The role of a PGC1-α variation in patient outcomes after severe traumatic brain injury

By

Emilie Loudenslager

Bachelor of Science in Nursing- Honors, University of Pittsburgh Honors College, 2020

Submitted to the Graduate Faculty of

The School of Nursing in partial fulfillment

of the requirements for the degree of

Bachelor of Science in Nursing - Honors

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH

SCHOOL OF NURSING

This thesis was presented

By

Emilie Loudenslager

It was defended on

April 9, 2020

And approved by

Sheila Alexander, PHD, RN, FCCM, University of Pittsburgh School of Nursing

Dianxu Ren, MD, PHD, University of Pittsburgh School of Nursing

Karin Reuter-Rice, PHD, CPNP-AC, FCCM, FAAN, Duke University School of Nursing

Yvette Conley, PhD, FAAN, Professor, University of Pittsburgh School of Nursing

The role of a PGC1-α variation in patient outcomes after severe traumatic brain injury

Emilie Loudenslager, BSN

University of Pittsburgh, 2020

A traumatic brain injury (TBI) is damage to the brain resulting from a blow or jolt to the head. Despite enduring similar extent of injuries and receiving similar treatment measures, some individuals recover much faster than others with little to no long lasting deficits. With mechanism of injury and environment of treatment being similar, these differences in recovery could indicate a potential genetic component. This study aimed to investigate the relationship between a single nucleotide polymorphism (SNP) of *PGC1-α* and outcomes following severe TBI. *PGC1-α* is heavily involved in several metabolic processes such as mitochondrial biogenesis, fatty acid oxidation, thermogenesis and several more. Despite this genes involvement in these processes, there has been no formal investigation into the role that *PGC1-α* plays following a TBI, warranting a need for a study of this nature.

Participants (n=429) were recruited from the University of Pittsburgh Medical Center (UPMC) Presbyterian Hospital following a severe TBI with a Glasgow Coma Score ≤ 8 and followed for 24 months post-injury to evaluate long term outcomes. Outcomes were evaluated using the Glasgow Outcome Scale (GOS), Neurobehavioral Rating Scale (NRS), and Disability Rating Scale (DRS). Allelic Discrimination of the SNP rs8192678 was performed using a taqman assay. Individuals with the CC genotype were found to have better outcomes on the NRS at the 3 month post-injury timepoint when compared to individuals with the CT/TT genotypes. These findings show that PGC1-α may play a role in TBI recovery and warrants further investigation of the role PGC1-α plays in recovery after TBI.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEGDEMENTS

Firstly, I would like thank everyone involved in the process of pulling this dissertation together and being so flexible throughout the whole process. Without the cooperation of the University of Pittsburgh School of Nursing and the University of Pittsburgh Honors College, this would not have been possible. I would next like to offer many thanks to my committee members Dr. Sheila Alexander, Dr. Dianxu Ren and and Dr. Karin Reuter-Rice for their time and support. Dr. Dianxu Ren was very generous in giving me his time and support during statistical analysis. I would next like to express much gratitude to the chair of my dissertation, Dr. Yvette Conley. Dr. Yvette Conley's support, encouragement and guidance was critical to my achievement; I couldn't of done this without her. I would also like to thank Sandra Deslouches for her constant assistance and support during my time in the lab; data collection wouldn't have been possible without her.

I would also like to extend my sincere gratitude to all of the participants and their families for taking part in this research study and the Brain Trauma Research Center for assisting with data collection- this wouldn't have been possible without them. Finally, I would like to thank my family and friends for their support on this project and throughout nursing school.

1.0 INTRODUCTION

A traumatic brain injury (TBI), is defined as an insult to the brain from an external mechanical force that disrupts normal brain function [4]. Every year, 2.87 million people visit the emergency room due to a TBI related injury, and recent statistics indicate that this number will only continue to increase as time goes on [3]. Between the years of 2006-2014, there was a steady increase in hospitalizations and deaths, 53% and 6%, respectively, relating to TBI [3,15, 16]. Unfortunately for many survivors, the effects of a TBI persist following their hospitalization and negatively affect their quality of life. As of 2015, roughly 2% of the U.S. population has been living with post-injury complications and disabilities following a TBI [12]. These complications vary in their incidence and in severity, but nonetheless demonstrate a need for improve treatment modalities following a TBI.

The insult that occurs on the brain during a TBI can result from two different modes- direct or indirect [4]. A direct assault would result from a bump or collision while an indirect assault would result from whiplash or a jolt. Depending on how the injury takes place, along with several other genetic and environmental factors, an individual's injury can range from mild to severe [4]. For the purposes of this proposed study, this paper will center on severe TBI. Several studies have found a high variability in outcomes despite similar extent and mechanism of injury between individuals, indicating that an individual's genotype may play a role in outcomes and post-injury quality of life [6, 19].

The damage that occurs following a TBI is thought to occur in two stages. The initial blow, or the traumatic event itself is referred to as the primary stage. During the primary stage, mechanical harm imposed on the brain causes tissue deformation and shearing/tearing of the

blood vessels, neurons, and glia; leading to necrotic cell death [8,9]. The secondary injury usually occurs hours to days following the primary injury as the formation of edema results in increased intracranial pressure (ICP) and secondary ischemia. Secondary ischemia causes failure of cellular ion pumps, resulting in an overload of sodium and calcium release. Failure of the ion pumps is exacerbated by the release of excitatory amino acids, such as glutamate and aspartate, leading to cell death. As cellular death occurs, mitochondrial dysfunction begins, resulting in the release of free radicals [9,12]. As free radicals, such as ROS, build up, the cerebral vascular function of the brain becomes impaired. This trauma reduces oxygenation of the brain and impairs the energy metabolism of the cells. As a result, the antioxidant system of the body attempts to convert the ROS to a less toxic derivative, but the large amounts of ROS deplete the antioxidants and leads to DNA fragmentation, inducing apoptosis and necrosis [18].

The purpose of this proposed study is to explore the relationship between a single nucleotide polymorphism (SNP; Gly482Ser; rs8192678) of PGC1-α and patient outcomes following severe TBI. *PGC1-α* is a transcriptional coactivator located on chromosome 4 (4p15.1) in humans [10]. The stability and transcriptional activity of *PGC1-α* is self-regulated via YingYang1 (YY1) and post-translational modifications, including, methylation, phosphorylation, acetylation and ubiquitylation. This gene plays a central role in mitochondrial biogenesis and respiration, adaptive thermogenesis, gluconeogenesis and many other important metabolic activities [1]. Recent works have highlighted the ability of *PGC1-α* to control global oxidative metabolism through two methods; the first by cellular remodeling through mitochondrial biogenesis and the second by organelle remodeling through the alteration of intrinsic properties of the mitochondria [1]. While this property would appear to cause an

increase in reactive oxygen species (ROS), evidence has suggested that *PGC1-α* is a powerful regulator of the removal of ROS by increasing the transcription of ROS detoxifying enzymes [1].

The expression of *PGC1-α* is highly inducible under physiologic cues such as exercise, cellular/oxidative stress, fasting, and cold temperature [1]. When induced under conditions of oxidative stress, *PGC1-α* moves from the cytoplasm to the nucleus to increase the transcription of ROS detoxifying enzymes to prevent/limit the damage caused by their release. Several studies have explored the role of the Gly482Ser SNP of *PGC1-α* in common chronic conditions such as, hypertension [15] and athletic ability [5,17], but there is very little data on the role of *PGC1-α* in patient outcomes following a TBI.

Even though there has been no formal research performed on *PGC1-α* 's role in TBI, studies have associated this gene with several chronic diseases and athletic ability/endurance [5,15,17]. A meta-analysis published in 2019 by Tharabenjasin, Pabalan, and Jarjanaz [18], included 14 studies that examined the role of Gly482Ser in athletic ability. Since exercise causes oxidative stress and *PGC1-α* is known to help reduce ROS, researchers wanted to determine which variant of *PGC1-α* is associated with increased athletic ability. After comparing the 14 studies, researchers found that out of the 14 significant outcomes, 7 survived the Bonferroni correction favoring the Gly allele for increased athletic ability while Ser had the least favorable, with Gly/Ser falling in the middle. Based on these findings, and due to the role *PGC1-α* plays in reducing ROS and the pathophysiologic process of a TBI, further investigation is warranted [5,9, 17, 18].

2.0 PURPOSE OF STUDY

The aim of this study is to explore the role of the Gly482Ser variation of Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha (*PGC1-α)* in patient outcomes following severe traumatic brain injury. Despite playing a role in several common disease processes, the role of the Gly482Ser variation has yet to be examined in the TBI population. This study examined outcome measures at 3, 6, 12, and 24 months after injury using the Glasgow Outcome Scale (GOS), the Disability Rating Scale (DRS), and the Neurobehavioral Rating Scale (NRS). Significant findings upon completion of this study would provide a foundation for a trajectory of research that has the ultimate goal to be able to identify individuals who have sustained a TBI who are at risk for poorer outcomes, potentially requiring more intense nursing management or who may benefit from *PGC1-alpha* targeted therapies to improve outcomes. The development of genotype-targeted therapies has potential for precision treatment of individuals who have sustained a TBI.

3.0 HYPOTHESIS

In accordance with the role this SNP plays in other disease processes and athletic ability [5,15, 17], the expected outcome is that the Ser/Ser (TT) homozygote genotype variant will show significantly poorer outcomes when compared the Gly/Gly (CC) homozygote genotype at the 6, 12, and 24 month measures, with Gly/Ser (CT) falling somewhere in the middle. It is also possible that individuals with the TT or CT genotypes will have poorer outcomes compared to the CC genotype, indicating that the T allele is bestowing tendency for poorer outcomes regardless of dosing. The NRS and DRS measures are anticipated to emulate the results of the GOS and contribute a better understanding of the correlation between PGC1-α and outcomes following a TBI.

4.0 MATERIALS AND METHODS

4.1 PARTICIPANTS

The University of Pittsburgh Institutional Review Board approved the parent study from which the biospecimens and data were stemmed from, and informed consent was obtained from all participants or next of kin admitted into the study. The parent study is an ongoing study that obtains data from patients admitted to the neurological intensive care unit at the University of Pittsburgh Medical Center Presbyterian Hospital. The parent study aims to examine the genomic and epigenomic changes that occur following severe traumatic brain injury through the collection and genetic analysis of blood and cerebrospinal fluid (CSF).

In order for individuals to be admitted into the study, the following inclusion criteria must be met: aged 16-80 years old, not brain dead, has endured a closed head injury with an admission Glasgow Coma Scale (GCS) ≤8 prior to receiving sedatives or paralytics, and draining CSF via an external ventricular drain (EVD) as standard of care. After verifying the presence of the criteria listed above and giving their informed consent, participants are admitted into the study, clinical data and biospecimens collected during the first 5 days during the acute phase and followed for 24 months post-injury to measure GOS, NRS, and DRS at 3,6, 12 and 24 months [3].

Upon receiving informed consent and determining eligibility, demographic information and initial GCS scores were extracted from the medical record. Staff assist in collecting blood and CSF specimens and alert research staff that samples are available for pick up to be processed in the genetic labs. Samples were processed according the lab protocol and frozen for future DNA extraction.

4.2 ASSESSMENTS

In order to meet eligibility criteria for the study, participants had to have a severe TBI which is indicated by a GCS ≤8 ; this score was obtained during the initial assessment of the patient upon their arrival to the hospital. The GCS measures coma severity based on eye-opening, verbal responses and motor response [2]. In order to record more long term data, participants were followed for two years post-injury. Evaluations were completed by a technician at the Brain Trauma Research Center (BTRC) at 3, 6, 12, and 24 months post-injury under the direction of a neuropsychologist.

The Glasgow Outcome Scale, Neurobehavioral Rating Scale, and the Disability Rating Scale were the outcomes of interest being evaluated in this study. The GOS evaluates a participants ability to function independently and their ability to care for themselves. The GOS is rated based on the following: 1 = death, 2 = persistent vegetative state, 3 = severe disability, 4 = moderate disability, and 5 = good recovery [9].

The NRS is a 27 item assessment that rates a participant's behavior on a scale of 0-7; 0 = deficit absent, 1 = very mild, 2 = mild, 3= moderate, 4 = moderately severe, 5 = severe, and 6 = extremely severe. Areas such as guilt, attention, emotional withdrawal, expressive deficits, disorganization, self-appraisal, anxiety and several other behaviors. Scores from each item are added together, allowing for a possible score range of 0-162. A score of zero represents no deficits while a score of 162 represents extremely severe deficits. Since the NRS requires individuals to be able to actively participate in the assessment, participants with a GOS of one and two are not included in this assessment [11].

The DRS is a tool used to evaluate functional outcomes and abilities following a TBI. Rating on a scale of 0-30, this assessment measures three categories of impairment through the

rating of seven subcategories. A score of zero indicates no disability while a score of 30 indicates death The three primary categories are impairment, disability and handicap while the seven subcategories are eye-opening, communication, motor responses, cognitive skill necessary for self-care, over-all dependence, physical/cognitive abilities and employment [6].

4.3 DNA EXTRACTION AND QUANTIFICATION

Extraction of DNA occurred from one of two sources for each sample utilized in this study. The preferred source was 10 mL of whole venous blood while the secondary source was from CSF that otherwise would have been discarded from the EVD system. DNA was extracted from the blood using a salting out protocol following centrifugation to isolate the white blood cells. DNA was extracted from CSF using the instructions provided from the manufacturer of the Qiamp Midi kit (Qiagen, Valencia, CA, USA). All DNA samples were stored at 4°C in 1x TE buffer [3].

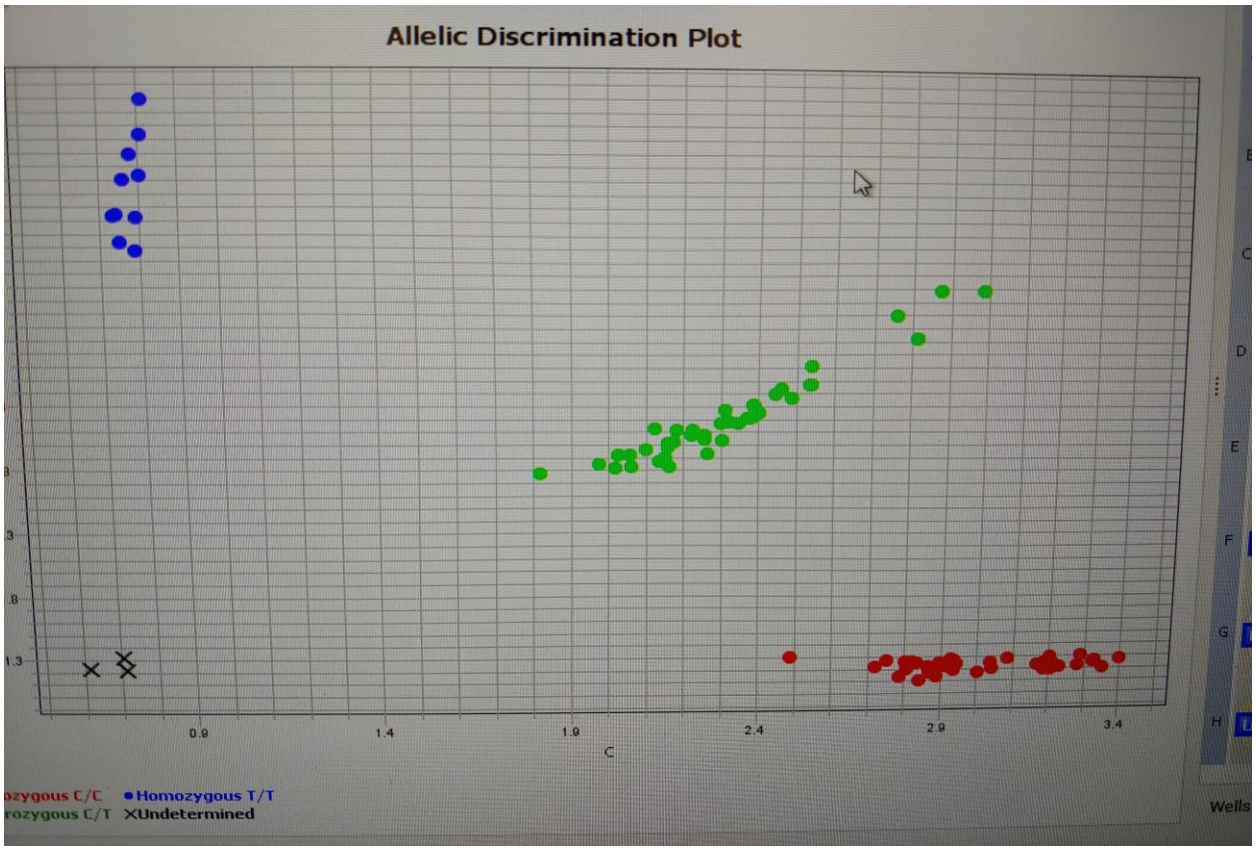
4.4 GENETIC DATA COLLECTION

Genotype analysis was carried out using a taqman allele discrimination assay for rs8192678 obtained as a made to order assay from Thermo Fisher Scientific. 2μl of a working dilution of genomic DNA and 23μl of master mix (see table 1 for composition) were combined and loaded into the PCR system under the following conditions for cycling: 35 cycles of 95°C for 30 seconds, 54°C for 60 seconds, 72°C for 40 seconds, then 72°C for 10 minutes followed by an indefinite hold at 10°C. Each allele was assigned a specific fluorescent symbol as shown in Figure 1.0; homogenous TT individuals were assigned VIC on the Y-axis while homogenous CC individuals were assigned FAM on the x-axis and heterozygotes having one VIC and one FAM labeled allele clustered in the middle.

Table 1. Master Mix Preparation

Sterile water	10µl
40X Concentration Assay Rs8192678	0.625µl
TaqMan Universal PCR Master Mix No AmpErase	12.5µl
Extracted DNA	2µl
	25µl per sample

Figure 1.0 Example of Taqman Results



4.5 STATISTICAL ANALYSIS

In this study, the SNP genotype was the independent variable while the GOS, NRS, and DRS scores at the 3, 6, 12, and 24 month time points were the dependent variables. The severity of injury- as indicated by the GCS, age, and sex were three potential covariates. The GOS was analyzed at each time point using the chi-squared test after being dichotomized into poor outcomes (GOS 1,2,3) and good outcomes (GOS 4,5). The DRS and NRS were analyzed by genotype by the one-way ANOVA test. An independent T-test and chi-squared test were run on the different variants to determine if a difference existed between them. Findings were considered significant if the p-value was ≤ 0.05. To explore the effects of the potential covariates (genotype, age, race, sex and initial GCS) on time points and measures trending significant, a multivariate regression analyses was conducted. 95% confidence intervals and odds ratios were also calculated.

5.0 RESULTS

The sample was composed of 429 participants with clinical data and samples. The average age of the sample was 37.38 years old (range 16-77) who were primarily Caucasian (n=367) males (n=336) with the CC genotype (n=200). There were no significant variations amongst the potential covariates involved in this study. GCS scores were dichotomized to breakdown the severity of the injury. Participants who had a score of 3-4 were compared to participants with a score of 5-8; 24.2% of participants had a score of 3-4 while 75.76% had a score of 5-8. Hardy-Weinberg equilibrium was met by the SNP (rs8192678). Additional demographic data is contained within Table 2 below.

Table 2. Sample Demographics

Characteristics	**TBI (n=429)**	**CC(n=200) 47.17%**	**CT(n=179) 42.22%**	**TT (n=45) 10.61%**	**P-value**
Age (years;mean ± SD)	**37.38 ± 16.64**	**38.32±17.39**	**36.67±16.17**	**37.51±15.47**	**0.63**
Sex					**0.56**
Male	**336(78.3%)**	**161(80.50%)**	**136(75.98%)**	**35(77.78%)**	
Female	**93(21.7%)**	**39(19.50%)**	**43(24.02%)**	**10(22.22%)**	
Race					**0.096**
Caucasian	**376(91.75%)**	**163(88.59%)**	**157(93.45%)**	**42(97.67%)**	
Not Caucasian	**33(8.25%)**	**21(11.41%)**	**11(6.55%)**	**1(2.33%)**	
Glasgow Coma Scale (GCS)					**0.90**
3-4	**104(24.2%)**	**47(23.50%)**	**43(24.02%)**	**12(26.67)**	
5-8	**325(75.76%)**	**153(76.50%)**	**136(75.98%)**	**33(73.33%)**	

Table 3 outlines the results of the dichotomized GOS chi-square test. There was no significant difference found between genotype and the frequency of mortality at each timepoint ($p \geq 0.05$). Table 4, also using the results of the dichotomized GOS chi-square test, showed no significant difference between genotype and frequency of poor outcomes

Table 3. Frequency of Mortality (GOS) by Genotype

GOS	CC (n=200)	CT (n=179)	TT (n=45)	P-value
3 month (n=387)	**64(35.16%)**	**44(26.99%)**	**13(30.95%)**	**0.263**
6 month (n=381)	**69(37.70%)**	**45(28.66%)**	**14(34.15%)**	**0.212**
12 month (n=358)	**71(41.52%)**	**47(31.33%)**	**14(37.84%)**	**0.167**
24 month (n=307)	**73(49.32%)**	**51(40.48%)**	**14(42.42%)**	**0.324**

Table 4. Glasgow Outcome Scale (GOS) Frequency of Poor Outcomes by Genotype

GOS	CC (n=200)	CT (n=179)	TT (n=45)	P-value
3 month (n=387)	**145(79.67%)**	**125(76.69%)**	**30(71.43%)**	**0.486**
6 month (n=381)	**132(72.13%)**	**101(64.33%)**	**26(63.41%)**	**0.246**
12 month (n=358)	**110(64.33%)**	**85(56.67%)**	**22(59.46%)**	**0.370**
24 month (n=307)	**100(67.57%)**	**75(59.52%)**	**23(69.70%)**	**0.307**

After analysis using the one-way ANOVA, there was no association found between genotype and the NRS; results of the analysis are shown in Table 5. Table 6 shows the one-way ANOVA analysis of the association between genotype and DRS. No significant differences were found upon analysis ($p \geq 0.05$).

Table 5. Neurobehavioral Rating Scale (NRS) Average by Genotype

NRS (mean ± SE)	CC (n=200)	CT (n=179)	TT (n=45)	P-value
3-month	**40.11 ± 1.09**	**41.19±1.55**	**46.33±3.95**	**0.12**
6-month	**40.47±0.91**	**40.18±0.95**	**37.61±1.59**	**0.40**
12-month	**41.03±1.38**	**39.28±0.97**	**40.95±2.88**	**0.58**
24-month	**39.83±1.69**	**41.93±2.03**	**42.17±2.78**	**0.68**

Table 6. Disability Rating Scale (DRS) Average by Genotype

DRS (mean ± SE)	CC (n=200)	CT (n=179)	TT (n=45)	P-value
3-month	**9.37 ± 0.76**	**8.70±0.73**	**8.07±1.30**	**0.67**
6-month	**6.87±0.68**	**6.54±0.67**	**5.93±1.18**	**0.81**
12-month	**4.94±0.65**	**5.78±0.76**	**3.43±0.89**	**0.30**
24-month	**5.07±0.86**	**4.27±0.72**	**3.67±0.96**	**0.62**

We found some marginal significance across genotypes after performing a multivariate analysis, controlling for age, race, gender and initial GCS, of the NRS at 3-months. This difference, as shown on Table 7, showed a marginal significant difference in both CC vs TT (p=0.057) and CC+CT vs TT (0.051). This indicates that individuals with the CC and CT genotype has better neurobehavioral outcomes at the 3 month time period than those with the TT genotype.

Table 7. NRS 3-month Multivariate Analysis

NRS 3 month	Coefficient	P-value
CC vs. TT	**-5.92**	**0.057**
CT vs TT	**-5.43**	**0.082**
CC+CT vs TT	**-5.68**	**0.051**
Age	**0.12**	**0.078**
Male vs Female	**-1.34**	**0.617**
Race	**-5.92**	**0.208**

6.0 Discussion

The aim of this study was to investigate the relationship between a variation of PGC1-α, rs8192678, and outcomes following a severe TBI. PGC1-α plays a role in several metabolic processes such as mitochondrial biogenesis, control of global oxidative processes, adaptive thermogenesis and other processes key to maintaining homeostasis [1]. Despite being implicated in several other disease processes, little research has been done to investigate the role this gene plays in recovery following a severe TBI [5,15,17].

Participants in this study mirrored the trends seen nationwide and in the Pittsburgh region in terms of demographics (Table 2) with Caucasian males being the most frequent demographic. Previous work has shown, the CC genotype is the most common (56.3%), CT is the second most common genotype (33.3%) and TT is the least common genotype (9.9%) [16]. This varies across ancestries- African Americans are far more likely to have the CC genotype (91.6%) while those from European ancestries are more likely to have the CT genotype (45.5%); TT is still the least common allele for both of these populations with a frequency of 0.8% and 13.3%, respectively [16]. The distribution of each genotype in this study mirrored these expected frequencies given the demographics of our study population (CC= 47.17%, CT= 42.22%, and TT=10.61%).

Unadjusted analyses across all GOS time-point measures showed no significant difference between the three genotypes in question. This could indicate that other factors, such as environment or covariates, could be a stronger predictor of outcomes rather than genotype alone. Analysis of the DRS also revealed no significant difference between the three genotypes at all measured time points. The lack of findings with for the DRS is likely another indicator that environment plays a large role in recovery. As exemplified in other studies [1], the expression of

PGC1-α is highly inducible under physiologic cues such as exercise, cellular/oxidative stress, fasting, and cold temperature; some of these physiological cues may be the topic of further research on this particular SNP in the future.

Upon initial analysis, the NRS showed no significant difference at all measured timepoints, however, multivariate analysis revealed a marginal significant difference at the three month time point across the three genotypes with TT individuals having poorer neurobehavioral outcomes. There was also a trend towards significance between the NRS and the age of the participants (p=.078). The lack of findings at the timepoints may have to do with the criteria for the NRS; participants must be alive to take the assessment, so those with a GOS of 1 and 2 are not included. This bias towards survivorship may suggest that the NRS is not the strongest measure of outcomes. Despite this bias, our results may indicate that TT could be a risk genotype for poor neurobehavioral outcomes and should be investigated further.

Based on our findings, it is possible that this gene could have greater implications for neurobehavioral outcomes rather than disability and death. Further research should be done to examine the role of this gene in terms of neurobehavioral recovery. Previous studies have not been in TBI, but have been in other phenotypes where energy would be expected to be an important component to the phenotype. An example of this is shown in a meta-analysis published in 2019 by Tharabenjasin, Pabalan, and Jarjanaz [18] that examined the role of this SNP in athletic ability. As an athlete exercises, ROS are produced and lead to the build-up of lactic acid in the muscles; since *PGC1-α* is known to reduce ROS, researchers were interested in examining if one variant of rs8192678 was associated with increased atheletic ability. After comparing 14 studies, researchers found that out of the 14 significant outcomes, 7 survived the Bonferroni correction favoring the Gly allele for increased athletic ability while Ser had the least

favorable, with Gly/Ser falling in the middle. These outcomes of this study mirror the outcomes found in our study; those with the Gly allele had better outcomes than those with the Ser allele, with Gly/Ser falling in the middle.

This study could be an important starting point in investigating the role PGC1-α plays in outcomes following TBI. Determining methods to improve outcomes for this population is vital in optimizing quality of life rather than just quantity. We did have a few limitations in this study. The first limitation was attrition related to the death of participants that impacted power for NRS analyses. Although our sample population is representative of the national trends of TBI occurrence, females and minorities may not be represented strongly within our sample and therefore our findings may not be generalizable. One final limitation was that by only investigating rs8192678, other genetic, clinical and environmental influences, were not included.

7.0 Conclusion

As more and more people survive TBIs, there is an increased need for continued improvements in treatment and outcomes for this population. This study offers information that could contribute to the understanding of how genetic variations can affect TBI outcomes. Further studies should be performed on rs8192678 to explore how environmental impacts, can affect the expression of this gene following a TBI. Based on this study, it appears as individuals who are Glycine (CC) homozygotes may have a better neurobehavioral outcome; further exploration on this gene may help to improve outcomes for carriers of this risk allele.

8.0 References

1. Austin, S., & St-Pierre, J. (2012). PGC1-Alpha and mitochondrial metabolism-emerging concepts and relevance in aging and neurodegenerative disorders. *Journal of cell science 125*, pp. 4963-4971. Doi: 10.1242/jcs.113662
2. Center for Disease Control and Prevention. (no date). Glasgow coma scale. Retrieved from https://www.cdc.gov/masstrauma/resources/gcs.pdf
3. Center for Disease Control and Prevention, National Center for Injury and Prevention. (2019). TBI-related death. Retrieved from: https://www.cdc.gov/traumaticbraininjury/data/tbi- deaths.html
4. Conley, Y.P., et al. (2014). Mitochondrial polymorphisms impact outcomes after severe traumatic brain injury. *Journal of neurotrauma 31*(1), 34-41. doi: 10.1089/neu.2013.2855
5. Eynon, N., Ruiz, J.R., Meckel, Y., Moran, M., & Lucia, A. (2011). Mitochondrial biogenesis related endurance genotype score and sports performance in athletes. *Mitochondrian 11*(1), 64-69. https://doi.org/10.1016/j.mito.2010.07.004
6. Gouvier, W.D., et al. (1987). Reliability and validity of the Disability Rating Scale and the Levels of Cognitive Functioning Scale in monitoring recovery from severe head injury. *Arch Phys Med Rehabil*. 68(2): p. 94-7.
7. Hasan, A., et.al. (2017). Mesenchymal stem cells in treatment of traumatic brain injury. *Front neurology, 8*, pg.28. doi: 10.3389/fneur.2017.00028
8. Huffman, J.C., Brennan, M.M., Smith, F.A., & Stern, T.A. (2010) Patients with neurologic conditions I. seizure disorders, cerebrovascular accidents and traumatic brain injury. In T.A. Stern, G.L. Fricchione, & J.F. Rosenbaum's (Eds).

Massachusetts general hospital of general hospital psychiatry (6th Eds.) pp. 237-253. Retrieved from: https://doi.org/10.1016/B978-1-4377-1927-7.00019-4

9. Jennett B, Bond M. (1975). Assessment of outcome after severe brain damage. *Lancet 1*, 480–484. DOIL 10.1016/s0140-6736(75)92830-5

10. Kaur, P., & Sharma, S. (2018). Recent advances on pathophysiology of traumatic brain injury. *Current neuropharmacology 16*(8), 1224-1238. doi: 10.2174/1871530318666180423121833

11. McCauley, S.R., et al., (2001). The neurobehavioural rating scale-revised: sensitivity and validity in closed head injury assessment. *J Neurol Neurosurg Psychiatry 71*(5): p. 643-51. doi: 10.1136/jnnp.71.5.643

12. National Center for Biotechnology Information. (2019). PPARGC1A PPARG coactivator 1 alpha [Homo *sapiens* (human). Retrieved from https://www.ncbi.nlm.nih.gov/gene/10891

13. National Institute of Neurological Disorders and Stroke. (2015) Hope through research: Traumatic brain injury. Retrieved from https://www.ninds.nih.gov/Disorders/All-Disorders/Traumatic-Brain-Injury-Information-Page

14. Niizuma, K.; Yoshioka, H.; Chen, H.; Kim, G.S.; Jung, J.E.; Katsu, M.; Okami, N.; Chan, P.H. (2010). Mitochondrial and apoptotic neuronal death signaling pathways in cerebral ischemia. *Biochim. Biophys. Acta1802*(1), 92–99. doi: 10.1016/j.bbadis.2009.09.002.

15. Rojek, A., Cielecka-Prynda, M., Przewlocka-Kosmala, M., Laczmanski, L., Mysiak, A., & Kosmala., W. (2014). Impact of PPARGC1A Gly482Ser polymorphism on ventricular structural and functional abnormalities in patients with hypertension. *Journal of human hypertension 28*, pp. 557-563.

16. Selfdecode. (no date). RS8192678. Retrieved from: https://selfdecode.com/snp/rs8192678/

17. Taylor, C.A., Bell, J.M., Coronado, V.G., & Heileyesis, T. (2017). TBI: Get the facts. Retrieved from: https://www.cdc.gov/traumaticbraininjury/get_the_facts.html

18. Tharabenjasin, P., Pabalan, N., Jarjanazi, H. (2019). Association of PPARGC1A gly482ser (rs8192678) polymorphism with a potential for athletic ability and sports performance: A meta-analysis. *PLoS one 14*(1), pp.1-18. .https://doi.org/10.1371/journal.pone.0200967

19. Toklu, H.Z., & Tumer, N. (2015) Oxidative stress, brain edema, blood-brain barrier permeability, and autonomic dysfunction from traumatic brain injury. In F.H. Kobeissy's (Eds.) *Brain neurotrauma: Molecular, neuropsychological and rehabilitation aspects.*. Retrieved from: https://www.ncbi.nlm.nih.gov/books/NBK299195/

20. Weaver, S.M., & Portelli, J.N. (2014). Neurobiological influences on recovery from traumatic brain injury: The role of genetic polymorphisms. *Current Pharmaceutical Design*, *20*(26), 4275-4283. DOI : 10.2174/13816128113196660657