Relationship Between Variation in the *TP53* Gene and Patient Outcomes Following Severe Traumatic Brain Injury

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Traumatic brain injury (TBI) is a leading cause of death and disability with millions of people living with long-term complications or disability related to TBI in the United States alone. This study examined the relationship between the p53 coding gene (*TP53*) and outcome variability following severe TBI. p53 has a known impact on neuronal apoptosis following TBI, which warrants investigation into *TP53* genetic variability as a prognosticator for TBI outcomes (assessed using the Glasgow Outcome Scale, Neurobehavioral Scale, and Disability Rating Scale.) Participants (n = 429) were recruited from the UPMC Presbyterian Hospital with a Glasgow Coma Score ≤8 and were followed for 24 months. The single nucleotide polymorphism (SNP) rs1042522 was analyzed using restriction fragment length polymorphism (RFLP) and digested with BstuI restriction enzyme. Individuals with the CC genotype (Arginine homozygotes) were at risk for poorer outcomes following TBI at the 24-month point when compared to CG/GG variants. The findings provide preliminary evidence that p53 plays a role in recovery following TBI and, if replicated, may warrant investigation into p53 targeted therapies for risk allele carriers.

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Preface

I would like to thank all of the people whom without their mentorship, this thesis would not have been possible. First, I would like to thank the University of Pittsburgh School of Nursing and Honors College for this opportunity. Next, I would like to offer my great appreciation to committee members Dr. Sheila Alexander, Dr. Dianxu Ren, and Dr. Nicole Osier for their guidance and time given in support of my research. I also would like to express my sincere gratitude to Sandra Deslouches for teaching, guiding, and assisting me countless hours in the lab during data collection. My thanks to Dr. Dianxu Ren for his generous assistance throughout statistical analysis. I want to extend a special thanks and deep appreciation to my dissertation director Dr. Yvette Conley. Without her continual encouragement, useful suggestions, and support throughout this entire process, I would not have been able to achieve any of this.

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1.0 Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability in the United States with approximately 2.5 million emergency department visits, 282,000 hospitalizations, and 50,000 deaths related to TBI annually [1]. The Center for Disease Control and Prevention (CDC) reported a 47% increase in TBI-related emergency department visits and 5% decrease in death rates from 2007 to 2013, suggesting an increase in the number of survivors of TBI [1]. Survivors often face chronic symptoms that reduce quality of life [2-6]. An estimated 5.3 million people in the United States alone are living with long-term complications or disability related to TBI [5].

TBI is defined as a disruption in normal brain function caused by external force to the head from either a direct assault (e.g. bump, collision) or indirect injury (e.g. jolt, whiplash). The extent of injury can range from mild to severe [1, 3]. This study focused on severe TBI. Despite similar extent and mechanism of injury, the difference in outcomes after TBI has been highly documented. There is high variability of both recovery rates and post-injury quality of life, suggesting that predisposing factors to outcomes following TBI may exist [6].

The neuronal damage following TBI occurs in two stages. The injury during the primary stage is sustained from the traumatic event itself. The external force to the head causes acceleration-deceleration or torsional forces on the neurons, damaging a localized site of glial cells, nerve fibers, and interrupting the blood brain barrier [3]. The secondary stage develops hours to days after the initial injury as inflammation is initiated at the site of the injury and expands to neighboring tissue, expanding the damage. Ischemia at the site initiates anaerobic respiration, thus accumulating lactic acid. The release of glutamate and other excitatory neurotransmitters causes ion channel leakage. Calcium leakage into the cell and mitochondria triggers free-radical

generation, exacerbating neuronal damage and cell death [3, 7]. Gene activation that promotes apoptosis is another cause of cell death following TBI. Apoptosis of neuronal cells is thought to be deleterious in this instance, though the mechanism of neuronal apoptosis has not yet been clearly identified [7, 8]. Research has been done looking at methods to decrease apoptosis after TBI [9, 10].

The purpose of study was to explore the relationship between the Arg72Pro single nucleotide polymorphism (SNP, rs1042522), of the p53 encoding gene (*TP53*) and patient outcomes following severe TBI. The gene *TP53* encodes the tumor protein called p53 which plays a critical role in apoptosis and DNA damage repair via cell cycle control [11]. The *TP53* gene is located on chromosome 17p13.1[12]. In unstressed cells, the p53 protein is kept inactive and at low levels by its negative regulator, MDM2 [13, 14]. A number of stressors are known to rapidly activate p53, such as DNA damage, hypoxia, oxidative and nutritional stresses, ribonucleotide depletion, and disruption of nucleolar function. Stimulation of p53 induces apoptosis by transactivating proapoptotic proteins including Noxa, PUMA, Bax, Bid, Bad, p53AIP1, and PERP, while also repressing antiapoptotic proteins Bcl-2 and Survivin [15]. p53 response also contributes to cell-cycle arrest or progression, senescence, DNA repair, autophagy, and cell metabolism [13, 14]. The p53 protein is located in the nucleus of cells throughout the body where it directly binds to DNA under both basal conditions and stress. However, p53 is significantly more bound after genotoxic insults which stabilize p53 [12, 13]. The stability and transcriptional activity of p53 are regulated by protein-protein interactions and post-translational modifications, including methylation, phosphorylation, acetylation and ubiquitylation [16].

Because of p53s proapoptotic activity, it is a known tumor suppressor gene and has been highly studied in human longevity, cancer risk and survival [12, 17]. Despite p53 beign well-

studied in cancer, few have looked at the relationship between p53 and TBI. It has been acknowledged that p53 contributes to neuronal cell apoptosis and autophagy, as well as transactivating genes that play a role in neuronal cell repair and regeneration, supporting warranting investigation into of the relationship between p53 and TBI outcomes [15, 18, 19]. Exploratory animal studies have also identified changes in expression of p53 following closed head injury [20], ischemic brain injury [21], lateral fluid percussion mediated injury [22], and p53 deficient mice [8, 23]. Specifically, these animal models have found an upregulation of p53 in the nucleus of injured cells following TBI [22].

Martínez-Lucas et al. was the first human study to look at *TP53* in patients with TBI. The study looked at the most common polymorphism in *TP53*, Arg72Pro (rs1042522), where a Cytosine (C) to Guanine (G) base change leads to replacement of arginine (Arg) with proline (Pro) at position 72 within the resulting gene product [8]. Previous research found that the Arg variant (CC) is at least five times more efficient at inducing apoptosis than the homozygous or heterozygous Pro variant (CG/GG) [24]. In a sample of 90 patients with TBI Glasgow Coma Scale (GCS) score ≤8, and the Glasgow Outcome Scale (GOS) was used at the time of discharge from the hospital and 6 months following. The homozygous Arg/Arg genotype was significantly associated with poorer outcomes when compared to the Proline variants. These findings warrant further investigation of p53 as an independent risk factor for outcomes after TBI [8].

2.0 Specific Aim and Purpose

The aim of this study is to further explore the Arg72Pro variation of *TP53* as a predictor of outcome variability following severe TBI. This study expands on the findings and addresses limitations of the Martínez-Lucas et al. study by including a larger sample size, looking at outcome measures at 3, 6, 12, and 24 months following injury, and including the disability rating scale (DRS) and the neurobehavioral rating scale (NRS) as more granular outcome measures. GOS, NRS, and DRS are commonly used reliable and valid measures of outcomes after TBI [25-27]. Following this study, it is the goal that the findings can be replicated and applied to develop and consider *TP53* genotype-targeted therapy for the TBI population. The development and use of precision health care could hold promise in preventing and treating symptoms in patients who sustained a TBI.

3.0 Hypothesis

In agreement with Martínez-Lucas et al. findings [8], the expected outcome is that the Arginine homozygote (CC) genotype will show significantly poorer outcomes compared to Proline variants (CG/GG) at the 6-month outcome measure. It is also expected to see continued difference in outcomes (GOS) at the 12-month and 24-month measures. It is anticipated that across the three time points, the Pro homozygotes will show the most desirable outcomes, the Arg homozygotes will show the least desirable outcomes. With the larger population, it is probable to find a stronger outcome variation between the heterozygous Arg/Pro individuals versus the homozygous individuals. The NRS and DRS measures are anticipated to mirror the results of the GOS and be able to better define the correlation between p53 and outcomes following TBI.

4.0 Methods and Measurements

4.1 Participants

The parent study from which the demographic data, clinical data, patient outcome data and biospecimens were derived was approved by the University of Pittsburgh Institutional Review Board. Next of kin provided written informed consent; consent was obtained at follow up visits when possible. This ongoing study includes patients admitted to the UPMC Presbyterian Hospital neuro trauma intensive care unit. Inclusion criteria were admitted for a closed head injury with a Glasgow Coma Score (GCS) ≤8 without effects of drugs, alcohol, paralytics or sedatives, age 16 to 80 years old, draining cerebrospinal fluid (CSF) via external ventricular drain as standard of care, and not brain dead.

4.2 Evaluation of Functional and Neurobehavioral Outcomes

Initial evaluations of patients were completed using the Glasgow Coma Scale (GCS). The GCS is a valid and reliable marker for traumatic brain injury, as it measures coma severity based on responsiveness of eye-opening, motor, and verbal responses. Subjects with a GCS ≤8 are classified as 'severe' traumatic brain injury [28]. After the initial injury, participants were followed for two years to complete outcome evaluations at 3, 6, 12, and 24 months [2]. A technician at the Brain Trauma Research Center (BTRC) completed the evaluations under the direction of a neuropsychologist.

The outcomes of interest for this study included the Glasgow Outcome Scale (GOS), Neurobehavioral Rating Scale (NRS), and Disability Rating Scale (DRS). The GOS is a valid and reliable measure [25], which objectively categorizes TBI outcomes based on their independence in daily functioning, as follows: 1 = death, 2 = persistent vegetative state, 3 = severe disability, 4 = moderate disability, and 5 = good recovery [29].

The NRS rates 27 items on a 7-level scale (0 = deficit absent, 1 = very mild, 2 = mild, 3 = moderate, 4 = moderately severe, 5 = severe, 6 = extremely severe) assessing behavioral manifestations of TBI. Some areas of interest include alertness, attention, fatigability, orientation, memory, motor behavior, expressive/reception language, mood disturbances, disinhibitory behavior or agitation, and capacity for self-insight [30]. The ratings for each item are tallied to create a summarive score. Possible scores range from 0, or no deficit, to 162, or deficits extremely severe. The NRS has an average inter-rater reliability of 74.3% and an average kappa statistic of 0.40 [2]. The NRS requires individuals to be alive and able to participate in the assessment, so participants with a of GOS 1 and 2 are not included. The sample size for NRS was n = 150 at 3 months, n = 175 at 6 months, n = 170 at 12 months, and n = 101 at 24 months.

The DRS is an assessment used in both the acute hospital setting and the community, to evaluate functional outcomes and ability following TBI. The assessment measures three categories of impairment, disability, and handicap by rating subcategories of eye-opening, ability to communicate, motor responsiveness, cognitive skill necessary for self care, overall dependence, physical and cognitive abilities, and employment. The score ranges from 0, or no disability, to 30, being death. The DRS has been found to be both reliable and valid with an inter-rater reliability ranging from 93-98% in the inpatient setting [31]. The DRS sample sizes were n = 256 at 3 months, n = 251 at 6 months, n = 224 at 12 months, and n = 159 at 24 months.

4.3 Genotype Data Collection

DNA samples for this study were extracted from one of two sources: blood (preferred) or cerebral spinal fluid (alternate). 10 mL whole venous blood was obtained via venipuncture, centrifuged to isolate the white blood cells, and extracted using a salting out protocol. CSF drainage was collected in a ventriculostomy bag as a part of routine clinical care, and the DNA was extracted using the manufacturers instruction for the Qiamp Midi kit (Qiagen, Valencia, CA, USA). All DNA samples were stored in 1x TE buffer at 4°C [2].

Restriction fragment length polymorphism (RFLP) was used to genotype participants. The primer set for rs1042522 was 5'-CTGGTAAGGACAAGGGTTGG-3' as forward and 5'-ACTGACCGTGCAAGTCACAG-3' as reverse. The 397 base pair fragment was amplified by polymerase chain reaction (PCR). PCR was performed with 2μl of diluted genomic DNA via the Denville Taq protocol which used a total volume of 36μl with the following cycling conditions: 35 cycles of 95°C for 30 seconds, 56°C for 36 seconds, 72°C for 40 seconds, followed by 72°C for 10 minutes and an indefinite hold at 10°C. For samples that did not work with the Denville Taq protocol, the Qiagen Taq protocol was used on a 30μl volume with the following cycling conditions: 95°C for 15 minutes, followed by 35 cycles of 94°C for 30 seconds, 54°C for 1 minute 30 seconds, and 72°C for 1 minute and 30 seconds, then 72°C for 10 minutes and an indefinite hold at 10°C.

The PCR fragments were then treated with 5 units of BstuI restriction enzyme, creating two short fragments (166 and 213 base pairs). A 2% agarose gel was run to separate the DNA fragments by size and to interpret the genotype of each participants DNA. Arg homozygotes (CC) showed a single band with no cut at 379 base pairs; Pro homozygotes (GG) showed two bands at

213 and 166 base pairs; and Proline heterozygotes (CG) showed all three bands at 379, 213, and 166 base pairs. Genotype data was manually entered and verified by a secondary individual to reduce risk of human error.

4.4 Statistical Analysis

The independent variable in this study is the SNP genotype. The Hardy-Weinberg equilibrium was tested on the SNP. The dependent variables are the GOS, DRS, and NRS at 3, 6, 12, and 24-month time points. Potential covariates include age, sex, and severity of injury (GCS). GOS was dichotomized into poor outcomes (GOS 1, 2, 3) versus good outcomes (GOS 4, 5). For GOS, chi-squared test was used to analyze each time point separately. One-way ANOVA was used to analyze DRS and NRS by genotype. To see if a difference existed between Arg (CC) and Pro variants (CG/GG), independent sample T test and chi-squared test were run. A p-value ≤ 0.05 was considered significant. Multivariate regression analyses were run on time points and outcome measures trending significant to explore effects of potential covariates of genotype, age, race, sex, and initial GCS. Odds ratios and 95% confidence intervals were calculated.

5.0 Results

A total of 429 participants with severe TBI were included in this analysis. The demographic characteristics of participants are outlined in Table 1. The average age was 37.38 years old (range 16-77); 78.3% were male; and 85.5% were Caucasian. Sex and race were both found to have significant variation across genotypes ($p \leq 0.05$). GCS scores were dichotomized (GCS 3-4; GCS 5-8) to further breakdown severity of TBI. Participants with scores 3-4 show the poorest outcome, whereas participants scoring 5-8 exhibit some response to stimulation. 24.4% of participants scored a GCS of 3-4. The SNP (rs1042522) met the Hardy-Weinberg equilibrium.

Table 1 Demographics of the participants included in study

Characteristics	**TBI (n=429)**	**CC (n=51) 11.9%**	**CG (n=157) 36.6%**	**GG (n=221) 51.5%**	**P-value**
Age (years; mean ±SE)	37.38±0.80	37.16±2.17	36.43±1.24	38.10±1.19	.637
Sex					.036*
Male	336 (78.3%)	38 (74.5%)	114 (72.6%)	184 (83.3%)	
Female	93 (21.7%)	13 (25.5%)	43 (27.4%)	37 (16.7%)	
Race					.014*
White/Caucasian	367 (85.5%)	39 (76.5)	129 (82.2%)	199 (90.0%)	
Nonwhite	33 (7.7%)	10 (19.6%)	12 (7.6%)	11 (5.1%)	
Glasgow Coma Score (GCS)					.203
3-4	104 (24.2%)	9 (17.6%)	45 (28.7%)	50 (22.6%)	
5-8	325 (75.8%)	42 (82.4%)	112 (71.3%)	171 (77.4%)	

Results from the chi-square test of the dichotomized GOS are outlined in Table 2. No significant difference was found between genotype and poor GOS at the 3-, 6-, 12-, or 24-month time points ($p \geq 0.05$), though it trended towards significance at 24-months. In Table 3, using One-way ANOVA, no association existed between genotype and NRS, though it trended towards significance at the 3-month time point. In Table 4, a significant association was found between genotype and DRS at the 24-month time point.

Table 2 Glascow Outcomes Scale (GOS) Frequancy of Poor Outcomes by Genotype

GOS	CC (n = 51)	CG (n = 157)	GG (n = 221)	P-value
3-month (n = 391)	37 (72.5%)	107 (68.2%)	159 (71.9%)	.746
6-month (n = 383)	31 (60.8%)	94 (59.9%)	135 (61.1%)	.987
12-month (n = 362)	28 (54.9%)	79 (50.3%)	112 (50.7%)	.743
24-month (n = 310)	25 (49.0%)	70 (44.6%)	104 (47.1%)	.117

Table 3 Neurobehavioral Rating Scale (NRS) Average by Genotype

NRS (mean±SE)	CC (n = 51)	CG (n = 157)	GG (n = 221)	P-value
3-month (n = 150)	40.86±2.05	44.16±2.18	39.50±.92	.081
6-month (n = 175)	40.95±1.80	40.50±.94	39.44±.90	.621
12-month (n = 170)	36.80±2.43	41.40±1.23	40.05±1.22	.233
24-month (n = 101)	38.89±1.97	40.89±1.71	41.87±1.99	.783

Table 4 Disability Rating Scale (DRS) Average by Genotype

DRS (mean±SE)	CC (n = 51)	CG (n = 157)	GG (n = 221)	P-value
3-month (n = 256)	9.56±1.46	9.74±.78	8.06±.68	.242
6-month (n = 251)	7.94±1.45	6.94±.71	5.98±.60	.315
12-month (n = 224)	5.96±1.50	5.55±.70	4.69±.66	.563
24-month (n = 159)	7.69±2.04	4.87±.86	3.50±.54	.048*

Table 5 summarizes all outcome measures for the Arg homozygotes (CC) and Pro heterozygotes and homozygotes (CG/GG). Outcome measures trended towards significance at the 24-month point, though no association was found between genotype variance and outcome. Results that showed marginal significance or significance were analyzed with multivariate regression analysis.

Table 5 Outcomes of Arginine homozygotes and Proline heterozygotes/homozygotes

	GOS			NRS			DRS		
	Arginine Poor n (%)	**Proline** Poor n (%)	**P-value**	**Arginine** mean±SE	**Proline** mean±SE	**P-value**	**Arginine** mean±SE	**Proline** mean±SE	**P-value**
3-month	37 (78.7%)	266 (77.3%)	.830	40.86±2.05	41.42±1.06	.421	9.56±1.46	8.83±.51	.282
6-month	31 (68.9%)	229 (67.8%)	.878	40.95±1.80	39.92±.65	.675	7.94±1.45	6.41±.46	.131
12-month	28 (63.6%)	191 (60.1%)	.649	36.80±2.43	40.68±.866	.694	5.96±1.50	5.10±.48	.332
24-month	25 (78.1%)	174 (62.6%)	.083	38.89±1.97	41.38±1.31	.279	7.69±2.04	4.14±.51	.079

We found a difference in the 24-month GOS between participatns with CG versus CC (OR=0.27; p=.014), and initial GCS 3-4 vs 5-8 (OR=5.16; p<.001) when controlling for potential covariates. Sex also showed marginal significance female vs male (OR=1.99; p=.56). Multivariate

analysis of NRS at 3-months (Table 7) showed only significant difference in outcomes in age (p=.025). Multivariate analysis of DRS at 24-month (Table 8) showed significant difference in both CG vs CC (B=-3.82; p=.021) and GG vs CC (B=-3.31; p=.048), and initial GCS poor vs good (B=3.08; p=.021).

Table 6 GOS 24-month Multivatiare Analysis for Poor Outcomes

GOS 24-months	OR (95% CI)	P-value
CG vs CC	0.27 (0.09-0.76)	.014*
GG vs CC	0.45 (0.16-1.25)	.126
Age	1.06 (1.04-1.08)	<.001*
White vs Nonwhite	0.32 (0.08-1.36)	.122
Female vs Male	1.99 (0.98-4.02)	.056
GCS 3-4 vs 5-8	5.16 (2.51-10.61)	<.001*

Table 7 NRS 3-month Multivariate Analysis

NRS 3-months	Coefficient	P-value
CG vs CC	3.62	.326
GG vs CC	-1.41	.695
Age	0.16	.025*
White vs Nonwhite	-5.85	.214
Female vs Male	-2.08	.436
GCS 3-4 vs 5-8	2.63	.404

Table 8 DRS 24-month Multivariate Analysis

DRS 24-months	Coefficient	P-value
CG vs CC	-3.82	.021*
GG vs CC	-3.31	.048*
Age	0.05	.194
White vs Nonwhite	-1.63	.529
Female vs Male	1.54	.176
GCS 3-4 vs 5-8	3.08	.021*

Table 9 summarizes the multivariate analysis of Arg homozygotes (CC) and Pro heterozygotes and homozygotes (CG/GG) for GOS and DRS 24-months. Both showed that Arg homozygotes had significantly worse outcomes than Pro variants (GOS p=.048; DRS p=.022). Initial GCS also showed significant association (GOS p<.001; DRS p=.023).

Table 9 GOS and DRS 24-month Multivariate Analysis of Arginine homozygotes and Proline heterozygotes/homozygotes

	GOS (Poor)		DRS	
	OR (95% CI)	P-value	Coefficient	P-value
Proline vs Arginine	0.36 (0.13-0.97)	.048*	-3.58	.022*
Age	1.06 (1.04-1.08)	<.001*	0.05	.191
White vs Nonwhite	0.34 (0.08-1.47)	.149	-1.54	.550
Female vs Male	1.86 (0.94-3.74)	.082	1.43	.199
GCS 3-4 vs 5-8	4.89 (2.40-9.97)	<.001*	2.96	.023*

6.0 Discussion

This study investigated the relationship between SNP rs1042522 of the p53 gene and outcomes following TBI. p53 plays a role in neuronal apoptosis following TBI, and variability in this gene has been shown in previous studies to have deleterious effects on TBI recovery [7, 8].

The demographic characteristics of these participants resemble nationwide trends in severe TBI: predominately male, and Caucasian [1]. The distribution of genotype frequency reflects expected frequencies based on reference SNP reports (e.g. dpSNP). Caucasians are more likely to carry the G allele (72.6% G) than African Americans (33.1% G) and other races.

Across all time points for GOS measures, unadjusted analyses indicated there were no significant differences between the three genotypes. This may show that genotype alone is not a strong predictor of outcome. However, when multivariate regression analysis was completed, CG was found to be a protective allele compared to CC (OR=.27; p=.014), as was GG (OR=.45; p>.05). This was a somewhat unexpected finding given the Martinez study. Their findings showed GG as indicator of better outcome, whereas our showed CG individuals had the best GOS across all time points [8]. The variance between our results could be from our larger sample size, or a result of participant drop out that prevented collecting GOS at all of the time points, for example participants who recovered well may have been less likely to need or attend clinic visits.

When comparing Arg homozygotes (CC) to Pro variants (CG/GG), Arg showed a trend towards significantly poorer GOS at 24-months (78.1% poor vs 62.6%; p=.083). No other time points showed significant difference, which again differs from the findings of Martinez, which found significant difference in GOS outcomes at the 6-month post-discharge between the Arg homozygotes and Pro homozygotes, and no significant difference in the heterozygotes [8]. This

may indicate that presence of Arg is not always an indicator of poor outcomes. However, upon multivariate regression analysis of Arg vs Pro variants, a significant difference was revealed. Arg was 2.7 times more likely to have a poor outcome than Pro (OR=0.36; p=.048). It also showed significant difference in GOS scored based on age and initial GCS, which is expected. Initial GCS had the strongest correlation (OR=4.89; p<.001) to GOS outcome.

NRS showed a trend towards significant difference at the 3-month point (p=.081), but no difference at any other time point. Upon multivariate analysis, only age was found to be significantly associated with NRS score (p=.025). The lack of findings for NRS may be because NRS requires participants to be alive and able to complete the assessment, so participants with a of GOS 1 and 2 could not be included. This could also show that NRS may not be the best measure for outcome following severe TBI because it looks more at granular outcomes, has a wide score range, and has a bias towards survivability.

DRS showed significant difference in outcomes at 24-months between the 3 genotypes, with GG as the best outcome. This supported our hypothesis. This could mean that individuals with GG may have a better prognosis following injury. When comparing Arg homozygotes to Pro individuals, it showed Arg as a slight risk for worse outcome at 24-months (p=.079). Multivariate analysis of the genotypes at 24-months showed that the CC genotype is more likely than both CG and GG to have a poor DRS score (p=.021, p=.048). Comparison of variants at 24-months showed Arg also showed risk for worse outcome compared to Pro (p=.022). Initial GCS also shows significant association with DRS score (p=.023).

Our data demonstrates that two years after TBI, having the Arg homozygote variant allele (CC) for rs1042522 may be associated with a worse prognosis and could be considered a risk allele. Past publications show Arg homozygotes (CC) induce neuronal apoptosis at least five times

more efficient than Pro homozygotes or heterozygotes (CG/GG) [24]. These findings could be used as a prognosticator following TBI, and could warrant preventative genetic testing in high-risk TBI occupations or individuals.

These findings could warrant the investigation of genotype-targeted therapy for p53 in the TBI population. A study done by Yang (2016) supports the administration of p53 inactivators following TBI. Their study delivered a p53 inhibitor, pifithrin-α oxygen analogue (PFT-α (O)), to lab control TBI-induced rats 5 hours post-injury to reduction of neuronal apoptosis. Results showed an improvement of motor and cognitive functions and supported p53 inhibitors as a targeted therapeutic strategy in TBI [32].

Limitations of our study are as follows: first, attrition related to participant drop out or death. Second, the results may not strongly represent minorities and females, though our sample profile resembled national occurrence of TBI. Finally, p53 and neuronal apoptosis is complex, and by looking at only rs1042522, environmental impacts and other gene influences was not included.

7.0 Conclusion

TBI affects many individuals and there is a need to better understand and improve outcomes for this population. This study offers data that contributes to the understanding of variations in outcomes following TBI. Rs1042522 of the *TP53* gene should further be tested as a prognosticator following TBI, with Arg homozygotes predisposed to a worse outcome. The findings of this study support future exploration on genotype-specific treatments in order to improve patient outcomes for risk-allele carriers.

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