The Role of the Dopamine Transporter (DAT) in the Development of PTSD in Preschool Children

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Population-based association studies have supported the heritability of posttraumatic stress disorder (PTSD). This study explored the influence of genetic variation in the dopamine transporter (DAT) 3' untranslated region variable number tandem repeat on the development of PTSD in preschool children exposed to Hurricane Katrina, diagnosed using a developmentally appropriate semistructured interview. A diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association, 1994), total symptoms, and specifically Criterion D symptoms were significantly more likely to be found in children with the 9 allele. This study replicates a previous finding in adults with PTSD. The specificity of this finding to the increased arousal symptoms of Criterion D suggests that dopamine and the DAT allele may contribute to one heritable path in a multifinality model of the development of PTSD.

Posttraumatic stress disorder (PTSD) is a complex disorder that develops in a significant proportion of individuals after exposure to one or multiple traumatic events. In addition to the criteria for exposure to a traumatic event, PTSD is also characterized by three distinct symptom clusters in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association, 1994). Criterion B includes symptoms of reexperiencing of the traumatic experience, Criterion C includes symptoms related to avoidance of reminders about the traumatic experience, and Criterion D includes symptoms of increased arousal such as sleep disturbances, irritability, hypervigilance, difficulty concentrating, and exaggerated startle response. Although trauma exposure is necessary for the development of PTSD, not all individuals exposed to traumatic events develop PTSD. There is increasing evidence that genetic vulnerability may play a role in the susceptibility to PTSD after trauma exposure.

Evidence supporting the role of genetic factors in the development of PTSD comes from several sources. Family studies examining the rates of PTSD in children of parents with and without PTSD indicate that the rates of PTSD are higher in children whose parents had PTSD than in similar-trauma-exposed children whose parents did not have PTSD (Sack, Clarke, & Seeley, 1995; Yehuda, Halligan, & Beirer, 2001). Twin studies provide additional support for a genetic component to the development of PTSD and have consistently found that 20–30% of the variance in the development of PTSD after trauma exposure is due to genetic factors (Stein, Jang, Taylor, Vernon, & Livesley, 2002; True et al., 1993; Xian et al., 2000). Similar to many psychiatric disorders the genetic contribution is most likely polygenic. Using case control and candidate gene studies, molecular genetic studies are beginning to identify associations between the development of PTSD and genes implicated in the stress response as well as genes involved in the activity and metabolism of dopamine, and other neurotransmitters. These genes include the dopamine transporter (DAT), dopamine receptor 2 (DRD2), dopamine beta-hydroxylase (DBH), the serotonin transporter (5HTT), Forkhead binding protein 5 (FKBP5), catechol-o-methyltransferase (COMT), and brain-derived neurotrophic factor (BDNF; Binder et al., 2008; Broekman, Olff, & Boer, 2007; Kilpatrick et al., 2007; Koenen, 2007; Lee et al., 2005). Few, if any of the studies have been replicated, and for genes such as DRD2, where more than one study has evaluated its association with PTSD, there have been inconsistent results (Gelernter et al., 1999). Therefore, although there is clear evidence for a genetic component to PTSD, specific genes and/or specific genetic
variations that influence the development of PTSD have yet to be convincingly and consistently reported.

Several lines of evidence support the role of dopamine in the etiology of PTSD including increased urinary and plasma levels of dopamine in individuals with PTSD, and a significant positive correlation between dopamine levels and severity of PTSD (Hammer & Diamond, 1993; Yehuda, Southwick, Giller, Ma, & Mason, 1992). Preclinical studies have indicated that of the three known major dopaminergic neuronal systems, the mesocortical/mesoprefrontal system (from midbrain to prefrontal cortex) is preferentially activated by stress compared to the other two systems. This is thought to have the effect of increasing the “gain” on the information processing system and increasing vigilance related to PTSD (see Vermetten & Bremner, 2002 for a review). Several genes involved in dopamine neurotransmission and metabolism including DAT, COMT, and DRD2 have been associated with the development of PTSD (Goldstein, Rasmussen, Bunney, & Roth, 1996; Rauch et al., 2000). Exploring the association between polymorphic variations in genes integral to dopamine metabolism and neurotransmission is therefore an appropriate approach to identify genetic factors contributing to the development of PTSD.

The DAT gene, also known as SLC6A3, is located on chromosome 5p15 and functions as the main regulator of dopamine levels in the synaptic cleft via reuptake through the transporter. DAT is expressed in a small number of neurons in the brain, particularly in the striatum, nucleus accumbens, and the ventral tegmental area (VTA; Ciliax et al., 1995; Garris & Wightman, 1994). Located within the gene is a variable number tandem repeat (VNTR) element of 40 base pairs in the 3′ UTR noncoding region of the gene. Individuals can have alleles of this VNTR ranging from 3 to 12 copies (Min Kang, Palmatier, & Kidd, 1999), but the majority of individuals have alleles with either 9 or 10 copies. Functional differences appear to exist specifically between the 9 and 10 alleles. In vitro studies have found that the 9 repeat allele has an elevated transcription rate when compared to the 10 repeat allele (Michelhaugh, Fiskerstrand, Lovejoy, Bannon, & Quinn, 2001). In vivo studies examining the influence of genotype on DAT binding have been inconsistent: Some find increased DAT binding with the 10/10 genotype, another study found decreased striatal binding with the 10/10 genotype, and a third study found no difference (Heinz et al., 2000; Jacobsen et al., 2000; Marinez et al., 2001). One previous study has examined the relation between this polymorphism and PTSD. In this study, Segman et al. (2002) identified an association between the 9 allele of the VNTR and the development of PTSD in a case control study of 102 chronic PTSD patients and 104 control individuals matched for combat and trauma exposure without PTSD.

Due to the significant evidence supporting the role of dopamine neurotransmission in PTSD and the previous finding of an association between the 9 allele of the DAT VNTR and PTSD we sought to replicate this finding. We examined the association of this polymorphism with the development of PTSD in a group of preschool children who experienced Hurricane Katrina and were subsequently assessed for PTSD using a developmentally specific semistructured interview.

**METHOD**

**Participants**

Participants were recruited primarily from weekly newspaper advertisements. A minority were recruited from flyers in a pediatrics’s office and contacts at community events. One hundred thirty-nine children were enrolled and their caregivers attended the first laboratory visit alone. Of these, 109 returned for the second visit with their children during which buccal swabs were obtained. This study was approved by the Tulane University School of Medicine Institutional Review Board.

Inclusion criteria included children who were (a) 3–6 years of age, (b) English-speaking, and (c) an inhabitant of the New Orleans metropolitan area at the time of Hurricane Katrina. Children could not participate if they had moderate mental retardation, autistic disorder, deafness, blindness, or were non-English speaking. These conditions were screened with questions for the caregiver over the phone during intake and a second level review of videotape of the children by an experienced child psychiatrist (M.S.). Moderate mental retardation was screened as a Peabody Picture Vocabulary Test score below 50. No children met these exclusion criteria. The primary female caregiver of each child participated with the children.

Of the 109 children enrolled in the study with exposure to Hurricane Katrina, the final analysis was conducted on 88 children. DNA samples that did not amplify consistently (8), individuals who refused to participate in genetic aspect of the study (2), or individuals with alleles different from the 9 or 10 alleles (11) were not included in the final analysis. Individuals who did not participate in the genetic aspect of the study or whose DNA did not amplify consistently did not differ in number of PTSD symptoms or number of criterion specific symptoms from those included in the final analysis. The genotype distribution of the three genotypes included in analysis did not differ from the Hardy-Weinberg equilibrium, \( \chi^2(1, N = 88) = 3.31, ns \) (Table 1). Our allele frequency is 0.70 for 10 allele and 0.60 for the frequency of 10/10 genotype and is consistent with a range of studies of the DAT transporter (Min Kang et al., 1999).

**Measures**

Posttraumatic stress disorder (PTSD) diagnosis and symptoms counts were assessed using the Preschool Age Psychiatric Assessment. Test–retest reliability of the Preschool Age Psychiatric Assessment is comparable to structured psychiatric interviews used to assess older children and adults, with an intraclass correlation coefficient of .56 and a kappa of .73 for the PTSD module. (Egger
The Preschool Age Psychiatric Assessment interview was performed by trained research assistants who were blind to genotyping information. Interviewers were trained on the content and scoring of the Preschool Age Psychiatric Assessment by a trainer from Duke University where the Preschool Age Psychiatric Assessment was created. Subsequent interviewers were trained on content and scoring rules by one of the local investigators (M.S.). New interviewers that joined the study observed experienced interviewers give three interviews and then coded two interviews while observing experienced interviewers and compared codes afterward. Next, they administered their first interview while being observed by a trainer and given immediate feedback. Next, the coding of every symptom of their next three interviews was completed with the advice of an experienced interviewer. Finally, throughout the study, the lead investigator (M.S.) met individually with interviewers weekly to watch their most symptomatic interviews on videotape to prevent drift, critique technique, and correct coding errors, with special attention focused on the Criterion B reexperiencing symptoms and the two avoidance symptoms.

Because of research that has suggested that DSM-IV criteria need substantial modifications to be valid for young children, we also diagnosed PTSD by the empirically validated alternative algorithm for young children (Scheeringa, Zeanah, Myers, & Putnam, 2003; Task Force on Research Diagnostic Criteria: Infancy and Preschool, 2003). The alternative algorithm required only one of the seven symptoms in Criterion C (avoidance and numbing symptoms) instead of three symptoms. Severity variables for Criteria B, C, and D were summed scores of the 5, 7, and 5 symptoms, respectively, that constitute those criteria.

From February 2006 through April 2008, buccal swabs were obtained from children during an ongoing study PTSD study in preschool children (M.S.). DNA was extracted from MasterAmp buccal swabs from Epicentre Biotechnologies (Madison, WI) using the MasterAmp DNA extraction solution following manufacturer’s recommendations. Genotyping for the DAT VNTR was performed using the polymerase chain reaction (PCR). The PCR products were then electrophoresed through a 2% agarose gel using standard protocols. Allele determination was made based on the size of fragments compared to known genotypes and size standards (Michelhaugh et al., 2001). The PCR was performed on a Bio-Rad C1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA) using the following conditions 5′ primer (forward): 5′-TGTGGTGTAGGGAACGGCCTGAG-3′, 3′ primer (reverse): 5′-CTTCCTGGAGGTCAAGGCTCAAGG-3′. The PCR was carried out in a 50 μL reaction with 10 pmol of each primer, 1.25 U of Ex Taq™ DNA Polymerase (TaKara Bio USA, Madison, WI), 1 × Ex Taq™ Buffer, 200μm dNTPS. Thermal cycling conditions were an initial denaturation for 5 minutes followed by 40 cycles of 94 for 30 seconds, 58 for thirty seconds, and 72 for 45 seconds. Ten microliters of final reaction was size fractionated on a 2% agarose gel with a standard 1kb DNA ladder for size determination. Alleles ranged from 3 copies to 12 copies; 100% of the samples were reamplified and analyzed twice. All genotyping was done blind to PTSD status. Samples that failed to amplify fragment size consistently or failed to amplify were eliminated from further analysis.

**Data Analysis**

As there is evidence that there are functional differences between the 9 and 10 alleles of the VNTR, and the number of individual rare alleles was small, we examined the relationship between PTSD and the DAT VNTR in individuals with 9/9, 9/10, or 10/10 genotypes. We excluded those with the rare alleles as the functional significance of those alleles is unknown and this is consistent with previous studies of this polymorphism (Durston et al., 2008; Karama et al., 2008). The PTSD diagnosis by genotype group was tested with logistic regression. Number of symptoms by genotype group was tested with nonparametric Wilcoxon rank sum tests because symptom count data was not normally distributed.

Gender, race, and age were examined for association with genotype. Analysis of variance examining total symptoms and symptoms specific to each criterion were examined for association with genotype. Analysis of PTSD symptoms was performed exploring 10/10 homozygotes compared to children with the 9 allele (9/9 and 9/10 genotypes).

**RESULTS**

**Genotype Data and Allele Frequencies**

There was no significant association between genotype and ethnicity, gender, or age. Analysis of genotype by PTSD symptoms within race categories, White and Black, resulted in no significant difference in mean total symptoms or symptom number by

<table>
<thead>
<tr>
<th>DAT Allele (N = 99)</th>
<th>Sample</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No. alleles</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
</tr>
<tr>
<td>10</td>
<td>139</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: Each subject (N = 99) has two alleles; therefore, there were 198 alleles in the total sample.
criterion indicating that these results are unlikely due to population stratification (Table 2).

**Genetic Association With PTSD Diagnosis and Symptoms**

Logistic regression results revealed that there is a significant difference in PTSD diagnosis according to genotype classification, odds ratio (OR) = 2.40, 95% CI = 1.02–5.67, Wald $\chi^2 (1, N = 88) = 4.00, p < .05$. Pairwise analysis indicates that individuals with the 9/9 genotype are approximately 6 times as likely to develop PTSD as individuals with the 10/10 genotype, OR = 6.00, 95% CI = 1.07–33.54, Wald $\chi^2 (1, N = 61) = 4.17, p < .05$. Using the alternative PTSD criteria there was a marginally significant association between the three genotypes and PTSD diagnosis, OR = 1.88, 95% CI = .97–3.67, Wald $\chi^2 (1, N = 88) = 3.45, p = .06$.

Individuals with the 9 allele (9/9 or 9/10 genotypes) had significantly greater total number of PTSD symptoms than homozygous 10 children and further analysis revealed that this was driven by Criterion D symptoms. Children with the 9 allele had significantly more Criterion D symptoms compared to homozygous 10/10 children, but there was no significant differences with Criterion B or Criterion C symptom counts (Table 3).

To explore ethnic stratification we analyzed PTSD symptoms within the two largest race categories, Black (n = 49) and White (n = 35), as the other categories did not contain sufficient numbers of individuals for meaningful analysis (n = 4). There were no significant differences in allele frequencies or PTSD symptoms by race (Table 2). When the analysis was performed within the Black race category, PTSD symptoms continued to be significantly associated with allele status. Black children with the 9 allele had significantly more PTSD symptoms ($M = 6.7, SD = 2.9$) compared to Black children with the 10 allele ($M = 4.6, SD = 2.4$; Wilcoxon rank sum test $z(49) = 2.42, p < .05$). White children with the 9 allele showed a similar pattern of more PTSD symptoms ($M = 5.9, SD = 3.4$) compared to White children with the 10 allele ($M = 4.8, SD = 2.3$), but it did not reach statistical significance. For the calculated effect size of 0.39 to reach statistical significance in the White children, a sample size of 37 per group would have been needed.

**DISCUSSION**

The pathophysiology underlying PTSD is complex and is likely explained by multifinality in which different pathways, including multiple genetic contributions, lead to different outcomes. Population-based association studies have supported the heritability of PTSD, and several specific genes have been associated with the development of PTSD. No previous studies had examined the genetic contribution of PTSD in preschool children, although heritability for anxiety disorders may be higher in younger age groups (Boomsma, van Beijsterveldt, & Hudziak, 2005). To date, associations of specific allele variants with PTSD had been shown in single studies or have had replication failures raising the possibility of false-positive findings. Our findings replicate those of Segman et al. (2002) and strengthen the hypothesis that DAT is implicated in the etiology of PTSD. Further, the finding that this association appears driven, for the most part, by Criterion D symptoms suggests a relatively more specific relationship between DAT and the neurobiology of PTSD.

Criterion D symptoms have been conceptualized as increased arousal phenomena including hypervigilance, concentration difficulty, irritability, exaggerated startle, and sleep disturbance. The underlying disturbance of these symptoms is thought to be due to individuals’ preoccupations with trauma-related internalizations that preclude normative orienting, attention, and self-regulation. This model is consistent with research that has found increased attention to threat stimuli in individuals with PTSD compared to individuals without PTSD in samples of children 9–17 years.

**Table 3.** Mean Number of Posttraumatic Stress Disorder Symptoms by Allele Status

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency</th>
<th>Total symptoms</th>
<th>Criterion B symptoms</th>
<th>Criterion C symptoms</th>
<th>Criterion D symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (%)</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
</tr>
<tr>
<td>9/10 or 9/9</td>
<td>36 40%</td>
<td>6.2  3.1</td>
<td>2.0  1.1</td>
<td>1.6  1.3</td>
<td>2.5  1.1</td>
</tr>
<tr>
<td>10/10</td>
<td>52 60%</td>
<td>5.1  2.5</td>
<td>1.8  1.0</td>
<td>1.1  1.1</td>
<td>1.9  1.0</td>
</tr>
<tr>
<td>Test statistic</td>
<td></td>
<td>$Z(N = 88) = 2.22^*$</td>
<td>$Z(N = 88) = 1.02$</td>
<td>$Z(N = 88) = 1.90$</td>
<td>$Z(N = 88) = 2.46^*$</td>
</tr>
</tbody>
</table>

*p < .05.*
old (Dalgleish, Moradi, Taghavi, Neshat-Doost, & Yule, 2001) and adults (Bryant & Harvey, 1997). The association of the DAT 9 allele, heightened attention, and PTSD appears to converge with evidence from healthy populations that the DAT 9 allele is associated with increased attention capacities. In a general population study of healthy 6- to 11-year-olds, children with the 9/10 genotype performed better on tests of selective attention and inhibitory capacity than children with the 10/10 genotype (Cornish et al., 2005). In another study of normal children aged 9–16, heterozygous children with the 9/10 genotype performed better on tests of reorienting attention compared to children homozygous for the 10 allele (Bellgrove et al., 2007). These studies when taken together indicate that the DAT 9 allele may confer heightened capacity for attention. As PTSD is associated with heightened attentional bias to threat, it appears logical that the DAT 9 allele would confer increased risk, relative to the DAT 10 allele, for the development of PTSD.

Several caveats to this study exist. Genotype was statistically associated with PTSD using the DSM-IV criteria, but was marginally statistically significant with the alternative criteria diagnosis. The fact that the genotype was significantly associated with continuous measures of the number of PTSD symptoms and the number of Criterion D symptoms may indicate that genotype status is more predictive of severity of PTSD rather than categorical diagnoses.

A potential limitation is that we did not correct for ethnic stratification in this study. Statistical analysis to adjust for ethnic stratification is done to prevent false-positive findings in studies when allele frequencies differ normatively between ethnic groups. However, ethnic stratification was not corrected for in this study, as analysis of mean total symptoms and criterion symptoms were not statistically different between Black and White race categories. For population stratification to present a statistical difficulty for association studies both differences in allele frequencies between racial groups and differences in rates of psychopathology have to exist. As we did not find any statistical difference between rates of PTSD symptoms between races, ethnic stratification in the analyses did not appear justified (Hutchison, Stallings, McGeary, & Bryan, 2005). Furthermore, analysis of the impact of genotype within our largest racial group, Black, remained statistically significant, and though analysis did not reach significance when examined within the White subgroup, this is most likely due to limited power of the relatively smaller sample size.

Our current finding of an association between the 9 DAT allele and PTSD symptoms is consistent with a previous study. However, we cannot exclude the possibility of the association being the result of other polymorphisms in linkage disequilibrium with the VNTR or another polymorphism in a nearby gene (Dragon et al., 2006; Feng et al., 2005). However, the consistency of findings of altered dopamine levels in PTSD, the functional significance of DAT in the ventral striatum (which is implicated in PTSD), the replication of this finding in a previous study, and the existence of a potential biological mechanism linking variation in DAT function as a result of this polymorphism and alterations in dopamine levels support our hypothesis. The association with Criterion D symptoms is intriguing, as no previous studies have examined the association of genetic factors with specific PTSD symptom criterion. It may be that part of the complexity of PTSD comes from differential genetic risk factors for particular criterion. If so, future genetic studies of PTSD ought to consider specifically exploring associations with the Criteria B, C, and D symptoms to delineate further the neurobiology of PTSD.

Clinical applications of genetic findings in this early stage of research are somewhat remote. However, genetic studies may ultimately identify vulnerable individuals and guide interventions or preventive approaches for those at risk for trauma exposure. With increasing numbers of studies finding associations between allele variants and psychiatric disorders, traction is building in the field for genetics to help define the underlying mechanisms leading to the development of psychopathology, relations between genetically determined neurobiology and disorders, and personalized approaches to interventions.

Future genetic studies may benefit from restricted data sets that focus on single trauma types, limited age ranges, and evaluation of specific criterion. Genetic studies of PTSD have the potential to delineate further the underlying neurocircuitry altered with traumatic exposure that results in the development of this complex, common, and impairing disorder. Given the role of the DAT in attention and the known role of attention to trauma reminders and PTSD, future studies ought to evaluate attention and orientation tasks as a function of DAT genotype in children with and without PTSD. These studies may further define the relation between variation in the DAT and the neurobiology underlying PTSD. Through an enhanced understanding of the neurobiological underpinnings of PTSD novel treatment modalities may be developed.

REFERENCES
