Genetic Influence on Cognitive and Behavioral Recovery after Pediatric Traumatic Brain Injury

**Background and Significance**

Traumatic Brain Injury (TBI) is a leading cause of morbidity and mortality in the United States, among both adults and children. The CDC estimates that about half a million children have to visit the emergency department every year as a result of a TBI (1).

Some of the most prominent deficits associated with a TBI are cognitive, and include memory deficits, deficits in executive functioning, decreased concentration and attention, and language impairment. The degree of deficit among individuals is dependent upon severity of the TBI, age at injury, and time post injury, however outcomes vary greatly between individuals with what appear to be similar injuries (2). Genetics likely have a role in the recovery process, and there is most likely a complex interaction between genetics, the environment, and the characteristics of the injury itself that contribute to the recovery process and long-term outcomes. Genes of interest for TBI outcomes include those that are a part of catecholaminergic systems. These systems are susceptible to injury and altered regulation after a TBI and are important for plasticity and repair, and also play a role in attention and memory function (3-4).

Since the presence of certain genetic alleles could have harmful or beneficial effects on a patient's recovery after TBI, it is important to identify which genes are important, and what the impact of different polymorphisms is on both short and long-term recovery. One gene of interest is the catechol-O-methyltransferase (COMT) gene. The COMT gene is on chromosome 22. COMT degrades catecholamines (dopamine, epinephrine and norepinephrine) via methylation (5). A frequently seen polymorphism of this gene is a substitution of methionine to valine at codon 158 (6). The valine polymorphism has a higher enzyme activity and the methionine has less enzyme activity. Less enzyme activity results in more neurotransmitter being present in cells and synapses. Many studies have been performed to investigate the link between COMT polymorphisms and executive functioning in healthy subjects. One study explored the link between the methionine/valine polymorphism and attentional control. Blasi et al. found that healthy subjects with two valine alleles performed more poorly on attentional control tasks than subjects with one methionine and one valine allele, and poorer still than those subjects with two methionine alleles (7). COMT is also associated with outcomes for individuals with neuropsychiatric disorders such as ADHD. Children who were homozygous for the valine allele performed more poorly on a delayed-match-to-sample task, a measure of working memory (8). Effects of the COMT polymorphism have been studied in adult TBI and it was found that individuals who were homozygotes for valine scored more poorly on tests of executive functioning than TBI patients who were methionine homozygotes (9). The effects of this polymorphism in children, particularly children who have suffered a TBI, have not been explored. This study will help determine if COMT polymorphisms play a role in TBI recovery in pediatric patients which would help predict outcomes and treatment plans for this population.

**Hypothesis and Specific Aims**
Specific Aim 1: To determine the association of COMT genetic polymorphisms with executive function 6 and 18 months after TBI or OI.

Hypothesis 1: The valine polymorphisms in COMT will predict poorer executive functioning in the experimental and control groups in both the short (6 months post-injury) and long-term (18 months post-injury).

Specific Aim 2: To determine the influence of TBI on the association of COMT polymorphisms with executive function outcomes 6 and 18 months after injury.

Hypothesis 2: TBI will amplify the adverse association of the valine polymorphism in both short (6 months post-injury) and long-term (18 months post-injury) executive functioning.

Methods

Subjects: Children who sustained an early childhood TBI between ages of 36-84 months were drawn from a cohort of patients that were previously enrolled in a multi-center study. The study evaluated cognitive, behavioral, neuropsychological, adaptive, and executive functioning at baseline (~ 1 month after injury), 6, 12, and 18 months after early childhood TBI of varying severities. The different severities of TBI were defined as follows: Mild TBI by a Glasgow Coma Scale (GCS) rating of 13-15 with no concordant MRI and/or CT findings, complicated mild was GCS 13-15 with associated MRI and/or CT findings, moderate TBI was GCS 9-12, and severe TBI was GCS 3-8. Individuals with a history of a neurological disorder, autism, or cognitive delays prior to injury were not eligible for the study. As part of the initial study an orthopedic injury control group (OI) was recruited, and will be used as a control group for this study. Two hundred twenty-one children participated in the initial study with 102 children in the TBI group and 119 in the OI group. 188 of those children were determined to be eligible for this study. Data has already been collected for 129 of the potential participants and we anticipate that approximately 150 genetic samples will be available for analysis at the start of the proposed study. The TBI group has 58 subjects and the OI group has 71 subjects. 31 of the TBI subjects had a complicated/mild TBI, 14 had a moderate TBI, and 13 had a severe TBI.

DNA Collection: Participants provided saliva samples for DNA extraction using the Oragene (DNA Genotek, Ottawa, Ontario, Canada) DNA Self-collection kit. The Gene Discovery Core Unit at CCHMC assisted in identifying the specific polymorphisms. The polymorphisms will then be matched to the appropriate subject’s executive functioning testing results.

Neuropsychological Evaluations: As part of the original study, multiple neuropsychological, cognitive, and social/environmental interaction evaluations were administered at 0 (average of 40 days post-injury), 6, 12, and 18 months after injury for both the TBI and OI groups. This study will focus on those tests that evaluated executive functioning outcomes. The three tests administered to evaluate executive functioning were BRIEF (Behavior Rating Inventory of Executive Function), NEPSY VF (NEPSY Verbal Fluency), and Shape School. The BRIEF is comprised of several clinical scales that evaluate different aspects of executive function as interpreted by the parent (10). Although the initial evaluation took place post-injury, the parents were asked to answer based on their child's behavior and activity before the TBI. The NEPSY
VF is completed by the child and evaluates the child's ability to shift between concepts (11). The Shape School is also completed by the child and measures the inhibition, attentional control, and task shifting aspects of executive functioning (12). The BRIEF results will be used as a primary outcome, with the NEPSY VF and Shape School measures being secondary outcomes.

**Analysis Plan**

JMP Genomics in SAS will be used to perform data analysis. Descriptive statistics will be performed to evaluate the distribution of the data. The COMT polymorphisms will be tested for Hardy-Weinberg equilibrium. In both TBI and OI children linear regression will be used to examine the relationship between executive functioning at 6 and 18 months post-injury and the COMT polymorphisms (Aim 1). Executive functioning will be the dependent variable and the COMT polymorphisms will be the independent variable. Linear regression will also be used to determine the combined influence of TBI and COMT polymorphisms on executive functioning outcomes (Aim 2). Executive functioning at 6 and 18 months post-injury will be used as the dependent variable and the interaction term of group (TBI vs. OI) by COMT polymorphism will be the primary independent variable. Power calculations were done individually for the TBI and OI groups using the program Quanto. The minor allele frequencies (MAF) for the COMT polymorphisms are 0.54 for the valine allele and 0.46 for the methionine allele (9).

Conservatively, assuming a range of MAFs from 0.1-0.5, we have 80% power to detect a variant that explains as little as 8% of the phenotypic variance, which equates to effect sizes between 0.40 and 0.71 in the TBI and OI groups.

**Expected Results and Rationale**

We expect that those children in both the TBI and OI groups that are homozygous for the valine allele will have the poorest scores in executive functioning tests compared to heterozygotes and children who are homozygous for the methionine allele. Furthermore, having experienced a TBI will exacerbate the effects of the valine allele and impair recovery from injury. We expect that impaired recovery will be evident in tests administered 6 months after injury and 18 months after injury. These predictions have been based on studies that have shown that being homozygous for the valine allele results in poorer outcomes in various measures of executive functioning in healthy adults. Studies have also investigated the link between this gene and TBI outcomes in adults, and found that those individuals who sustained a TBI and were homozygous for the valine allele scored lower on executive functioning tasks than their TBI counterparts who were homozygous for the methionine allele. With this study we hope to investigate to what extent the same effect is true in children and begin to understand what can be done clinically to minimize negative outcomes associated with a certain allele.

**References**


5. COMT catechol-O-methyltransferase


