme, and TT homozygotes are slow metabolizers. [23] For pharmacologic considerations, GG homozygotes should be given an increased dose of the drug, GT heterozygotes should be given the therapeutic dose of the drug, and TT homozygotes should be given a decreased dose and the drug has the potential to be toxic to the patient. [23]

From the analysis of the data, it was found that there was no significant interaction between genotype and propofol. Although, when controlling for propofol, it was found that rs3745274 GG and GT genotypes were less likely to have worse outcomes when compared to the TT genotype at 3-month GOS. The GG genotype phenotypically are rapid metabolizers – this means that patients will have active metabolites of particular drugs in their system for a smaller amount of time. In terms of drug administration, this means that this population will need increased doses to get the expected effect. Phenotypically, those with the GT genotype are normal metabolizers. It was also found that, when controlling for propofol, the rs2279343 variant AA and AG genotypes were less likely to have worse outcomes when compared to the GG genotype at 3-month GOS. For severe TBI patients in the ICU, this could lead to decreased sedation and increased ICP levels which can impact the patient's recovery. This could also lead to an increased risk of complications. Worse outcomes refer to having a lower GOS, which means that patients need constant help with activities of daily living, unable to be independent, and their level of restriction has changed since before their TBI. Also, this means that people with the TT genotype, phenotypically slow metabolizers, can have a worse outcome. This could relate to possible adverse drug reactions due to having decreased metabolism of the drug, as well as decreased systematic clearance. In severe TBI patients, this may lead to increased sedation which

can increase the risk of delirium and cognitive dysfunction. Even though the interaction between genotype and propofol are not significant, the slow metabolization of propofol caused by this variant could cause propofol infusion syndrome to occur. While collecting data, there were no noted instances where propofol infusion syndrome occurred, but there were many instances where the dose or choice of sedative was changed throughout the patient's ICU stay due to unoptimized sedation, whether that be the patient being too sedate or not sedated enough. The most common sedatives used in these patients were morphine, fentanyl, and propofol. Also, standing orders for propofol at the University of Pittsburgh Medical Center includes checking serum triglyceride levels 48 hours after admission if the patient is receiving propofol, and then daily if the patient is receiving a propofol infusion greater than 60 mcg/kg/minute for more than 24 hours, or if the patient is less than 21 years old. If triglyceride levels are abnormally high, this is another reason for the discontinuation of propofol.

It is also interesting to note, the impact of the rs3745274 variant on 3-month GOS, was not found on 6-month GOS, which shows that long-term function regarding drug metabolism and *CYP2B6* can improve. Also, severe TBI patients usually have longer than normal hospital stays and may sometimes need inpatient rehabilitation too. This can increase the amount of time patients get particular medications that are metabolized through *CYP2B6*. If these drugs are not being adequately metabolized this can impact outcome. Once the patient leaves the hospital or rehabilitation, the amount of drugs they receive might decrease. This could be another explanation for why *CYP2B6* has a significant impact on 3-month GOS, but doesn't for 6-month GOS.

When controlling for the genotype, propofol is found to be significant to have worse 3-month and 6-month DRS. This means that when receiving the drug, patients are more likely to

have decreased independence, ability to perform activities of daily living, as well as decreased communication abilities. This could be due to potential adverse drug reactions. Studies have shown that using propofol at a higher dose than recommended, or for a long period of time could lead to increased cognitive impairment, as well as other adverse effects such as cardiac, kidney, liver, and pancreatic dysfunction. Having a neurological insult adds to the potential for this to happen. If these events do happen to patients this can greatly increase their length of stay in the hospital and the need of additional treatments. Both of these can also lead to complications to occur. This remains independent of patient's genotypes, showing that this relationship is most likely due to drug dosage and time given.

Limitations of this study includes charting systems, mechanism of injury, and polysubstance use. The data collected on drugs used was collected from 2000 – 2016. As data was being pulled from the charts, getting information from the early 2000s was difficult due to charting being mostly on paper and only a few documents being copied over to the computer system. Even though these documents did provide information on the sedative the patient received, it was difficult to find in some cases and in other cases there was not any information found – leading to the participant not being able to be included in this study. Patients also experienced different mechanisms of injury and extent of damage caused by injury. Every patient had a severe TBI, but the way in which the injury occurred can impact the patient's hospital course and time of recovery. As well as the fact every patient had different co-morbidities that could potentially compromise their recovery. Although, there are standard treatments that all patients with severe TBI receive, which includes intubation and sedation. This study also only focused on the inclusion of propofol – if the patient received it at any time throughout their stay or not – but many of the patients received other sedatives, diuretics, anti-epileptics, and other

medications throughout their stay. This polysubstance use could also impact the metabolism of propofol, as well as the chances of adverse reactions and complications that the patient could experience. There could also be a combined effect that could potentially impact the patient's recovery both positively and negatively. Another limitation was a small sample size and lack of diversity among the participants, although it is reflective of the TBI population in the area.

Since this study only focused on the inclusion of propofol future research in this area could investigate the difference in outcomes when receiving high-dose propofol versus low-dose propofol, as well as the time period the patient receives propofol. This research could provide more insight on recommended propofol dosages based on genotype. Not relating to propofol, but treatment for severe TBI includes intubation and sedation. All patients were either intubated pre-hospital or in the hospital's emergency department upon admission. It would be interesting to investigate the difference in outcome of patients who were not intubated on the scene, whether it is due to a difficult airway or not presenting with particular symptoms, versus those intubated before transportation to the hospital.

These findings are indicative that genetic polymorphisms are important to promote favorable patient outcomes. More research about particular medication dosages need to be done, but this serves as a basis to show that genotyping patients to tailor medication dosages can potentially promote patient outcomes and recovery.

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