I, Julia M Smith, hereby submit this original work as part of the requirements for the degree of Master of Arts in Psychology.

It is entitled:
Genetic and environmental influences on executive functioning 12 months after pediatric traumatic brain injury

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Genetic and environmental influences on executive functioning 12 months after pediatric traumatic brain injury

A thesis submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Psychology, College of Arts and Sciences by

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Abstract

Deficits in executive function (EF) skills, or “higher order” cognitive processes, are particularly pervasive and problematic following pediatric traumatic brain injury (TBI), affecting academic, social, and functional outcomes. Extant studies indicate that the environment plays a role in the recovery process from pediatric TBI, including parental practices. In addition, a growing body of evidence suggests that an individual’s genetic makeup may also affect the recovery process. Studies have found an association between the dopamine receptor-4 (DRD4) 7-repeat allele and deficits in EF commonly seen after TBI; specifically, the DRD4 7-repeat allele has been associated with executive dysfunction in healthy children and is a well-known risk factor for the development of Attention Deficit Hyperactivity Disorder (ADHD). Research has also indicated that children with this allele are highly sensitive to the effects of experience or intervention, meaning that positive or negative effects of environmental factors (e.g., family functioning, therapeutic interventions) may be amplified in children with this allele. The present study examined the association of the 7-repeat allele and environment with neurocognitive and behavioral outcomes after pediatric TBI. For the present study, 113 participants (51 TBI and 62 orthopedic injury controls) were assessed 12 months post-injury and provided saliva samples for DNA extraction. Instruments assessing EF and environmental factors (i.e., parenting style, home environment) were collected. Regression analyses were utilized to examine the associations of genetics and TBI outcomes and possible interactions among hypothesized predictors of recovery. It was hypothesized that TBI and family environment would significantly moderate the association between the DRD4 7-repeat allele and EF, such that the association between the allele and EF would be amplified. It was further hypothesized that the presence of TBI, in addition to the quality of parenting style and home environment, would further amplify the
associations of the DRD4 7-repeat allele with EF measures. We found evidence to partially support gene by environment interactions within our pediatric population; however, significant differences were found only in the 7-repeat negative group, which was counter to the original hypotheses. Our findings suggested that family environmental factors significantly influenced efficiency scores in two modified Stroop task subtests. Compared to the 7-repeat positive participants, in both models the 7-repeat negative participants did significantly better in the more positive family environment. We did not find evidence to support the hypothesis of gene by injury interactions, nor evidence to support the hypothesis of triple interactions. These findings were counter to our initial hypotheses and indicate that further work needs to be done to better understand the association of genetics with recovery after pediatric TBI. Larger studies are needed to determine the exact link between genotype and TBI recovery, and how this information can be used to inform prognosis and develop individualized treatment protocols.
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Genetic and Environmental Influences on Executive Functioning 12 Months after Pediatric Traumatic Brain Injury

Traumatic brain injury (TBI) is one of the leading causes of morbidity and mortality in children in the United States, with over $1 billion in total annual health care costs (Schneier, Shields, Hostetler, Xiang, & Smith, 2006) and affecting an estimated 475,000 children ages 0-14 annually (CDC; Langlois, Rutland-Brown, & Thomas, 2006). Long-term impairments in pediatric TBI are associated primarily with neurocognitive and behavioral problems that develop after injury (Taylor, 2004). A barrier to providing optimal care and longer-term management following pediatric TBI is that both the expression of these impairments and the responsiveness of such impairments to therapy are highly variable, even in children who sustain seemingly similar head injuries (Taylor, 2004). Therefore, recent research effort has focused on understanding how such variability may be related to moderating factors such as genetics (e.g., Kurowski, Martin, & Wade, 2012) and home environment (e.g., Gerring & Wade, 2012).

Neurocognitive deficits in TBI are associated with impairments in long-term functioning after TBI across multiple settings, including home, school, and community. Deficits in executive function (EF) skills, or “higher order” cognitive processes, such as working memory, problem solving, inhibitory control, planning and execution, are particularly pervasive and problematic, affecting academic, social, and functional outcomes (Ganesalingam et al., 2011). Children who acquire a TBI at an early age and those with severe injuries have been shown to be more vulnerable to developing EF deficits (V. Anderson & Catroppa, 2005; Ewing-Cobbs, Prasad, Landry, Kramer, & DeLeon, 2004). However, children with less severe TBI can also experience long-term cognitive deficits (Catroppa, Anderson, Morse, Haritou, & Rosenfeld, 2007; Yeates, et
al., 2012). For some children, these deficits are pervasive and can last for years post-injury (Nadebaum, Anderson, & Catroppa, 2007).

Extant studies indicate that the environment plays a role in the recovery process from pediatric TBI. Specifically, socioeconomic resources, social supports, and better family functioning have been shown to buffer or reduce the adverse effects of severe TBI on EF (Kurowski et al., 2013; Yeates et al., 2004). Parental practices have also been shown to have a significant impact on EF recovery in children with TBI (Potter et al., 2011). However, environmental factors appear to have greater effects on behavioral, social and academic recovery than on EF skills and/or neurocognitive outcomes (e.g. constructional skills, naming, word reasoning, etc.) following a pediatric TBI (Taylor et al., 2002; Yeates et al., 2004; Yeates et al., 2002). Environment, as expressed in parenting styles, also appears to have a greater effect on EF outcomes in complicated mild/moderate TBIs than in severe TBIs in children who received an early TBI (Potter et al., 2011).

In addition to environmental factors, a growing body of evidence suggests that an individual’s genetic makeup may also affect the recovery process through several different pathways. Genetic factors may influence immediate or short-term recovery through pathophysiological factors, such as modulation of injury extent, response to neurotrauma, and initial recovery from injury. Additionally, genetic factors may also influence long-term consequences of TBI, such as cognitive capacity and neurobehavioral outcomes (e.g., EF behaviors) (McAllister, 2010). Genetic research on TBI recovery is relatively sparse; the extant literature has focused on the relationship between the known biological and behavioral outcomes of a genetic marker and the associated outcome measure being explored. For example, some researchers have focused on genes associated with cholinergic function (e.g., catecholamine), as
these genes have been shown to be associated with changes in neurocognitive outcomes; other researchers may focus on genes associated with neural repair (e.g., Apolipoprotein E [ApoE]), which may be more appropriate for a survival analysis.

It is important to note that research investigating the role of genetics in recovery from TBI has a number of challenges related to isolating the relationship between genetics and behavioral outcomes. Due to the need for an extremely large number of cases required to conduct a genome wide approach, in which outcomes are correlated with the whole genome without focusing on specific genes or genetic markers, studies examining the relationship between TBI and genetics have adopted a single gene approach instead (see for reviews: McAllister, 2010; Weaver, Chau, Portelli, & Grafman, 2012). However, the effects of genes can be complex, and single genes rarely have been shown to have large independent effects in general. Even in known relationships, genetic effects found in genetic association studies are typically small and issues with replicability are common (Lohmueller, Pearce, Pike, Lander, & Hirschhorn, 2003; Muñafó & Flint, 2004). It is also quite likely that a number of genetic factors interact to drive phenotypic presentation, along with influences from environmental factors.

*Effects of genes on TBI: BNDF & ApoE*

When a traumatic brain injury occurs, a complex cascade of cellular and subcellular events occurs that contributes to both acute and chronic cognitive outcomes for TBI survivors. Currently, genetic factors can be conceptualized and categorized by the cognitive domain they affect or are likely to affect. These domains include pre-injury risk factors for TBI, response to neurotrauma, repair and plasticity, and pre- and post-injury cognitive capacity and reserve (McAllister, 2010). Much of the current research on genetics and TBI outcomes have focused on
genes related to repair and plasticity and only in the adult population. The genetic factors that correspond with these two broad processes are brain-derived neurotrophic factor (BDNF) and ApoE.

BDNF is involved with both early and late long-term potentiation, which is an important process in the creation and maintenance of memory. Studies in adults examining a single-nucleotide polymorphism (SNP) that results in the change in amino acid from valine to methionine (Val66Met substitution) suggest that polymorphisms in the BDNF gene may influence cognitive performance shortly after mild TBI in adults (McAllister et al., 2012). Another study suggested that patients with TBI and healthy adult controls may not be influenced by polymorphisms in the same way. In healthy adult populations, Met carriers tend to perform more poorly on tests of EF and have reduced prefrontal cortex (PFC) gray matter volume compared to Val homozygotes (Egan et al., 2003). However, in a study with an adult TBI sample, Krueger and colleagues (2011) found that the Met allele may serve as a protective factor against declines in EF following a TBI.

Much of the current TBI and genetics research has investigated the effect of ApoE in an adult population, which is a glycolipoprotein that may be important for neural repair and “clean up” following a TBI. Several studies have found evidence that the e4 allele is associated with poor outcomes after TBI (Chiang, Chang, & Hu, 2003; Nathoo, Chetty, Van Dellen, & Connolly, 2003). However, much of the current genetic research has focused on the adult, rather than pediatric, TBI populations. As of 2014, only five genetic association studies specific to pediatric TBI were found, all of which studied outcomes associated with ApoE (Kurowski, Martin, & Wade, 2012; Reuter-Rice, Eads, Berndt, & Bennett, 2015). There was significant heterogeneity in study designs and mixed findings, but three of the five studies, using the Glasgow Outcome
Scale (GOS), found that the ApoE e4 allele was associated with poorer outcomes 6-12 months after a pediatric TBI (see Kurowski, Martin, & Wade, 2012 for a review). One of these studies (Teasdale, Murray, & Nicoll, 2005), found an interaction between age and outcome that suggested younger children who carry the ApoE e4 allele may be at greater risk for unfavorable outcomes following a pediatric TBI compared to older children. These studies provide preliminary evidence that genes, in particular the ApoE gene, may affect EF outcomes for children following a TBI.

While current pediatric TBI and genetics research has focused on the ApoE gene, research from the adult literature suggests that genetics related to pre- and post-injury cognitive capacity and reserve may also be associated with EF impairments following TBI. For this domain, much of the current research has focused on genes involved in the dopaminergic and serotonergic pathways, which are pathways often implicated with cognitive and social impairments following TBIs (McAllister, 2010; Weaver, Chau, Portelli, & Grafman, 2012). For EF, due to its association with the PFC, studies suggest that genetic polymorphisms may influence functioning by affecting dopamine and serotonin levels, as well as neuronal maturation and growth. Of these genetic factors, genes that affect dopamine activity have been repeatedly associated with EF and outcomes following TBI (McAllister, 2010; Weaver, Chau, Portelli, & Grafman, 2012).

Effects of genes on TBI: Dopamine

Dopamine is an important neurotransmitter involved in several critical roles of the central nervous system, including motor control, cognition, reward, and emotion (Oak, Oldenhof, & Van Tol, 2000; Vallone, Picetti, & Borrelli, 2000). The relationship between dopamine and EF has
been well-established (Logue & Gould, 2014). Catechol-O-methyl transferase (COMT) is an enzyme that degrades catecholamines (e.g., dopamine, epinephrine, and norepinephrine) and their proteins are encoded by the COMT gene (Grossman, Emanuel, & Budarf, 1992). The COMT Val158Met has been associated with EF, such that healthy homozygous carriers of the Met allele have been shown to have EF advantages compared to carriers of a Val allele (Egan et al., 2001). However, the relationship may also be affected by other dopamine genes. For example, studies have found that EF advantages associated with the Met allele were moderated by absence or presence of the DRD2 TAQ1 AI allele (Reuter et al., 2005). In addition, these effects may also differ by gender. Solís-Ortiz and colleagues (2010) found in a study of the interaction between estrogen and COMT polymorphisms that women with Val/Val genotypes performed better than Met allele carriers. Studies on the COMT polymorphism in an adult TBI population have found a trend that the COMT Met allele was associated with better performance on the Wisconsin Card Sorting Task, but not with performance on other measures of EF (Lipsky et al., 2014). Carriers of the Met allele performed comparably to healthy controls, whereas carriers of the Val/Val allele performed below average. This suggests that reduced EF typically found in healthy Val carriers may be further reduced following a TBI.

**Effects of DRD4 on executive function**

Another dopamine gene that has been associated with EF is the dopamine receptor-4 gene (DRD4). The DRD4 gene is located near the telomere of chromosome 11p with a variable nucleotide tandem repeat (VNTR) polymorphism in exon III. Of its alleles, which can range from 2 to 10-repeats, the most common subtype contains 4-repeats, but 2- and 7-repeat alleles are also known to commonly exist. Together, these three forms make up over 90% of the observed population. The incidence of these alleles varies widely by ethnicity and race, but
globally the 4-repeat variant occurs at about 64%, followed by the 7-repeat at about 20% of the population (Chang, Kidd, Livak, Pakstis, & Kidd, 1996; Lichter et al., 1993). Studies have found an association between variants in the DRD4 gene and deficits in EF that resemble those commonly seen after TBI (see below), but little to no research has been done to date directly on the TBI population. Much of the current research has focused on DRD4 as a genetic factor for EF performance in children and its association with attention-deficit hyperactivity disorder (ADHD).

The DRD4 7-repeat allele has been associated with executive dysfunction and externalizing behaviors in children. The expression of the DRD4 gene is largely in the PFC and affects the function of the executive attention network, a brain network that is an integral part of the EF network (Meador-Woodruff et al., 1996; Oak, Oldenhof, & Van Tol, 2000). The executive attention network includes the anterior cingulate cortex and the lateral PFC; both of these areas receive strong projections from the ventral tegmental area (VTA), an area rich in dopaminergic cell bodies. These areas have been shown to play an important role in executive attention tasks, such as rapid visual information processing and the resolution of conflicts between brain regions and competing stimuli (Botvinick et al., 1999; Coull et al., 1996; MacDonald et al., 2000; Posner and Petersen, 1990). Dopamine is an important modulator of the executive attention network and dopamine receptors are particularly dense in the cingulate. Animal models have shown that carriers of the 7-repeat allele are less responsive to dopamine (Asghari et al., 1995). The result appears to be suboptimal dopamine signaling in the PFC when compared to its more frequent 4-repeat expression. Research suggests that this phenomenon is related to a blunted ability in 7-repeat carriers to reduce cyclic AMP levels in the brain, an important second messenger derived from ATP used for intracellular signal transduction.
Individuals with the 7-repeat allele may need as much as three times the amount of dopamine to function at an optimal level (Asghari et al., 1995). This presumed lack of sufficient dopamine is hypothesized to result in attention and higher order deficits that are the hallmark features of attention-deficit hyperactivity disorder (ADHD), the disorder most highly associated with the DRD4 7-repeat allele. A meta-analysis by Faraone and colleagues (2001) found that the 7-repeat allele was twice as prevalent in ADHD probands. Later research has shown that 25-50% of genetic risk can be accounted for by the gene, making it a particularly salient target for research (Grady et al., 2003). The behaviors that research have most often associated with this gene coincide with the symptoms used in the diagnostic criteria for ADHD, which include inattention, impulsivity, and dysfunctional reward-seeking and motivational behaviors (Fossella et al., 2002; Froehlich et al., 2007; Kramer et al., 2007).

The DRD4 7-repeat allele has not only been linked with diagnoses of ADHD in childhood, but its expression has also been shown to be moderated by early risk and environmental factors. Becker and colleagues (2010) conducted a longitudinal study that found regulatory problems related to difficult temperament (e.g., irritability, distractibility, dysphoric mood) in infancy increased the risk of an ADHD diagnosis in adolescence for children with the allele but not for children without the allele. Martel and colleagues (2010) found that the allele moderated family environmental effects on ADHD, such that children who carried the DRD4 7-repeat allele were only at increased vulnerability of developing ADHD when in combination with inconsistent parenting. Their study has added to the growing literature indicating that the relationship between the 7-repeat allele and ADHD may by better described through a differential susceptibility model reflecting a heightened sensitivity to experiences from one’s environment among carriers of the gene (see Belsky et al., 2009).
Behavior in children recruited from the general population with the DRD4 7-repeat allele has also been found to be moderated by types of parenting styles. H. Smith and colleagues (2012) focused specifically on effortful control (EC) behavior in children, which included many behaviors related to executive functions (e.g. attention, inhibition, reward, motivation and planning), and whether the DRD4 7-repeat allele moderated the association between parenting style (operationalized as positive or negative) and EC in children. Their results showed that children with the DRD4 7-repeat allele displayed lower EC in relation to negative parenting than children without the allele. In addition, Berry and colleagues (2013) longitudinally studied the effect of the 7-repeat allele and early maternal sensitivity on attention level of early adolescent children. They found that children who carried the 7-repeat allele and experienced early maternal insensitivity demonstrated the highest levels of inattention as middle-school students. There was also evidence that children with the 7-repeat allele who experienced the most sensitive early maternal care were associated with lower levels of inattention in middle childhood. The magnitude of the absolute genetic effect increased as they got older, demonstrating that children appeared to be on inattention “trajectories” that could have clear behavioral and academic implications for these individuals over time. These findings support a broader genetics framework proposed by Belsky and colleagues that the DRD4 7-repeat allele is better classified as a “plasticity gene” rather than a “vulnerability gene,” such that carriers of the DRD4 7-repeat gene are not only at risk of poor outcomes in early negative family environments, but have the best outcomes in early positive family environments when compared to peers that do not carry the 7-repeat allele (for review of potential plasticity genes, see Belsky et al., 2009).

With growing evidence that the presence of the DRD4 7-repeat allele may indicate that a child’s behavior can be improved given the proper environment, researchers have begun
exploring the effect of targeted behavioral interventions that teach positive parenting skills. A large study ($N = 237$) out of the Netherlands used a randomized controlled trial to test the role of the 7-repeat allele in explaining variability in response to a behavioral intervention for parents and their children with externalizing behavioral problems (Bakermans-Kranenburg, Van, Pijlman, Mesman, & Juffer, 2008). Specifically, they examined the externalizing behavior of children following a video-feedback intervention targeting positive parenting and sensitive disciplining. They found that the intervention was most effective in children with the DRD4 7-repeat allele who also had parents that showed the largest increase in positive discipline use. This was the first study of its kind to explore measured gene by observed environment interaction, and its findings suggest that children may respond differently to intervention effects depending on their genetic differences.

With a lack of genetics research in a pediatric population on TBI recovery (Kurowski, Martin, & Wade, 2012), there is a critical need to better understand the role genetics plays during recovery after pediatric TBI. To our knowledge, no study has yet examined the DRD4 7-repeat allele in a pediatric TBI population. This allele, due to its association with attention and behavioral problems in healthy children and those with ADHD, as well as its responsiveness to environmental factors including parenting practices, is a particularly salient target for pediatric TBI research. The ultimate goal of the current project is to identify children who are likely to experience poor functional outcomes following TBI and to develop treatments to optimize their recovery and functional independence.

*Hypothesis 1- TBI Moderation*: It is hypothesized that TBI will significantly moderate the association between the DRD4 7-repeat allele and EF, such that the association between the allele and EF will be amplified in children with TBI.
Hypothesis 2- **Environmental Moderation**: It is hypothesized that family environment will significantly moderate the relationship between the DRD4 7-repeat allele and EF, due to the allele’s sensitivity to environmental factors, such that the association between the allele and EF will be amplified in poor environments.

*Exploratory Hypothesis 3- Joint Moderation*: In light of Hypothesis 1 and 2, it is further hypothesized that the presence of TBI, in addition to the quality of parenting style and home environment, will further amplify the associations of the DRD4 7-repeat allele with EF measures.

**Methods**

**Participants**

Individuals who had sustained an early childhood TBI, defined as between the ages of 36 and 84 months (3-7 years), were recruited from a cohort of patients that were enrolled previously in a multi-center study (3 sites: Cincinnati, Cleveland, and Columbus, OH). The study prospectively 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 Glasgow Coma Scale (GCS) and imaging findings were used to define TBI severity as follows: complicated mild as GCS 13-15 with associated CT and/or MRI findings, moderate TBI as GCS 9-12, and severe TBI as GCS 3-8. The GCS score assigned to the child was the lowest one recorded. The initial study also recruited an orthopedic injury (OI) control group for comparison. Inclusion in the OI group required a documented bone fracture in an area of the body other than the head that required an overnight hospital stay, as well as the absence of any evidence of loss of consciousness or other findings suggestive of brain injury. We
also used the OI group as a comparison group in our study of the association of the DRD4 polymorphisms with recovery from pediatric TBI. Exclusion criteria included histories of child abuse, neurological disorder, autism, or intellectual disability prior to injury or if English was not the primary language spoken in the home.

*DNA Collection and Analysis:* Participants provided saliva samples for DNA extraction. The Oragene (DNA Genotek, Ottawa, Ontario, Canada) DNA Self-collection kit was used. Saliva was self-collected by spitting into an Oragene cup. DNA was extracted using the manufacturer’s recommended procedure. Briefly, the sample was incubated at 50 degrees Celsius for 1 hour, DNA was purified using the Oragene-DNA Purifier solution (OG-L2P), and then precipitated using 95-100% ethanol solution. The genotypes of the DRD4 polymorphism were determined by polymerase chain reaction (PCR). VNTR genotyping of the DRD4 genes were performed using slight modification of previously described protocols (Ebstein et al., 1996; Hamarman, Fossella, Ulger, Brimacombe, & Dermody, 2004; Stein et al., 2005).

DRD4 polymorphisms were tested for Hardy-Weinberg equilibrium, a principle stating that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors, using JMP genomics software as part of the SAS program. The Hardy Weinberg Equilibrium was not violated ($x^2 = .04, p = .84$), suggesting that our genetic sample is representative of the general population. In keeping with past published research, genetic group was dummy-coded as either 0 (absence of a 7-repeat allele) or 1 (presence of at least one 7-repeat allele) (Bakermans-Kranenburg & van Ijzendoorn, 2006; Berry et al., 2013; H. Smith et al., 2012). Of the 113 participants who provided DNA samples, 31.9% were DRD4 7-repeat positive and 68.1% were DRD4 7-repeat negative.
Measures

To assess EF, measures assessing a range of parent reported global EF and neuropsychological testing of mental flexibility, selective attention, cognitive flexibility and processing speed skills were administered at all time points. These included a parent-report instrument of executive functioning behaviors (the BRIEF, Behavior Rating Inventory of Executive Function), an instrument of mental flexibility (NEPSY Verbal Fluency), and a modified Stroop Test designed to evaluate EF skills (The Shape School). To assess family environmental factors, an instrument of the quality and quantity of stimulation and support available in the home environment (the EC-Home), a questionnaire on self-reported parenting style (Parental Practices Questionnaire), and independently coded ratings of parental Warmth, Responsiveness and Negativity from recorded video sessions were used. The EC-Home was collected at a baseline home visit and the PPQ and video sessions were administered at all time points. Information regarding socioeconomic status (SES; defined as Z-scores that combined parental education and median census track income by zip code) and injury severity was also collected from the caregiver and/or hospital records at the baseline assessment and constitute the injury and demographic factors to be included in the analysis. See Table 1 for a summary of instruments administered and included in the present study.

Executive Function Skills

To assess behavioral outcomes related to attention and everyday EF, a parent-report instrument of executive functioning behaviors (the BRIEF, Behavior Rating Inventory of Executive Function) was administered (Derogatis & Spencer, 1982). The BRIEF is currently in wide use as a questionnaire for assessing behavior related to executive functioning in children.
For children ages 5 to 18, a school-age version is used (BRIEF) and for children ages 2-5 years, a preschool version is used (BRIEF-P). The two versions are highly similar but adjusted to reflect age-appropriate behaviors related to EF. They use many of the same questions and domains of EF, and each version yields an overall measure of EF, the Global Executive Composite (GEC). Of the genetics cohort at the 12 month visit, 40 participants were in the BRIEF-P age range (17 TBI, 23 OI) and 69 participants were in the BRIEF age range (32 TBI, 37 OI). The BRIEF was identified as a Common Data Elements supplemental instrument by the Pediatric TBI Outcomes Workgroup (McCauley et al., 2012). Because the BRIEF is a parent rating of EF skills, the 12 month ratings were based on the parent’s interpretation of the child’s EF skills at that time. For the present analyses, the Global Executive Composite scores were used as a dependent variable.

To assess EF at time of evaluation, the NEPSY and Shape School instruments were administered to the child at the 12 month assessment. The NEPSY Verbal Fluency (VF) subtest (Korkman, Kirk, & Kemp, 1998) is a measure of mental flexibility that requires children to shift from one conceptual set to another. Children are instructed to name as many age appropriate items from a conceptual set, such as types of food or animals, as they can. They are given 60 seconds to name as many items as possible and then switch to the next set. The total response score from this subtest was used as a dependent variable in the analyses.

The Shape School test (Espy, 1997) is a modified Stroop task that measures inhibition and executive control in a format appropriate for preschool aged children (ages 3-6 years). The tasks utilize a story book format and age appropriate concepts of color, facial expression and shape to test EF skills, such as inhibition and switching set. The Shape School has been found to be sensitive to developmental changes in executive functions. It includes raw scores of correct and incorrect responses by concept set, as well as efficiency scores using correct responses.
divided by time. The efficiency subtest scores (# correct responses minus incorrect responses/time) on tasks of inhibition, switching, and a task combining both were used in analyses. It should be noted that some children who completed the first subtest (Inhibit) were not able to complete all of the subtests as the difficulty increased (Inhibit: \( N = 106 \), Switch: \( N = 96 \), Combined: \( N = 93 \)). In addition, for the Shape School Switch and Combined subtests, children included in the analyses had significantly better pre-morbid EF function (as assessed by BL BRIEF ratings), higher average IQ, and were older compared to children who were not able to complete the subtests.

*Environmental Measures*

To assess the quality of home environment, the Early Childhood- Home Observation for Measurement of the Environment (EC-HOME, Caldwell & Bradley, 2000) was administered at baseline. This instrument involved an in-home visit by an assessor who rated observed levels of parental stimulation and support for the child. Both objective observations of stimuli found within the home and discussions about child-related activities with parents were included in the scoring of the instrument. Factors of interest included in the instrument are learning materials, developmental stimulation, physical environment and parental supportiveness. A total HOME score is calculated by summing ratings across eight domains. Higher scores indicate greater levels of structure, stimulation and support that have been rated in the home environment. Research has shown the HOME to be a reliable and valid predictor of cognitive development in children that can assess factors not wholly captured by SES (Caldwell & Bradley, 2000). In the current study, total HOME scores and SES were significantly correlated \( (r = .56, p = <.01) \), suggesting that these measures were related but nonequivalent. Variance inflation factor (VIF)
and tolerance estimates suggest multicollinearity was within the acceptable range ($VIF > 2.50$ and $tolerance < .40$).

To assess self-reported parenting style, the Parenting Practices Questionnaire (PPQ) was also administered at 12 months (Robinson, Mandleco, Olsen, & Hart, 1995). The PPQ assesses self-reported engagement in three types of parenting styles: authoritative, authoritarian, and permissive (Baumrind, 1966). The permissive parent allows the child to regulate his or her own activities, avoids control, and uses reason and manipulation but not power to parent the child. The authoritarian parent shapes and controls the child in accordance with a set standard and often restricts the child’s autonomy. The authoritative parent directs the child in a rational manner, encourages give and take, and both autonomy and disciplined conformity are valued. Permissive and authoritarian parenting styles are generally considered maladaptive, whereas authoritative parenting is considered an effective parenting style. The instrument consists of 62-items presented in a 5-point Likert scale. We used the raw total score for each of these three dimensions to characterize parenting styles affecting the family environment as environmental measures.

An objective assessment of parenting behaviors was also collected at 12 months using video-taped and coded parent-child interactions. The parent–child interactions consisted of two averaged five-minute interaction sessions that were coded based on structured rating system validated by Landry and colleagues (Landry, Chapieski, Richardson, Palmer, & Hall, 1990; Landry, Miller-Loncar, Smith, & Swank, 2002; Landry, Smith, Miller-Loncar, & Swank, 1997; Landry, Smith, Swank, Assel, & Vellet, 2001). The included coded interactions were from two “free play” sessions, where the parent was instructed to spend time with his/her child as if they were at home. Developmentally appropriate toys and parent magazines were available in the
room. Parent behavior was rated on a 5-point scale (higher scores indicating more positive behavior) along the dimensions of parental warmth, contingent responsiveness, and negativity. Parental warmth was rated based on the presence and intensity of verbal and nonverbal warmth, affection, and positive regard toward the child. Contingent responsiveness ratings reflected the degree of the parent’s sensitivity and responsiveness to the child’s behavior. Negativity was rated as expressions of disapproval of the child or the child’s attributes, activities, products, or choices, as well as sassy, sarcastic, rude, or impudent speech. The interactions were divided into two 5-minute segments that were coded independently and averaged to increase the stability of the measures. Ratings of 15% of the tapes by the entire rating team revealed a satisfactory level of interrater reliability, with intraclass correlation coefficients ≥ 0.80 for all codes. Procedures for maintaining reliability between blinded and un-blinded raters minimized any bias due to the awareness of some of the raters of the nature of the child’s injury (TBI vs. OI). We used mean parent scores of Warmth, Contingency and Negativity from the two play sessions as environmental measures. The Negativity score was dichotomized as presence (score of 4 or less) or absence (score of 5) of Negativity due to the low occurrence of negative parent-child interactions recorded during the play sessions.

Results

Statistical approach

All statistical analyses were conducted using SPSS or SAS. In this study, the three EF outcome instruments were: BRIEF-GEC, NEPSY Verbal Fluency, and the Shape School. The three environmental instruments were: EC-HOME, PPQ, and the coded parent-child interaction videos. Due to a high level of skew related to low levels of negativity, the variable for parent
negativity from the coded parent-child interaction videos was made dichotomous (parent rating of 5 coded as 0 [No Negativity] and parent rating of ≤4 coded as 1 [Some Negativity]). Other independent variables in the model included gene variant, injury group, age at injury, as well as the interaction terms. Models controlled for age at injury, sex, race (white versus non-white), and SES. Because of potential genetic variation by race (Chang et al., 1996; Lichter et al., 1993), race was retained as a covariate in all models. Additionally, because of the potential effects of SES on outcomes and the significant difference between TBI and OI groups, it was also included as a covariate in all analyses. Since age at injury and sex could be accounted for by normed standard scores in BRIEF-GEC and age at injury in NEPSY VF, but was not accounted for in the raw efficiency scores of the Shape School subtests, both age at injury and sex were entered in to all models regardless of outcome measure and trimmed if not found to be significant predictors in the model.

Simple statistics such as means, standard deviations, and frequencies were used to summarize the data and group comparisons (TBI and OI) were made using independent t-tests. The main focus of the analysis was on the association of the DRD4 7-repeat allele with executive functioning outcomes at 12 months post-injury and the moderating effects of TBI and/or family environment. More specifically, gene (Absence versus Presence) x injury type (TBI versus OI) and gene x environment interactions were tested in separate models. In each model, covariates (age and/or sex if significant predictors, race and SES in each model in that order) were entered first, followed by injury type, allele variant, environmental moderator and the cross product term of the gene x hypothesized moderator (injury or environment). For exploratory triple interactions, the triple interaction of gene x injury x environment was included as the final term to examine the exploratory hypothesis of joint moderation effects. Exploratory triple interaction
models were analyzed first (hypothesis #3). For insignificant models, the triple interaction term was trimmed and double interaction models were run that included a gene x injury (hypothesis #1) or gene x environment (hypothesis #2) term. Finally, main effects of injury, gene and environment were also explored.

Analysis of demographics

Two hundred twenty-one children participated in the initial study (102 in TBI group, 119 in OI group). One hundred seventy four children completed 12-month assessments (85.3% of the TBI group, 73.1% of the OI group). Participants from the original study were recruited to participate in a follow-up study during middle school and invited to provide salivary DNA samples at the same assessment. In the follow-up study, 135 participants provided saliva samples for DNA extraction. Of those, only one participant’s data could not be used in the DRD4 analyses due to unreadable genetic data. There were 12 participants for whom genetic data were available but who did not complete 12-month follow up visits in the earlier study. Across sites, there were no significant demographic differences between participants that chose to provide genetics samples and those that did not (all p-values >.05). In the present sample, 51 participants who had sustained a TBI were included in the analysis (13 severe, 11 moderate, and 27 complicated mild). An un-complicated mild TBI group that provided genetic data and completed the 12 month visit (N = 9), defined as a GCS 13-14 with normal CT and/or MRI findings, was excluded from the analysis. An examination of mean scores revealed that their scores did not reflect a dose-response relationship as the other TBI groups and OI group demonstrated and were removed. Therefore, of the 135 participants that provided genetic data, 113 were included in the final analyses. The Differential Ability Scales (DAS) (Elliot, 1990) were administered to establish global cognitive abilities of the participants. At the 12-month assessment, both the TBI
and OI groups had mean IQ scores in the average range; however, mean IQ in the OI group was marginally higher than the TBI group \((p = .05)\). Attrition was not significantly related to group, age at injury, sex, race, or SES. However, it should be noted that significantly more children were included in the 12 month analyses from the Cincinnati site due to lower attrition rates compared to the other two sites. See Table 2 for demographic and injury characteristics of the genetics cohort at 12 months.

The number of participants in the present study \((N = 113)\), although modest in size, is consistent with sample sizes used in recent genetic studies that found a significant relationship between similar alleles and cognitive outcomes in either adults or children (Bakermans-Kranenburg et al., 2008; McAllister et al., 2008). Data for analysis was taken from the 12-month evaluation post-injury, with the exception of EC-HOME from the baseline home visit.

**Preliminary analyses**

We conducted a preliminary examination of the data, first focusing on the distributions. Of the EF measures, NEPSY Verbal Fluency, Shape School Switch and Shape School Combined scores were normally distributed with no issues in regard to skew or kurtosis noted. The distributions of the BRIEF-GEC scores were skewed \(\text{skew} = 1.25; \text{kurtosis} = 2.75\). Specifically, one participant scored greater than three standard deviations above the sample mean \((t = 107)\). Examination of the data in light of the participant’s history and their level of functioning led to the determination that the high GEC score was likely valid and representative of the high degree of post-injury executive dysfunction that the participant experienced. Therefore, it was decided that the score would be retained; however, should a model be significant with the BRIEF-GEC as the dependent variable, it would be rerun with this outlier coded as 80 (two standard deviations
above the mean) to determine whether the outlier alone was driving the significance. This change
would improve the distribution to appear more normal ($\text{skew} = .73; \text{kurtosis} = .01$). The Shape
School Switch subtest was noted as leptokurtic ($\text{skew} = .86; \text{kurtosis} = 4.46$), meaning most of
the scores were centered around the mean.

Of the environmental measures, Warmth, Contingency, Authoritarian, Permissive and
HOME scores were normally distributed with no issues in regard to skew or kurtosis noted.
Negativity scores were highly skewed ($\text{skew} = -3.04; \text{kurtosis} = 11.56$) with most scores showing
low negativity. The variable was dichotomized as 1 (score of 5.00 meaning no Negativity) and 0
(score <5.00 meaning some Negativity demonstrated) to improve the distribution ($\text{skew} = -1.75;
\text{kurtosis} = 1.10$). Authoritative parenting style scores were significantly negatively skewed ($\text{skew}
= -1.16; \text{kurtosis} = 1.86$) with more scores falling in the upper range.

As for demographics, race was also skewed ($\text{skew} = -1.30; \text{kurtosis} = -.32$) with the
sample being predominately White, but no other issues with skew or kurtosis were noted. Other
than the BRIEF-GEC score addressed above, there were no concerns about problematic outliers.

Correlational analyses were conducted to examine multicollinearity among the predictors.
Although the literature provides no consensus for assessing multicollinearity based on
correlation coefficients, it has been suggested that correlations that exceed .50 pose a risk for
multicollinearity (Bonate, 1999). For the current study, correlation coefficients among the
environmental measures ranged from -.40 to .48. While this indicated a substantial amount of
shared variance, it did not warrant concern about multicollinearity or the need to combine the
environmental factors into a single factor. However, there was one exception among the
environmental variables where the correlation coefficient exceeded .50. Warmth and
Contingency scores from the coded parent-child interaction videos were significantly correlated
(r = .85). Warmth and Contingency were still entered and examined separately in interaction models. It was decided that if a model was found significant it would be rerun using an averaged score of the two variables (note: no models were found to be significant). For the dependent variables that were included in the current study, correlation coefficients ranged from -.29 to .34 when compared across measures. The Shape School subtests were more highly correlated (r = .47-.50) with each other, which is to be expected given their similar constructs. It has been posited that using tolerance and VIF statistics is more accurate than examining correlation coefficients when assessing for multicollinearity (Shieh & Fouladi, 2003). Therefore, we applied the criteria of Variance Inflation Factor (VIF) greater than 2.50 and tolerance less than .40 (Allison, 1999) as an indication of multicollinearity; using this criteria, no cases of multicollinearity were identified with the independent variables, with the exception of Warmth and Contingency.

Regression analyses: Exploratory triple interactions

We first calculated models that examined the exploratory gene x injury x environment interactions. Models using the BRIEF-GEC were examined first, followed by Verbal Fluency and the Shape School subtests (Inhibit, Switch, and Combined). These initial models yielded no significant findings with BRIEF-GEC or the Shape School subtests as the dependent variables. With Verbal Fluency as the EF outcome measure, there was a significant gene by injury by environment interaction with the dichotomous Negativity scores from the coded parent-child interaction videos (unstandardized β = 6.87, t (93) = 1.98, p = .05).

Using differences of least squares means to understand the nature of the interaction among injury type, gene variant, and parental negativity, dichotomous Negativity scores for the
TBI and OI groups were used. Follow-up analyses using ANOVA or ANCOVA were not possible due to small cell sizes.

However, a follow-up ANCOVA revealed that cell sizes were not equal between groups and that in the Some Negativity groups, cell sizes ranged from two to seven participants, whereas in the No Negativity groups, cell sizes were larger (range: 11-32). Differences of least square means revealed that the significant triple interaction ($\text{Estimate} = -0.43$, $t (93) = -2.19$, $p = .03$) involved the OI 7-repeat positive Some Negativity group, which only had two participants that scored below average on NEPSY VF ($\text{Estimate} = 3.28$). Therefore, this estimate is not considered to be valid representations of this OI group in the greater population. Follow-up regressions that held either injury group or gene variant as constant revealed only a significant injury x environment interaction in the DRD4 7-repeat positive group ($\text{unstandardized } \beta = 9.45$, $t (26) = 3.39$, $p = <.01$), suggesting this interaction was driving the significance of the model and that the significant interaction is not meaningfully interpretable. See Figure 1.

*Regression analyses: Gene x injury interactions*

We next calculated regression models that examined gene by injury interactions. Results failed to yield significant findings with the BRIEF-GEC, NEPSY VF or the Shape School scores as the dependent variables ($p = >.05$).

*Regression analyses: Gene x environment interactions*

We next calculated regression models that examined gene by environment interactions. Results failed to yield significant findings with the BRIEF-GEC or NEPSY VF scores as the dependent variable ($p = >.05$). For the Shape School subtests, we found two significant gene x environment interactions.
With the Shape School efficiency scores from the Inhibit subtest as the EF outcome measure, there was a significant gene x environment interaction with the Parenting Practices Questionnaire (PPQ) Permissive subscale score \((\text{unstandardized } \beta = .40, t (97) = 2.31, p = .02)\). To better understand the nature of the interaction among gene and Permissive parenting style, an analysis of simple slopes was conducted. Permissive parenting scores for the 7-repeat positive and 7-repeat negative groups were estimated at one standard deviation above and below the mean for Permissive Parenting scores.

At one standard deviation below the mean Permissive Parenting score, it was estimated that Shape School Inhibit scores for the 7-repeat positive group were 0.22 points lower than that of the 7-repeat negative group, which was a marginally significant difference \((p = .05)\). This suggested that lower levels of permissive parenting style were associated with higher efficiency scores in the 7-repeat negative group. At one standard deviation above the mean, the difference in Shape School Inhibit scores between the 7-repeat positive and 7-repeat negative groups was .15 points, which was not significantly different \((p = .17)\).

Simple slopes analysis further indicated that the slope of the line for the 7-repeat negative group \((\text{Estimate} = -0.31, t (97) = -2.79, p = .01)\) was statistically significant from zero, meaning that Shape School Inhibit efficiency scores depend on permissive parenting style, with lower efficiency scores expected among those with higher levels of permissive parenting style. The line of the 7-repeat positive group did not significantly differ from zero \((\text{Estimate} = .09, t (97) = .71, p = .47)\), which suggested that among 7-repeat positive participants, Shape School Inhibit scores did not differ based on level of permissive parenting style. See Figure 2.

With the Shape School efficiency scores from the Switch subtest as the EF outcome measure, there was a significant gene by environment interaction with the EC-HOME score.
(unstandardized $\beta = -.01$, $t (87) = -2.18$, $p = .03$). To better understand the nature of the interaction among gene and home environment, an analysis of simple slopes was conducted. EC-HOME scores for the 7-repeat positive and 7-repeat negative groups were estimated at one standard deviation above and below the mean for EC-HOME scores.

At one standard deviation below the mean HOME score, it was estimated that Shape School Switch efficiency scores for the 7-repeat negative group were .05 points lower than that of the 7-repeat positive group, which was not a significant difference ($p = .25$). At one standard deviation above the mean, the difference in Shape School Switch efficiency scores between the 7-repeat positive and negative groups was .09 points, which was a marginally significant difference ($p = .06$). This suggested that more positive home environment levels were associated with higher efficiency scores in the 7-repeat negative group.

Simple slopes analysis further indicated that the slope of the line for the 7-repeat negative group ($Estimate = .01$, $t (87) = 3.09$, $p = <.01$) differed significantly from zero, meaning that Shape School Switch efficiency scores depend on home environment, with higher efficiency scores expected among those with more positive home environment. The line of the 7-repeat positive group did not significantly differ from zero ($Estimate = <.01$, $t (87) = .31$, $p = .76$), which suggested that among 7-repeat positive participants, Shape School Switch efficiency scores did not differ based on home environment. See Figure 3.

Main effects: Injury

There were two significant regression models that found a main effect of injury on EF outcomes. With the BRIEF-GEC as the EF outcome measure, there was a significant main effect of injury type (unstandardized $\beta = 7.47$, $t (104) = 3.23$, $p = .002$). With the Shape School efficiency scores from the Switch subtest as the EF outcome measure, there was a main effect of
injury type \( (unstandardized \beta = -10, t (91) = -2.95, p = .004) \). Results failed to yield significant main effects of injury with the NEPSY VF or other Shape school scores (Inhibit and Combined) as the dependent variables \( (p = .05) \).

**Main effects: Gene**

Results failed to yield significant main effects of gene with the BRIEF-GEC, NEPSY VF, or the Shape School scores as the dependent variables \( (p = .05) \).

**Main effects: Environment**

With the BRIEF-GEC as the EF outcome measure, there was a significant main effect of Permissive Parenting Style \( (unstandardized \beta = 12.30, t (104) = 4.98, p = <.001) \). With the NEPSY VF as the EF outcome measure, there was a significant main effect of Permissive Parenting Style \( (unstandardized \beta = -1.58, t (106) = -2.37, p = .020) \) and HOME score \( (unstandardized \beta = .16, t (105) = 3.47, p = .001) \). Results failed to yield significant main effects of environment with the Shape School scores as the dependent variables \( (p = .05) \).

**Discussion**

The current study sought to examine potential genetic and environmental moderators, and their interactions, as predictors of EF outcomes following recovery from an early pediatric TBI. In doing so, we aimed to identify children who are likely to experience poor functional outcomes following TBI and to develop treatments to optimize their recovery and functional independence.

We found evidence to partially support gene by environment interactions within our pediatric population; however, significant differences were found only in the 7-repeat negative group, which was counter to our original hypotheses. Our findings suggested that family
environmental factors (permissive parenting style and home environment), significantly influenced efficiency scores in two of the Shape School subtests. Compared to the 7-repeat positive participants, in both models the 7-repeat negative participants did significantly better in the more positive family environment (i.e., low permissive parenting style and high home environment). We did not find evidence to support our first hypothesis of gene by injury interactions, nor evidence to support our third hypothesis of triple interactions following the finding that the significant gene x injury x environment model was invalid due to small cell sizes. These findings were counter to our initial hypotheses and indicate that further work needs to be done to better understand the association of genetics with recovery after pediatric TBI.

To our knowledge, this is the first study to evaluate the association of the DRD4 7-repeat allele with EF after pediatric TBI. It builds on prior work that has associated the DRD4 7-repeat allele with EF in healthy children and those diagnosed or at risk of developing ADHD (Faraone, Doyle, Mick, & Biederman, 2001; Froehlich et al., 2007). In prior work, the DRD4 7-repeat allele was associated with poorer EF (Fossella et al., 2002; Froehlich et al., 2007; Kramer et al., 2007; Langley et al., 2014), with a robust gene by environment interaction that suggests negative early childhood environment is associated with poorer EF outcomes (Becker et al., 2010; Berry et al., 2013; Martel et al., 2011). In contrast, our study demonstrated that absence of the 7-repeat allele and positive early childhood environments is associated with better EF outcomes. We also did not find evidence of possible interactions related to EF outcomes following TBI. There may be several potential explanations for these findings.

To our knowledge, DRD4 function in a pediatric or adult TBI cohort has not been examined. Therefore, the 7-repeat allele may behave differently in this population. Krueger and colleagues (2011) evaluated the effect of the Val66Met polymorphism of the BDNF gene on
recovery of EF following TBI in adults and found that the Met variation, which was associated with EF deficits in healthy populations, was protective against declines in EF following TBI. A similar protective function may be occurring with the DRD4 7-repeat allele. The effects of TBI on DRD4 function are unclear and need to be researched further.

Although the DRD4 7-repeat allele is generally associated with poorer EF, studies have also found that carriers of the 7-repeat allele diagnosed with ADHD perform better on some neuropsychological measures than children diagnosed with ADHD without the allele. This suggests there may be a protective factor among those who develop ADHD, compared to other DRD4 variants (Manor et al., 2002; Swanson et al., 2000). However, researchers have also found the opposite results using similar instruments and population (Langley et al., 2014). Furthermore, research has shown that children with ADHD are at increased risk of sustaining a TBI or orthopedic injury (DiScala, Lescohier, Barthel, & Li, 1998; Lyon, Baker, & Gren, 2009). Therefore, our sample of injured children may represent a subset of children already at risk of developing poor EF or ADHD. Future studies should evaluate EF in both pediatric TBI and those with comorbid ADHD diagnoses, as well as normally developing children.

Additionally, the measures used likely evaluate slightly different aspects of EF that may be influenced by developmental differences in this young cohort. For instance, in the current study we used one parent rating measure of EF behaviors (BRIEF) and two neuropsychological measures (NEPSY VF and Shape School), while prior studies have relied primarily on externalizing behaviors, symptomatology of ADHD, or continuous performance tests (Bakermans-Kranenburg & van IJzendoorn, 2006; Berry et al., 2013; Kebir, Tabbane, Sengupta, & Joober, 2009), respectively). The BRIEF-GEC is likely most similar to other studies that used behavioral outcomes, such as the Child Behavioral Checklist, but the global score was used and
may not capture the externalizing and inattentive behaviors that have been associated with DRD4 gene by environment interactions. The NEPSY VF, a verbal fluency task, and the Shape School, a modified Stroop task, may not assess aspects of EF that are sensitive to the gene by environment effects. Lipsky and colleagues (2005) studied the association of the COMT Cal158Met genotype with EF following TBI in an adult population and found significant deficits on perseverative errors during the Wisconsin Card Sorting task, but did not find significant differences on the Stroop Color and Word Test, Trail Making Test, and two tests of verbal fluency (Controlled Oral Word Association Test and Animal Naming). Kebir and colleagues (2009) conducted a review of EF neuropsychological results associated with the DRD4 7-repeat allele and found that the continuous performance test (CPT) and derived tasks were the most used instruments in studies. As for results, the most consistent result appeared to be the association of high reaction time variability with the 7-repeat allele absent groups, which may be specific to those diagnosed with ADHD. They reported that speed of processing, set-shifting and cognitive impulsiveness were less frequently investigated but seemed to be worse in the 7-repeat allele carriers. Finally, they noted no effect of genotype was found on response inhibition (e.g., stop and go/no-go tasks). More research is needed to explore whether these gene and assessment findings are also found within a pediatric TBI population. Further issues related to EF assessment are discussed in the limitations section below.

Although several previous studies have demonstrated an association of the 7-repeat allele with poor EF, there are some conflicting reports (Bellgrove et al., 2005; Manor et al., 2002; Swanson et al., 2000). Other variants of the DRD4 VNTR polymorphism, specifically, the 2-repeat, may be less responsive to dopamine and therefore is more similar to the 7-repeat than the 4-repeat (Wang et al., 2004). Therefore, the current classification of homozygous or
heterozygous presence of the 7-repeat allele may be too simplistic of a classification. Additionally, it is more likely that a complex interaction across several genes will better explain the genetic association with outcomes. Genes involved in other neuro-signaling, inflammatory, or neuroplasticity pathways (e.g., COMT, BDNF, ApoE, etc.) may interact with the DRD4 variants to determine EF outcome after pediatric TBI. Genetic factors may regulate acute biological responses shortly following TBI, but alteration of typical genetic expression through methylation and acetylation due to neurotrauma can also affect the expression of surrounding genes and the overall phenotype. Epigenetic factors associated with neurotrauma are currently not well understood, but are likely to be an area of focus in the future and should also be explored with DRD4 expression following TBI. Finally, multiple factors likely influence recovery after TBI, including environmental, genetic, and other individual characteristics (see Figure 4). Comprehensive studies including these recovery factors, in addition to more complex genetic interactions, are needed in future research.

Limitations

This study should be regarded as an exploratory or pilot study. Although this is a large study relative to other studies of pediatric TBI, much larger studies are likely needed to understand the influence of the DRD4 polymorphism on EF outcome after pediatric TBI. This study consisted primarily of complicated mild/moderate TBI and due to small cell size related to presence of the 7-repeat allele, subpopulation analyses were not examined. Larger studies that include a broad range of injury severity are needed to better elucidate the interaction between severity and genotype. Additionally, other functional polymorphisms within the DRD4 gene or genetic variants in other genes may influence recovery after pediatric TBI. Further research has also supported that biomarkers related to trauma (e.g., interleukins) may also play a role in adult
and pediatric TBI recovery (for reviews see McAllister, 2010; Reuter-Rice et al., 2015). Thus, larger studies examining the contribution of a set of polymorphisms, genes and/or biomarkers are likely needed in the future. In addition, consideration should be given to the recruitment of an uninjured control or comparison group in light of these findings. In the present study, an OI group was used that may share similar biomarker exposure related to trauma (e.g., inflammation, infection, etc.), and it is unclear how epigenetic factors may influence DRD4 function in these cohorts. Further research is needed in order to understand the potential influence of these biomarkers on epigenetics and neurocognitive outcomes in children.

EF is also a difficult construct to measure, with much debate over what cognitive abilities comprise EF (see Barkley, 2012). There is also a well-known discrepancy between neuropsychological EF outcomes and behavioral/functional outcomes (Hughes, 2011; Lezak et al., 2012). Furthermore, many difficulties present in the testing of adult EF are made more problematic by the substantial inter-individual variability of skills seen in preschoolers due to the rapid and variable develop of important cognitive skills during this age. Additionally, many tests of EF are multi-dimensional and require both executive and non-executive cognitive abilities to complete. Therefore, task impurity is a concern in many EF tasks (Miyake et al., 2000). For example, while the NEPSY VF task is meant to assess a child’s ability to switch mental set, it is also contingent upon a child’s language skills, as well as other associated EF skills like attention. Estimated levels of pre-injury EF was also not controlled for in this study and should be included in future analyses.

The use of summary scores as outcome variables, such as those used in the NEPSY VF models, may mask personal and situational factors that result in impaired performance. For young children, this can include greater distractibility, shorter attention spans, motivational
issues, and poorer sense of testing requirements. Despite using raw scores, similar issues can also affect the efficiency scores of the Shape School subtests. It has also been proposed that the unique environment during an assessment, such as one-on-one support and encouragement that help keep a child on-task, may mean that the assessor acts as the “frontal lobes” of the child and affect scores (Stuss & Alexander, 2000). Due to the rapidly developing skills and disparity between abilities in the age range studied in the present study, there is also a lack of appropriate and comprehensive EF measures available to study this population, as well as good norms. P. Anderson and Reidy (2012) suggested that most EF tests designed for preschoolers may be considered “experimental,” such that they are often not commercially available, have limited reliability and validity information, and use inappropriate convenience norms. Therefore, continued development of reliable, valid, and comprehensive EF assessments are necessary to further our understanding of EF outcomes in very young children that can also be used for reassessment as children enter into school-age and adulthood.

Finally, this study looked at EF outcomes only at 12 months post-injury. Due to the lack of research on DRD4 function in a pediatric TBI population, it is unclear if this is an appropriate amount of time for the effects of a TBI to be evident in EF outcomes for those with and without the 7-repeat allele. Previous research has looked at the effects of environmental moderators over time or at later than 12 months (e.g., Becker et al., 2010). For example, Berry and colleagues (2013) used data from the NICHD Study of Early Child Care and Youth Development to examine the gene by environment interaction over time. Using latent growth models, they found a significant interaction between the 7-repeat allele and early maternal sensitivity that predicted inattention trajectories across middle childhood (from prekindergarten to fifth grade). Of interest to the present study, ratings of inattention across groups were far more restricted in
prekindergarten ratings compared to later time points. They also found that the magnitude of the absolute genetic effect increased over time as children’s inattention trajectories diverged. This suggests that EF outcome differences for children who have sustained TBIs in early childhood, carry the 7-repeat allele, and have negative family environmental factors, may not be revealed until later in childhood. Thus, the gene by environment interaction in a pediatric TBI population should also be examined over time and into later childhood.

**Conclusions**

There is limited research examining the effects of genetics on pediatric TBI outcomes, and this study expands the knowledge base with a relatively large sample that has been followed 12 months post-injury. The study provides preliminary evidence of a gene by environment interaction in a pediatric population for children lacking the DRD4 7-repeat allele, but did not find significant moderating effects for EF outcomes in a pediatric TBI population. Larger studies are needed to determine the exact link between genotype and TBI recovery, and how this information can be used to inform prognosis and develop individualized treatment protocols. This pilot study suggests that genetics potentially influence TBI outcomes and warrants further investigation.
Bibliography


Solís-Ortiz, S., Pérez-Luque, E., Morado-Crespo, L., & Gutiérrez-Muñoz, M. (2010). Executive functions and selective attention are favored in middle-aged healthy women carriers of


Table 1
Instruments Administered

<table>
<thead>
<tr>
<th>Domain/Construct</th>
<th>Instruments</th>
<th>Source</th>
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<tr>
<td><strong>Executive Function Skills</strong></td>
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<td></td>
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<tr>
<td>Observed behavior</td>
<td>BRIEF/BRIEF-P</td>
<td>Parent</td>
</tr>
<tr>
<td>Planning/Problem Solving</td>
<td>NEPSY VF</td>
<td>Child</td>
</tr>
<tr>
<td>Inhibition and Attentional Control</td>
<td>Shape School</td>
<td>Child</td>
</tr>
<tr>
<td><strong>Environmental Instruments</strong></td>
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<td>Home environment</td>
<td>EC-HOME</td>
<td>Parent interview and in-home</td>
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<td>observation</td>
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<td>Parental Practices Questionnaire</td>
<td>Self-report parent questionnaire</td>
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<td>Measure of Parent-Child Interaction</td>
<td>Coded video observation</td>
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<td>Socioeconomic status</td>
<td>Income and Education</td>
<td>Parent interview</td>
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*Note. BRIEF (-P) = Behavior Rating Inventory of Executive Function (Preschool version), NEPSY VF = NEPSY Verbal Fluency test, EC HOME= Early Childhood Home Observation for Measurement of the Environment.*
Table 2
*Demographics and Injury Characteristics of Genetics Cohort at 12 Months*

<table>
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<th>TBI</th>
<th>OI</th>
<th>Overall</th>
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<tbody>
<tr>
<td><strong>N (%)</strong></td>
<td>51 (45.1%)</td>
<td>62 (54.9%)</td>
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<tr>
<td><strong>Current Age (X, [SD])</strong></td>
<td>6.39 (1.11)</td>
<td>6.21 (1.10)</td>
<td>6.29 (1.09)</td>
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<tr>
<td><strong>Age at Injury (X, [SD])</strong></td>
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<td>5.09 (1.07)</td>
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<tr>
<td><strong>DRD4 7-repeat allele (% Presence)</strong></td>
<td>17 (33.3%)</td>
<td>19 (30.6%)</td>
<td>36 (31.9%)</td>
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<tr>
<td><strong>Gender (% Male)</strong></td>
<td>30 (58.8%)</td>
<td>32 (51.6%)</td>
<td>62 (54.9%)</td>
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<tr>
<td><strong>Ethnicity (% White)</strong></td>
<td>38 (74.5%)</td>
<td>49 (79.0%)</td>
<td>87 (77.0%)</td>
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<tr>
<td>*<em>SES (X, [SD])</em></td>
<td>-.11 (.98)</td>
<td>.25 (.93)</td>
<td>.09 (.97)</td>
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<tr>
<td>*<em>IQ (X, [SD])</em></td>
<td>96.61 (15.30)</td>
<td>104.61 (13.10)</td>
<td>101 (14.63)</td>
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</tbody>
</table>

*Note. *Significant difference between groups, *p* < .05. TBI = traumatic brain injury, OI = orthopedic injury, N = sample size, X = mean, SD = standard deviation, SES = socioeconomic status, IQ = intelligence quotient.*
Figure 1. Gene x Injury x Environment interaction with Verbal Fluency scores as the dependent variable and the dichotomous Negativity scores from the coded parent-child interaction videos as the environmental variable (unstandardized $\beta = 6.87$, $t (93) = 1.98$, $p = .05$). However, a follow-up ANCOVA revealed that cell sizes were not equal between groups and that in the Some Negativity groups, cell sizes ranged from two to seven participants, whereas in the No Negativity groups, cell sizes were larger (range: 11-32). Differences of least square means revealed that the significant triple interaction ($Estimate = -0.43$, $t (93) = -2.19$, $p = .03$) involved the OI 7-repeat positive Some Negativity group, which only had two participants that scored below average on Verbal Fluency ($Estimate = 3.28$). Note: TBI = traumatic brain injury, OI = orthopedic injury, 7 Repeat + = presence of a DRD4 7-repeat allele, 7 Repeat - = absence of a DRD4 7-repeat allele.
Figure 2. Gene x Environment interaction with Shape School Inhibit subtest scores as the dependent variable and the Parenting Practices Questionnaire (PPQ) Permissive subscale score as the environmental variable (unstandardized $\beta = .40$, $t (97) = 2.31$, $p = .02$). An analysis of simple slopes was conducted and permissive parenting scores for the 7-repeat positive and 7-repeat negative groups were estimated at one standard deviation above (High Permissive) and below (Low Permissive) the mean for Permissive Parenting scores. At one standard deviation below the mean Permissive Parenting score, it was estimated that Shape School Inhibit scores for the 7-repeat positive group were 0.22 points lower than that of the 7-repeat negative group, which was a marginally significant difference ($p = .05$). At one standard deviation above the mean, the difference in Shape School Inhibit scores between the 7-repeat positive and 7-repeat negative groups was .15 points, which was not significantly different ($p = .17$). Simple slopes analysis further indicated that the slope of the line for the 7-repeat negative group ($Estimate = -0.31$, $t (97) = -2.79$, $p = .01$) was statistically significant from zero. The line of the 7-repeat positive group did not significantly differ from zero ($Estimate = .09$, $t (97) = .71$, $p = .47$). Note: 7 Repeat + = presence of a DRD4 7-repeat allele, 7 Repeat - = absence of a DRD4 7-repeat allele.
Figure 3. Gene x Environment interaction with Shape School Switch scores as the dependent variable and the EC-HOME scores as the environmental variable (unstandardized $\beta = -.01$, $t (87) = -2.18$, $p = .03$). An analysis of simple slopes was conducted and EC-HOME scores for the 7-repeat positive and 7-repeat negative groups were estimated at one standard deviation above (High Home) and below (Low Home) the mean for EC-HOME scores. At one standard deviation below the mean HOME score, it was estimated that Shape School Switch efficiency scores for the 7-repeat negative group were .05 points lower than that of the 7-repeat positive group, which was not a significant difference ($p = .25$). At one standard deviation above the mean, the difference in Shape School Switch efficiency scores between the 7-repeat positive and negative groups was .09 points, which was a marginally significant difference ($p = .06$). Simple slopes analysis further indicated that the slope of the line for the 7-repeat negative group ($Estimate = .01$, $t (87) = 3.09$, $p < .01$) differed significantly from zero. The line of the 7-repeat positive group did not significantly differ from zero ($Estimate = <.01$, $t (87) = .31$, $p = .76$). Note: 7 Repeat + = presence of a DRD4 7-repeat allele, 7 Repeat - = absence of a DRD4 7-repeat allele, EC-HOME = Early Childhood Home Observation for Measurement of the Environment.
Figure 4. Mediators/moderators of traumatic brain injury (TBI) recovery. Note: TBI = traumatic brain injury, SES = socioeconomic status, PT = physical therapy, OT = occupational therapy, ST = speech therapy. Credit for graph to Dr. Brad Kurowski, Cincinnati Children’s Hospital Medical Center (unpublished).