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Haptoglobin Phenotype and Apolipoprotein E Polymorphism: Relationship to Post-Traumatic Seizures and Neuropsychological Functioning after Traumatic Brain Injury

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Abstract

The relationship between genetic predisposition to reduced iron capacity and apolipoprotein E (APOE) with post-traumatic seizures (PTS) and neuropsychological outcomes was investigated in patients with traumatic brain injuries (TBI) from a prior valproate clinical study. Haptoglobin (Hp) concentration/phenotype and APOE genotype was determined in 25 PTS and 26 controls (no PTS) subjects ~10 year after TBI. Hp phenotype was also determined in previously collected frozen samples for 25 additional PTS and 32 no PTS subjects. There was no relationship between Hp phenotype or APOE genotype and occurrence of PTS. APOE genotype was not related to neuropsychological outcome; however when adjustments were made for differences in educational levels, APOE ε4 subjects did worse especially on verbal intellectual and verbal memory skills. In contrast to our hypothesis, those with Hp 1-1 (high affinity binder of hemoglobin), scored somewhat worse on verbal IQ and Tapping D at 1 and 12 month after injury.

Keywords

Traumatic Brain Injury; Post-Traumatic Seizures; haptoglobin; apolipoprotein E

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1. Introduction

Traumatic brain injury (TBI) is a major cause of long-term disability in the United States. Although more individuals survive traumatic brain injury than in the past, TBI results in a cascade of biochemical responses that result in survivors having residual physical, cognitive, emotional and/or behavioral impairments. The etiology of secondary brain injury is multi-factorial, with likely inter-related processes that include mitochondrial energy failure, excessive generation of reactive oxygen species (ROS), activation of destructive enzymes such as poly (ADP-ribose) polymerase (PARP), membrane disruption, neuronal death, thrombosis due to intravascular coagulation in small vessels, increased synaptic concentrations of excitatory amino acids, and activation of innate inflammatory responses [1]. There have also been significant advances in the understanding of the role of genetics in outcome after TBI, especially with the association with apolipoprotein (APOE) ε4 allele [2].

Head trauma causes about 5% of all cases of epilepsy. Epileptic seizures occur in over 20% of the people whose traumatic brain injury includes intracranial hematoma, depressed skull fracture, immediate seizure, or penetrating injury [3]. Injury that involves blood in contact with the cortex has a significantly increased risk of post-traumatic seizures [4]. The role of iron in generation of ROS and tissue injury is well established. Hemoglobin is the richest source of iron in the body and capable of producing ROS [5]. The importance of blood or blood components (e.g., hemoglobin) to the process of epileptogenesis is suggested by the finding that intracranial injections of hemoglobin/iron results in increased oxidative stress and seizures [6,7]. Panter et al. demonstrated that intracranial injections of hemoglobin, lysed erythrocytes, or iron salts result in chronic, focal spike activity in rodents [8]. Sadrzadeh et al. showed that iron released from free hemoglobin molecule was responsible for neuronal damage in cats [9]. Therefore, the inadequate clearance of hemoglobin from the brain following head trauma could result in accumulation of iron in the brain, oxidative-induced tissue injury and ultimately development of seizure disorders.

The neutralization and removal of extracellular hemoglobin is affected primarily by haptoglobin, an α_2-glycoprotein [10]. The main biological function of haptoglobin is to bind free hemoglobin, stabilize it (i.e., prevent the dissociation of the heme group) and to speed its removal through the reticuloendothelial system to prevent iron loss and kidney damage during intravascular hemolysis [10]. Haptoglobin contains two β (heavy), and two α (light) chains. Humans are polymorphic for haptoglobin (Hp), with three major phenotypes: Hp 1-1, Hp 2-2, and the heterozygous Hp 2-1[11]. The β chains are identical in all haptoglobin phenotypes and the variations are due to the presence of different α chains. Hp 1-1 expresses only the α1 chain and is the smallest (86 KDa). The Hp 2-1 and 2-2 express α2 chains, which, due to an unequal crossing-over and partial duplication, can form polymers of 86-300 KDa (Hp 2-1), and up to 900 KDa (Hp 2-2) [11]. Although the main function of Hp is to remove free hemoglobin, there are profound functional differences among Hp phenotypes. For instance, Hp 1-1 binds hemoglobin with high affinity and is the most effective in suppression of inflammatory response oxidative stress associated with extracellular hemoglobin [10]. In contrast, Hp 2-2, due in part to its large molecular mass, is the least effective.

Apolipoprotein E (APOE) is produced in the brain in response to injury (for review see [12]). Experimental studies have demonstrated that APOE functions as an antioxidant, anti-inflammatory, anti-excitotoxic and neurotropic. In addition, polymorphisms of APOE play an important role in neuropathological finding in patients with head injuries. Of the three common alleles, ε2, ε3 and ε4, the presence of ε4 has been shown to have a less favorable outcome in some previous studies. A prospective study of 106 patients with moderate to severe brain injury found that patients carrying the ε4 allele had a 2.4 relative risk of seizures [13]. Studies have

found that carrying the ε4 allele is associated with worst outcome in some studies of patients with TBI [14-17], but not all studies [13,18,19].

The objective of this study was to investigate the relationship between the genetic predisposition of reduced efficiency in clearing free hemoglobin iron (or reduced iron handling capacity) following hemorrhagic injury and apolipoprotein ε4 allele to post-traumatic seizure and neuropsychological outcomes.

2. Methods

2.1 Patients

Participants were recruited from the three hundred seventy nine eligible patients who had taken part in a double-blind placebo controlled clinical study evaluating the use of valproate (VPA) for prophylaxis of post-traumatic seizures (PTS) at the University of Washington Harborview Medical center (UWHMC) from February 1991 to December 1995 [20]. Eligibility for the VPA trial required a risk factor for PTS, specifically one of the following: intracranial hematoma, cortical contusion, depressed skull fracture, acute post-traumatic seizure, or a penetrating brain injury. The study was a randomized, double-blind parallel design with patients assigned to receive therapeutic concentrations of phenytoin for one week or valproate for up to 6 months. Patients were loaded with study drug within 24 hours of injury. Eighty-four % of patients were followed until their death or the end of the 2-year observation period. Fifty-six patients developed late PTS within two years with no difference among the treatment arms in the occurrence. Approximately 10 years after their injury, we were able to contact 25 of the 50 surviving subjects with late PTS and 26 subjects in a control group. The control group was selected from the patients who also had taken part in the VPA Study [20] but did not develop PTS. The control group was matched for age (<40 years vs. ≥ 40 years, sex and PTS risk factors [21]. Despite a lack of effect of treatment on PTS found in the VPA trial [20], the control group was also matched based on which treatment arm they received in the VPA trial. The study protocol and consent forms were approved by the University of Washington Human Subjects Division. All subjects gave their informed consent prior to their inclusion in the 10 year follow-up.

2.2 Study Design

A brief interview with the subjects was performed to obtain baseline information on seizure history, diet, current alcohol use and concurrent medications that they were taking. Neuropsychological testing was performed as described below and a sample of blood was collected. Serum was used for haptoglobin concentration and phenotyping analyses, as described below. The remaining blood cells were used for determination of APOE genotype. In addition, we were able to determine haptoglobin phenotype in serum samples previously collected and frozen for an additional 25 subjects who developed PTS and an additional 32 matched control subjects. Haptoglobin phenotype was not determined in the remaining 5 PTS subjects due to either a lack of sample availability or poor quality of the frozen sample. The samples were previously collected 3.1 ± 1.5 days after TBI (range: 1-6 days) as part of the VPA trial.

2.3 Sample Analysis

Serum haptoglobin concentrations were measured using an automated immunonephelometer, following manufacturer's instruction [22]. Haptoglobin was phenotyped in serum using the method described by Smithies with minor modifications [23]. Haptoglobin binds hemoglobin in a 1:1 ratio, and hemoglobin under certain conditions behaves as a peroxidase. The hemoglobin-Hp 1-1 has a molecular mass of 150 KDa. Hemoglobin-Hp 2-1 has a molecular mass range of 150 KD to 364 KDa and separates as a band at 150 KDa with many higher

molecular weight bands. Hemoglobin-Hp 2-2 has a molecular mass range of 234 KDa to 964 KDa and separates with many high molecular weight bands. Briefly, hemolysate was prepared by hemolysing washed red blood cells in 15 volumes of cold water and centrifuged (35,000×g). A mixture of serum (10 μL), hemolysate (0.3 μL), 5× sample buffer (4 μL) and water (5.7 μL) was electrophoresed on a 4-20% gradient (polyacrylamide) minigel (1 mm thick). The electrophoresis was run at 125 volts for 30 min and 250 volts for 4hr (constant voltage) at 4 C. The gel was washed in distilled water and placed in the peroxidase staining solution. The staining solution contains two parts mixed equally. Part A: 200 mg leucomalachite green, 20 mg EDTA, 10 ml acetic acid (glacial) and 15 ml distilled water, and part B, 0.6% H_2O_2. The gel was then developed for about 4-6 min until bands appear, scanned and analyzed in a computer.

Apolipoprotein (APOE) genotypes were determined in blood cells using the method described by Chapman et al [24].

2.4 Neuropsychological Testing

Neuropsycholocological testing was done at 1, 6 and 12 months as part of the original VPA trial protocol [25]. Briefly, motor function was evaluated by the Finger Tapping Test for dominant and non-dominant hands [26]. Memory was assessed by the Selective Reminding Test, Sum of Recall [27]. Measures of visual-spatial performance skills included the Wechsler Adult Intelligent Scale Performance Intelligence Quotient (PIQ)[28]. For the 10 year follow-up in the 51 subjects who returned, neuropsychological testing included the Wechsler Abbreviated Scale of Intelligence (WASI -FSIQ) and the Processing Speed Index (PSI) of the Wechsler Adult Intelligence Scale [29]. Ten year follow-up also included the Selective Reminding Test which was included in the 1, 6 and 12 month assessments. Subjects who were neurologically too impaired to be tested were assigned a score one unit worse than the worst observed and subjects who died were assigned one unit worse than that.

2.5 Statistical Analysis

Chi-square tests and Mann-Whitney tests were used for statistical analysis of demographic and injury characteristics, haptoglobin phenotype and APOE genotype distributions between PTS and control subjects. Statistical analysis of the neuropsychological testing was performed using t-tests, ANOVA; and multiple regression of the neuropsychological test scores on genetic markers, seizure group, TBI severity using time to follow commands and education or age. Adjusted means were calculated from the regression analyses by substituting the overall mean for all variables except the grouping of interest in the regression equations.

3. Results

Table 1 shows demographic and injury characteristics of the 50 subjects who experienced at least one epileptic seizure (PTS) (including the 25 subjects who returned for a follow-up visit at approximately 10 years and 25 subjects for whom previously frozen serum samples were available) compared to those without epileptic seizures (no PTS). There were no significant differences between the groups on demographic or injury characteristics. There was no statistically significant difference in haptoglobin serum concentration in those subjects who had developed PTS compared to controls (no PTS). There was no relationship between haptoglobin phenotype (Table 2) or APOE genotype (Table 3) and occurrence of PTS. There was also no relationship between haptoglobin phenotype and haptoglobin concentration. The results of the APOE genotype, haptoglobin phenotype and neuropsychological test are given in Table 4 and 5, respectively. Those subjects with APOE ε4 genotype had higher education prior to injury (p=.004). APOE genotype was not related to outcome at any time without adjustment for seizures, TBI severity or education (p-values between .11 and .95 with no

adjustment for multiple comparisons). However, with adjustment for seizures, TBI severity and education, subjects with APOE ε4 genotype performed significantly worse on VIQ at 1 month and 1 year post injury (each $p < .05$ without adjustment for multiple comparisons) and on a verbal memory test at 1, 6 and 12 months post injury (each $p < .05$ without adjustment for multiple comparisons). Those subjects with Hp 2-2 were older than the other 2 groups. Those with Hp 1-1, which should indicate better clearance of hemoglobin, scored worse on VIQ and Tapping D at 1 and 12 months after injury (each $p<.05$ after adjustment for seizures, severity, and age but without adjustment for multiple comparisons) with occasional other tests showing similar trends.

4. Discussion

The role of oxidative stress in neurological disorders is well established. Iron and iron compounds (such as hemoglobin) can enhance oxidative damage in the body via generation of reactive oxygen species. The brain is especially susceptible to oxidative damage due to its high oxygen consumption, its high lipid content, and low antioxidant capacity. Therefore, free iron or hemoglobin via oxidative damage can have a role in the etiology of some of the neurodegenerative disorders. Indeed, increased accumulation of iron in the brain and defective antioxidants have been linked to both Parkinson's and Alzheimer's Diseases [30]. Markers for oxidative stress such as lipid peroxidation, DNA damage, protein damage and increased nitrotyrosine have been documented in Parkinson's Disease [31].

Panter et al [32] found a highly significant association between low or absent plasma haptoglobin concentrations and the occurrence of seizures in several family groups with idiopathic epilepsy. In another study looking at the association of haptoglobin phenotypes and the frequency of seizure in patients with refractory epilepsy, Sadrzadeh et al. [33] found a significant association between Hp 2-2 phenotypes and the frequency of seizures. That is, 67% of epileptic patients with Hp 2-2 phenotype experienced one or more seizure attacks per month as compared to 11% seen in those with Hp1-1. Saccucci et al. found similar under-representation of the Hp 1-1 genotype in two populations of children with generalized epilepsy compared to controls [34].

Haptoglobin is a plasma α-2 glycoprotein that in addition to its major role in removing free hemoglobin from circulation following intravascular hemolysis, can also function as an antioxidant (especially Hp 1-1) by binding hemoglobin and preventing hemoglobin induced oxidative tissue damage [35]. Serum and cerebrospinal fluid haptoglobin concentrations are increased significantly after TBI [36]. Although the exact mechanism for the latter phenomenon is not known, it might be related to the inflammatory processes that follows the injury. Haptoglobin is a positive acute phase protein and its serum concentrations should go up and stay elevated during inflammatory response to injury.

In our patient population, the nature of injury to the brain is different from those seen following chronic micro hemorrhagic events. In TBI, the physical damage to the brain may be the reason for seizure activities seen in those patients. Haptoglobin cannot protect the brain tissue against the initial physical injury. Even though good haptoglobin function may prevent some hemoglobin-induced damage, such an effect may be too little too late when the injury can involve direct physical damage to the brain. If one is thinking of preventing PTS using an agent that binds or clears blood, the results presented here suggest the effect of the agent needs to be much stronger than the differences between the different Hp phenotypes.

Prior findings on the relationship of APOE alleles and outcome after TBI have been mixed, with quite a few studies, even small ones, finding a relationship with global outcome as measured by the Glasgow Outcome Scale (GOS) or Glasgow Outcome Scale —Expanded

(GOSE) and about an equal number, including the largest study with 984 participants, finding no association. The single prior study looking at the relationship with PTS found a relative risk of 2.4 (95% confidence interval, 1.15-5.07, p=0.03) associated with APOE ε4. The single study looking at neuropsychological functioning found no relationship. Our study failed to confirm an association between the ε4 allele and PTS, however our sample size was considerably smaller as we were only able to obtain APOE for the 51 subjects returning at 10 years post-injury.

The results of the relationship between APOE ε4 and neuropsychological performances are interesting and worthy of comment. Those with APOE ε4 alleles had significantly better pre-injury educational levels and their unadjusted performance was not different from the no APOE ε4 group. However, since higher educational levels typically are associated with better cognitive functions, they may be masking TBI effects. Unlike the Chamelian et al. study [37], after adjustment for educational attainment, we did find somewhat poorer neuropsychological functioning among those with the ε4 allele in the areas of verbal intellectual and verbal memory skills, although the differences would not be significant with adjustment for multiple comparisons. These measures are relatively more robust to the effects of injury. This brings up the question of whether the poorer cognitive skills are the result of the injury or pre-dated the injury. Studies done to date, including this one, have not looked at APOE status and cognitive performance in a comparison group without TBI or other neurological condition. A study with such a comparison, or even a study looking at cognitive function in relation to APOE status in healthy individuals, would shed light on whether poorer pre-injury verbal abilities in those with APOE ε4 could explain the finding of the current study. While better educational levels of the APOE ε4 group may be entirely a chance occurrence, consideration should also be given to whether this is due to a selection bias caused by loss of APOE ε4 subjects with lower education to mortality or functional limitations of survivors precluding willingness to participate in our research. It would be advisable for future research to be attentive to potential subject selection biases that may be contributing to inconsistent results in the literature.

Although blood in close association with the brain is a prominent risk factor for seizures after TBI and several other conditions and injection of iron or heme into the brain serves as a laboratory model for PTS, to our knowledge, this is the first study to examine whether differences in haptoglobin phenotype are related to the risk of developing PTS. No such relationship was found. This raises the question of whether the development of post-traumatic seizures is mediated by free hemoglobin/iron or whether this experiment of nature just uses “too small a dose”

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References

[1]. Chesnut RM. Secondary brain insults after head injury: clinical perspectives. New Horiz 1995;3:366–75. [PubMed: 7496744]

[2]. Diaz-Arrastia R, Baxter VK. Genetic factors in outcome after traumatic brain injury: what the human genome project can teach us about brain trauma. J Head Trauma Rehabil 2006;21:361–74. [PubMed: 16915011]

[3]. Temkin NR. Antiepileptogenesis and seizure prevention trials with antiepileptic drugs: meta-analysis of controlled trials. Epilepsia 2001;42:515–24. [PubMed: 11440347]

[4]. Willmore LJ. Post-traumatic epilepsy: cellular mechanisms and implications for treatment. Epilepsia 1990;31(Suppl 3):S67–73. [PubMed: 2226373]

[5]. Sadrzadeh SM, Graf E, Panter SS, Hallaway PE, Eaton JW. Hemoglobin. A biologic fenton reagent. J Biol Chem 1984;259:14354–6. [PubMed: 6094553]

[6]. Reid SA, Sypert GW, Boggs WM, Willmore LJ. Histopathology of the ferric-induced chronic epileptic focus in cat: a Golgi study. Exp Neurol 1979;66:205–19. [PubMed: 114405]

[7]. Willmore LJ, Sypert GW, Munson JV, Hurd RW. Chronic focal epileptiform discharges induced by injection of iron into rat and cat cortex. Science 1978;200:1501–3. [PubMed: 96527]

[8]. Panter SS, Sadrzadeh SM, Hallaway PE, Haines J, Anderson VE, Eaton JW. Hypohaptoglobinemia: a possible predisposition to epilepsy. Trans Assoc Am Physicians 1984;97:56–62. [PubMed: 6535352]

[9]. Sadrzadeh SM, Anderson DK, Panter SS, Hallaway PE, Eaton JW. Hemoglobin potentiates central nervous system damage. J Clin Invest 1987;79:662–4. [PubMed: 3027133]

[10]. Javid J. Human haptoglobins. Current topics in hematology 1978;1:151–192. [PubMed: 400530]

[11]. Black JA, Dixon GH. Amino-acid sequence of alpha chains of human haptoglobins. Nature 1968;218:736–41. [PubMed: 4172407]

[12]. Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 1994;17:525–30. [PubMed: 7532337]

[13]. Diaz-Arrastia R, Gong Y, Fair S, Scott KD, Garcia MC, Carlile MC, Agostini MA, Van Ness PC. Increased risk of late posttraumatic seizures associated with inheritance of APOE epsilon4 allele. Arch Neurol 2003;60:818–22. [PubMed: 12810485]

[14]. Teasdale GM, Nicoll JA, Murray G, Fiddes M. Association of apolipoprotein E polymorphism with outcome after head injury. Lancet 1997;350:1069–71. [PubMed: 10213549]

[15]. Friedman G, Froom P, Sazbon L, Grinblatt I, Shochina M, Tsenter J, Babaey S, Yehuda B, Groswasser Z. Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. Neurology 1999;52:244–8. [PubMed: 9932938]

[16]. Lichtman SW, Seliger G, Tycko B, Marder K. Apolipoprotein E and functional recovery from brain injury following postacute rehabilitation. Neurology 2000;55:1536–9. [PubMed: 11094110]

[17]. Chiang MF, Chang JG, Hu CJ. Association between apolipoprotein E genotype and outcome of traumatic brain injury. Acta Neurochir (Wien) 2003;145:649–53. [PubMed: 14520543]discussion 653-4

[18]. Nathoo N, Chetry R, van Dellen JR, Connolly C, Naidoo R. Apolipoprotein E polymorphism and outcome after closed traumatic brain injury: influence of ethnic and regional differences. J Neurosurg 2003;98:302–6. [PubMed: 12593615]

[19]. Teasdale GM, Murray GD, Nicoll JA. The association between APOE epsilon4, age and outcome after head injury: a prospective cohort study. Brain 2005;128:2556–61. [PubMed: 16033781]

[20]. Temkin NR, Dikmen SS, Anderson GD, J WA, Holmes MD, Cohen W, Newell DW, Nelson P, Awan A, Winn HR. Valproate therapy for prevention of post-traumatic seizures: a randomized trial. J Neurosurg 1999;91:593–600. [PubMed: 10507380]

[21]. Koehler, J. Biostatistics. University of Washington; Seattle: 1994. Risk of late unprovoked seizures in patients with moderate/severe head injuries.

[22]. Van Lente F, Marchand A, Galen RS. Evaluation of a nephelometric assay for haptoglobin and its clinical usefulness. Clin Chem 1979;25:2007–10. [PubMed: 116779]

[23]. Smithies O. Zone electrophoresis in starch gels: group variation in the serum proteins of normal adults. Biochem J 1955;61:629–641. [PubMed: 13276348]

[24]. Chapman J, Estupinan J, Asherov A, Goldfarb LG. A simple and efficient method for apolipoprotein E genotype determination. Neurology 1996;46:1484–5. [PubMed: 8628509]

[25]. Dikmen SS, Machamer JE, Winn HR, Anderson GD, Temkin NR. Neuropsychological effects of valproate in traumatic brain injury. Neurology 2000;54:895–902. [PubMed: 10690983]

[26]. Reitan, R.; Wolfson, D. The Halstead-Reitan Neuropsychological Test Battery: Theory and Clinical Interpretation. Vol. 2nd ed. Neuropsycholology Press; Tucson: 1993.

[27]. Buschke H. Selective reminding for analysis of memory and learning. Journal of Verbal Learning and Verbal Behavior 1973;12:543–50.

[28]. Wechsler, D. Manual for the Wechsler Adult Inteligence Scale. The Psychological Corp; New York: 1955.

[29]. Wechsler, D. WAIS III Administration and Scoring Manual. The Pyschological Corporation. Harcourt Brace and Company; San Antonio: 1997.

[30]. Halliwell, B.; Gutteridge, JMC. Free radicals in biology and medicine. Vol. 3rd ed. Oxford University Press; New York: 2000. p. 175-178.

[31]. Halliwell, B.; Gutteridege, JMC. Free radicals in biology and medicine. Oxford University Press; New York: 2000. p. 741

[32]. Panter SS, Sadrzadeh SM, Hallaway PE, Haines JL, Anderson VE, Eaton JW. Hypohaptoglobinemia associated with familial epilepsy. J Exp Med 1985;161:748–54. [PubMed: 3981086]

[33]. Sadrzadeh SM, Saffari Y, Bozorgmehr J. Haptoglobin phenotypes in epilepsy. Clin Chem 2004;50:1095–7. [PubMed: 15161734]

[34]. Saccucci P, Verdecchia M, Piciullo A, Bottini N, Rizzo R, Gloria-Bottini F, Lucarelli P, Curatolo P. Convulsive disorder and genetic polymorphism. Association of idiopathic generalized epilepsy with haptoglobin polymorphism. Neurogenetics 2004;5:245–8. [PubMed: 15490286]

[35]. Melamed-Frank M, Lache O, Enav BI, Szafranek T, Levy NS, Ricklis RM, Levy AP. Structure-function analysis of the antioxidant properties of haptoglobin. Blood 2001;98:3693–8. [PubMed: 11739174]

[36]. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 1996;42:1589–600. [PubMed: 8855140]

[37]. Chamelian L, Reis M, Feinstein A. Six-month recovery from mild to moderate Traumatic Brain Injury: the role of APOE-epsilon4 allele. Brain 2004;127:2621–8. [PubMed: 15496436]

Table 1

Description of Patients

	PTS Subjects n=50	No PTS n=58	p
Age mean (sd)	36 (17)	40 (15)	0.72
Gender (% male)	88	90	1.0
% Surgery for Subdural Hematoma	22	22	1.0
% Dural Penetration by injury	30	24	0.52
Non-reactive Pupils			0.37
% none	83	91	
% ≥ 1	17	9	
Early Seizures 1-7 days	6	3	0.66
Time to Follow Commands			0.32
% 1-6 days	58	69	
% ≥ 7 days	42	31	

Table 2

Haptoglobin (Hp) Concentration and Phenotype in PTS Cases and Controls (No PTS)

	PTS Subjects n=50	No PTS n=58	Expected Ratio for Seattle Area[b] [36]
Hp (mg/dl)[a]	200 ± 90	192 ± 71	---
Hp Phenotype			
Hp 1-1	11 (22 %)	7 (12 %)	14%
Hp 2-1	22 (44%)	28 (48%)	48%
Hp 2-2	17 (34%)	23 (40%)	38%

[a] normal range for Haptoglobin (100-300 mg/dl), p = 0.57

[b] There is not a statistically significant difference in Hp phenotype distribution in patients who developed PTS compared to those who did not. (χ^2 = 0.250, DF=2, p=0.883)

Table 3

APOE Genotype and PTS

	PTS Subjects	No PTS
ε2,3 or ε3,3	18 (72.0 %)	18 (69.2 %)
ε2,4 or ε3,4 or ε4,4	7 (28.0 %)	8 (30.8%)

In most populations the allele frequency for APOE2, APOE3 and APOE4 are 0.075, 0.774 and 0.15. Therefore we would expect 72% of patients to not carry the APOE4 allele. There was no statistically significant difference in the frequency of APOE4 allele in patients who developed PTS compared to the control non PTS patients. (χ^2 = 0.047, DF=1, p=0.828)

Table 4

APOE Genotype and Neuropsychological testing

	ε2,3 or ε3,3		**ε2,4 or ε3,4 or ε4,4**		**p**	
n	36		15		0.200	
Age (yrs)	33.9 (11.8)		38.2 (10.1)		0.004	
Years Education	12.1 (2.2)		14.6 (2.7)		1.00	
% Male	89		87		0.76	
TBI Severity (TFC)						
% > 24 hr	53		40			
% 1-24 hr	28		53			
% < 1 hr	19		7			
1 month	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
SRCL	65	68	61	54	0.64	0.048
VIQ	96	99	95	88	0.92	0.050
PIQ	86	89	86	79	0.94	0.196
Tapping D	45	46	43	38	0.67	0.137
Tapping ND	41	43	40	35	0.69	0.126
6 month						
SRCL	79	81	76	71	0.65	0.027
VIQ	107	109	108	102	0.73	0.065
PIQ	104	107	109	102	0.38	0.332
Tapping D	50	51	49	47	0.63	0.187
Tapping ND	46	46	47	46	0.65	0.897
12 month						
SRCL	79	83	79	71	0.92	0.012
VIQ	104	109	108	99	0.47	0.042
PIQ	104	108	113	107	0.11	0.852
Tapping D	50	51	48	46	0.60	0.227
Tapping ND	46	45	47	48	0.53	0.530
10 Years						
SRCL	76	77	75	71	0.95	0.236
PSI Index	90	92	92	86	0.71	0.246
FSIQ	102	104	107	101	0.44	0.480

Unadjusted: p value is results of t-tests

Adjusted: Neuropsych from regression controlling for seizure, TFC, education and then APOE genotype

Table 5

Haptoglobin Phenotype and Neuropsychological testing

	Hp 1-1		Hp 2-1		Hp 2-2		p	
n	18		50		40			
Age (yrs)	36 (17)		36 (15)		43 (16)		0.02	
Years Education	11.9 (1.6)		13.0 (2.6)		12.6 (2.7)		0.16	
% Male	89		90		88		0.92	
TBI Severity (TFC)							0.51	
% > 24 hr	47		47		55			
% 1-24 hr	27		29		32			
% < 1 hr	27		24		13			
1 month	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
n	17		49		40			
SRCL	44	46	46	44	52	56	0.61	0.13
VIQ	82	84	86	85	93	94	0.13	0.04
PIQ	75	76	76	74	83	84	0.48	0.14
Tapping D	35	35	38	36	43	44	0.29	0.04
Tapping ND	33	33	36	35	38	39	0.64	0.25
6 month								
n	16		43		36			
SRCL	57	59	69	65	66	69	0.37	0.40
VIQ	88	90	100	99	103	103	0.03	0.06
PIQ	83	85	96	95	99	100	0.12	0.11
Tapping D	42	42	45	44	48	50	0.38	0.09
Tapping ND	40	40	42	41	43	44	0.72	0.46
12 month								
n	11		36		27			
SRCL	55	59	71	69	72	73	0.16	0.25
VIQ	87	90	102	101	106	105	0.015	0.048
PIQ	81	86	102	101	105	105	0.03	0.08
Tapping D	37	40	46	44	50	51	0.04	0.02
Tapping ND	39	41	43	41	45	46	0.40	0.32
10 year								
n	10		22		17			
SRCL	71	72	79	78	74	74	0.36	0.50
PSI	87	88	93	93	90	90	0.69	0.70
FSIQ	97	97	105	105	106	106	0.38	0.39

Unadjusted Analysis: p from ANOVA

Adjusted Analysis; p from regression controlling for seizures, TFC, age and then HP