JOURNAL OF CHILD AND ADOLESCENT PSYCHOPHARMACOLOGY
Volume 24, Number 8, 2014

Pp. 454–457
DOI: 10.1089/cap.2014.0077

Brief Report

Race Moderates the Association of Catechol-*O*-methyltransferase Genotype and Posttraumatic Stress Disorder in Preschool Children

Kathryn L. Humphreys, PhD, EdM, Michael S. Scheeringa, MD, and Stacy S. Drury, MD, PhD

Abstract

Objective: The present study sought to replicate previous findings of an association between the Catechol-*O*-methyltransferase (*COMT*) *val158met* polymorphism with posttraumatic stress disorder (PTSD) and symptomatology in a novel age group, preschool children.

Methods: *COMT* genotype was determined in a sample of 171 3–6-year-old trauma-exposed children. PTSD was assessed with a semistructured interview. Accounting for sex, trauma type, and age, genotype was examined in relation to categorical and continuous measures of PTSD both controlling for race and within the two largest racial categories (African American [AA] and European American [EA]).

Results: Race significantly moderated the association between genotype and PTSD. Specifically, the genotype associated with increased PTSD symptoms in one racial group had the opposite association in the other racial group. For AA children the *met/met* genotype was associated with more PTSD symptoms. However, for EA children, *val* allele carriers had more PTSD symptoms. Whereas every AA child with the *met/met* genotype met criteria for PTSD, none of the EA children with the *met/met* genotype did. This genetic association with *COMT* genotype, in both races but in opposite directions, was most associated with increased arousal symptoms.

Conclusions: These findings replicate previous findings in participants of African descent, highlight the moderating effect of race on the association between *COMT* genotype and PTSD, and provide direct evidence that consideration of population stratification within gene-by-environment studies is valuable to prevent false negative findings.

Introduction

Research on the associations of genetic polymorphism variants with psychiatric disorders has been beleaguered by replication failures. Critics have further noted a pattern in which initial studies demonstrate larger effects than subsequent studies examining gene–phenotype associations (Ioannidis et al. 2001). However, replication studies in well-selected and carefully controlled cohorts, especially those that consider developmental factors, are necessary to advance the integration of genetic research and developmental psychopathology.

The relevance of dopamine function in the development of posttraumatic stress disorder (PTSD) has been supported in multiple studies (Stam 2007). Previously we, and others, have demonstrated an association between the dopamine transporter gene (*DAT*) and PTSD (Segman et al. 2002; Drury et al. 2009; Chang et al. 2012; Drury et al. 2013); however, exploration of additional genes known to regulate dopaminergic neurotransmission is expected to provide additional support for the role of dopaminergic function in PTSD and enhanced insight into the pathophysiology of PTSD. Catechol-*O*-methyltransferase (COMT), a gene on chromosome 22q11 (Grossman et al. 1992), contains a frequently examined functional single nucleotide polymorphism rs4680, or *COMT val*158*met*. The *met* allele results in *COMT* enzymatic activity that is 40% less active than the *val* allele (Chen et al. 2004), leading to decreased dopamine turnover in the synaptic cleft, particularly in the prefrontal cortex, where *COMT* is the primary regulator of extracellular dopamine turnover (Sesack et al. 1998).

Two previous studies in adults have demonstrated an association between the *met* allele of *COMT val158met* genotype and PTSD. One study was in an African sample (Kolassa et al. 2010) and the other study was in a Caucasian sample (Boscarino et al. 2012). Given the strong evidence linking dopaminergic function and PTSD, this study sought to replicate the association of *COMT* genotype and PTSD findings in a cohort of well-characterized preschool trauma-exposed children. We hypothesized that the *met/met*

Tulane University School of Medicine, New Orleans, Louisiana.
Funding: This research was supported by National Institute of Mental Health grant (R01 MH065884) to Dr. Scheeringa and an APIRE research award from the American Psychiatric Association and K12HD043451 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development (to Dr. Drury). The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health & Human Development or the National Institutes of Health.

genotype would be associated with elevated risk for PTSD and higher PTSD symptoms, and also explored whether the type of trauma exposure moderated this relationship.

Methods

Participants

Participants were a subset of subjects recruited from a larger project (R01 MH65884-01A1) of 284 trauma-exposed children for whom genetic data was obtained. Recruited children were exposed to a range of traumatic events. Specifically, children were characterized as those exposed to single traumatic events, repeated traumatic events, or Hurricane Katrina (Scheeringa et al. 2012). DNA collection began 1 year after study initiation, as such DNA was collected from 176 consecutively enrolled subjects. Analyses were conducted on 171 children, which included 31 single event, 31 repeated event, and 109 Hurricane Katrina survivors. DNA samples that did not amplify consistently ($n=3$) and individuals without complete PTSD data ($n=2$) were not included in the analyses.

The Tulane University School of Medicine Institutional Review Board approved this study. Participants were required to have experienced at least one life-threatening trauma when the child was old enough to remember it with a narrative recall (at least 3 years old). Exclusion criteria included head trauma with a Glasgow Coma Scale score ≤ 7 in the emergency room, mental retardation, autistic disorder, blindness, deafness, and coming from a foreign language-speaking family. The primary female caregiver of each child also participated.

Measures

Demographic information (e.g., age, sex, race) was obtained via parent report. For child's race, parents selected Black, White, mixed, and other.

PTSD diagnosis and symptom counts were assessed using the Preschool Age Psychiatric Assessment, performed by trained research assistants who were blind to genotyping information. PTSD was diagnosed by the empirically validated alternative algorithm for young children (Scheeringa et al. 2003), in which only one of the seven symptoms in Criterion C (avoidance and numbing symptoms) was required instead of three symptoms. Severity variables for Criterion B (re-experiencing), C, and D (increased arousal) were summed scores of the 5, 7, and 5 symptoms respectively, which constitute those criteria.

Genotyping

DNA was extracted from MasterAmp buccal swabs from Epicentre Biotechnologies (Madison, WI) using the MasterAmp DNA extraction solution following manufacturer's recommendations. Polymerase chain reaction (PCR) was performed using the following primers (Massat et al. 2004): 5′-ACT GTG GCT ACT CAG CTG TG-3′ and 5′-CCT TTT TCC AGG TCT GAC AA-3′. PCR was performed in a 50 μL reaction with 10 pmol of each primer, 1.25 U of Ex Taq™ DNA Polymerase (TaKara Bio USA), 1× Ex Taq Buffer, and 200 μm deoxynucleotide triphosphates (dNTPS). Thermal cycling conditions were an initial denaturation for 5 minutes followed by 33 cycles of 94 for 30 seconds, 58 for 30 seconds, and 72 for 45 seconds; 20 μL of the PCR was digested with Nla III (New England Biolabs) and size fractionated on a 4% agarose gel. Allele status was determined by fragment size, and all samples were run in duplicate and coded by individuals blind to all other data.

Data analysis

Sex, age, and trauma exposure type were included as covariates for all analyses. The relationship between the *COMT val158met* genotype and total PTSD symptoms and symptoms within each cluster (i.e., B, C, and D) were examined using generalized linear models specifying Poisson log linear distribution. PTSD diagnosis was examined using logistic regression.

Results

Genotype data and allele frequencies

There were no significant associations identified among trauma group, race, age, or sex with PTSD symptoms.

COMT genotype distribution was *met/met* ($n=12$), *val/met* ($n=99$), and *val/val* ($n=62$) in the complete sample. Genotypes did not deviate from Hardy–Weinberg equilibrium. Genotype was not significantly associated with sex or age (see Table 1). Given the small sample size of the groups that identified as ''mixed'' race ($n=11$) and ''other'' ($n=8$), we conducted all following analyses in children who identified as either ''Black'' (hereafter referred to as African American [AA]) or ''White'' (hereafter referred to as European American [EA]) ($n=152$). Significant differences in genotype frequencies were found between racial groups, as the EA group had a higher frequency of *met* alleles than the AA group, consistent with observed allele frequencies (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=4680). Given the existence of significant differences in allele frequencies, and the potential for the impact of population stratification, analyses were conducted including race as a moderator of the association between *COMT* genotype and PTSD symptomatology.

COMT *and PTSD symptomatology: Examining race as a potential moderator*

Race was unrelated to PTSD diagnosis, PTSD total symptoms, and symptoms within each symptom cluster (ps ranged from 0.60 to 0.95). Notably, the logistic regression for *COMT* genotype by PTSD diagnosis did not terminate because of quasicomplete

Table 1. Race, Sex, and Age by *COMT* Genotype ($N=171$)

	met/met	*val/met*	*val/val*	χ^2 *or F*
Sex				0.16, $p=0.92$
Male	8	61	37	
Female	4	37	24	
Age				
Mean (SD)	5.74 (1.20)	5.23 (1.05)	5.14 (1.12)	1.55, $p=0.22$
African American				5.42, $p=0.07$
PTSD	5	25	21	
No PTSD	0	29	24	
European American				5.87, $p=0.05$
PTSD	0	15	6	
No PTSD	6	17	4	

Total sample size for sex and age was the full sample of 171 subjects. The African American and European American groups comprised a subset of 152 of the subjects.

COMT, Catechol-*O*-methyltransferase; PTSD, posttraumatic stress disorder.

separation in the data (all AA children with *met/met* genotype met criteria for PTSD; no EA children with the *met/met* genotype met criteria for PTSD) (see Table 1).

A significant *COMT* genotype by race interaction was found in predicting total PTSD symptoms, Wald χ^2 (2,142) = 15.45, $p < 0.001$. Subsequent analyses within symptom clusters demonstrated that the genotype by race interaction was not significant for Criterion B symptoms, Wald χ^2 (2,142) = 2.94, $p = 0.23$, or Criterion C symptoms, Wald χ^2 (2,142) = 4.22, $p = 0.12$. However, a significant genotype by race interaction was found for Criterion D symptoms, Wald χ^2 (2,142) = 10.47, $p = 0.005$. Given the repeated post-hoc tests, the family wise α level was adjusted to $p < 0.017$ (dividing 0.05 by 3 [for the three symptom clusters]) to determine statistical significance. Even following this adjustment, the above-cited p value for the interaction of genotype and race for Criterion D symptoms remained statistically significant.

COMT *and PTSD symptomatology: Within racial subgroups*

Based on 1) concerns about population stratification in case control studies, 2) the presence of differences in allele frequencies between AA and EA groups in our sample, and 3) the detection of a significant moderation of the examined effect by race for both the diagnosis and symptom level analyses, post-hoc tests within each of the two largest race categories, AA ($n = 104$) and EA ($n = 48$), were conducted with both total PTSD symptoms and Criterion D symptoms.

AA analysis. Analyses within the AA group demonstrated that *COMT* genotype was significantly associated with total PTSD symptoms, Wald χ^2 (2,97) = 12.44, $p = 0.002$. Individuals with *met/met* had significantly more PTSD symptoms than either other genotype ($ps < 0.014$) (Fig. 1A). A significant effect was found for Criterion D symptoms, Wald χ^2 (2,97) = 7.84, $p = 0.02$ (Fig. 1B). Pairwise post-hoc comparisons demonstrated that *met/met* individuals had significantly more Criterion D symptoms than individuals with the *val/val* genotype ($p = 0.04$), whereas neither group significantly differed from the *val/met* group, which was intermediate in Criterion D symptoms.

EA Analysis. Within the EA group, *COMT* genotype significantly predicted total PTSD symptoms, Wald χ^2 (2,41) = 10.99, $p = 0.004$ (Fig. 1A). In this group, however, the *val/val* genotype group was associated with more PTSD symptoms, and significantly differed from both *met* groups ($ps < 0.013$). A significant genotype effect was found for Criterion D symptoms, Wald χ^2 (2,41) = 6.19, $p = 0.045$) (Fig. 1B). The *met/met* genotype group, associated with the most symptoms in AA children, had significantly fewer Criterion D symptoms than both *val* groups (i.e., *val/val* and *val/met*) ($ps < 0.05$) in EA children.

Discussion

Our findings replicated previous studies in African adults that demonstrated an association between the *met* allele of *COMT val158met* and PTSD (Kolassa et al. 2010). However, the differential effect of *COMT* genotype in AA compared to EA preschool children highlights the existence of racial heterogeneity in relation to whether a particular allele is associated with elevated risk following trauma exposure. Our results demonstrated a significant interaction between race and genotype where the genotype that conferred risk for PTSD in one racial group appeared to confer protection from PTSD in the other racial group. Specifically, the *met/met* genotype was associated with elevated PTSD risk in AA preschool children, whereas it was associated with the lowest risk in EA individuals. In both racial groups, the association between *COMT* genotype and PTSD was driven by Criterion D symptoms.

Our finding within the EA group differed from a study of adult Caucasian participants in whom the presence of a *met* allele was correlated with an increased risk of lifetime PTSD diagnosis compared with controls (Boscarino et al. 2012). However, in this study a cumulative risk index of four different genetic variants was used, such that data specific to the *COMT* genotype and PTSD were not included, and limited the ability to directly compare those results with the current findings.

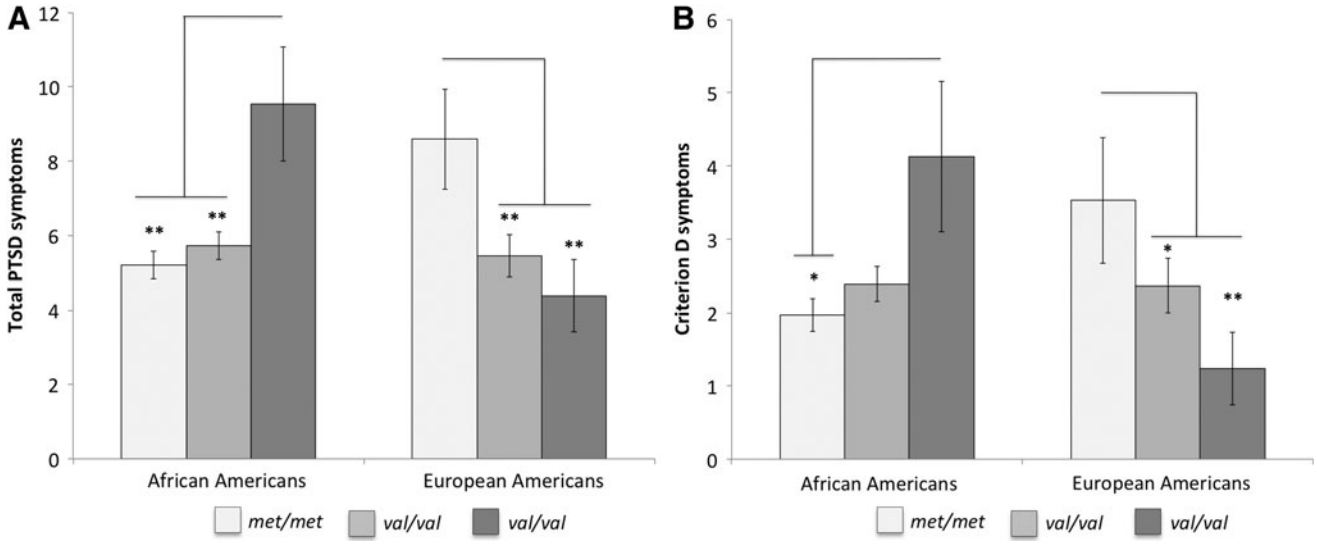

FIG. 1. Total posttraumatic stress disorder (PTSD) symptoms **(A)** and Criterion D symptoms **(B)** by race and Catechol-*O*-methyltransferase (*COMT*) genotype. *$p < 0.05$. **$p < 0.01$.

Population stratification represents a critical challenge for the field of psychiatric genetics. Whereas initial studies raised concerns about false positive associations caused by genetic admixture, our findings, and reviews by others (Cardon and Palmer 2003), suggest that population stratification can also mask gene–phenotype associations. The present study provides an example in which a false negative association between *COMT* and PTSD may have been concluded, had analyses not been performed that examined race as a moderator. The finding that none of the EA children with the *met/met* genotype were diagnosed with PTSD whereas all of the AA children with the *met/met* genotype were diagnosed with PTSD resulted in secondary analyses that demonstrated a significant moderation of the relationship between genotype and PTSD by race. Our findings dovetail with the recent meta-analytic work on the impact of another commonly studied gene variant, with known significant racial differences in allele frequencies, the serotonin transporter length polymorphism (5-HTTLPR), where associations were also significantly moderated by race (van IJzendoorn et al. 2012).

Several limitations should be noted. The results for the *met/met* genotype should be interpreted with caution given the small sample size ($n = 11$). Additionally, research in human genetics that emphasizes racial differences requires caution (Berg et al. 2005), as historical use of such real or perceived differences has provided justification for bias and discrimination. Racial group was defined by caregiver report; however, there is evidence that self-identified race/ethnicity is highly correlated with ancient geographic ancestry (Tang et al. 2005). Nevertheless, the underlying mechanism for this differential association of *COMT* genotype and PTSD within both racial groups is unclear, and merits further investigation, particularly in light of the findings of an association between this same genotype and PTSD in other studies.

Clinical Significance

The inclusion of potential moderators, including racial group, in the study of candidate genes in the prediction of psychopathology, are essential for advancing our understanding of risk and resilience in terms of individual differences and complex biological systems. Although trauma is a potent stressor on the brain, only a subset of individuals appears susceptible to PTSD. Identifying these individuals, as well as the potential pathways through which susceptibility is conferred, requires consideration of genetic, developmental, and environmental factors. These same factors can be equally useful when considering targeted interventions following trauma exposure. Our study adds additional support to the importance of dopaminergic neurotransmission in the pathophysiology of PTSD, and also suggests that both racial and developmental differences in the underlying neurobiology of PTSD exist. Given the effect of multiple psychopharmacologic agents on dopamine metabolism, future studies should carefully consider racial, developmental, and genetic differences.

Disclosures

No competing financial interests exist.

References

Berg K, Bonham V, Boyer J, Brody L, Brooks L, Collins F, Guttmacher A, McEwen J, Muenke M, Olson S, Wang VO, Rodriguez LL, Vydelingum N, Warshauer-Baker E: The use of racial, ethnic, and ancestral categories in human genetics research. Am J Hum Genet 77:519–532, 2005.

Boscarino J, Erlich, Hoffman, Zhang: Higher FKBP5, COMT, CHRNA5, and CRHR1 allele burdens are associated with PTSD and interact with trauma exposure: implications for neuropsychiatric research and treatment. Neuropsychiatr Dis Treat 131, 2012.

Cardon LR, Palmer LJ: Population stratification and spurious allelic association. Lancet 361:598–604, 2003.

Chang SC, Koenen KC, Galea S, Aiello AE, Soliven R, Wildman DE, Uddin M: Molecular variation at the SLC6A3 locus predicts lifetime risk of PTSD in the Detroit Neighborhood Health Study. PLoS One 7, 2012.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR: Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): Effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807–821, 2004.

Drury SS, Brett ZH, Henry C, Scheeringa M: The association of a novel haplotype in the dopamine transporter with preschool age posttraumatic stress disorder. J Child Adolesc Psychopharmacol 23:236–243, 2013.

Drury SS, Theall KP, Keats BJB, Scheeringa M: The role of the dopamine transporter (DAT) in the development of PTSD in preschool children. J Trauma Stress 22:534–539, 2009.

Grossman MH, Emanuel BS, Budarf ML: Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11.1→q11.2. Genomics 12:822–825, 1992.

Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG: Replication validity of genetic association studies. Nat Genet 29:306–309, 2001.

Kolassa IT, Kolassa S, Ertl V, Papassotiropoulos A, De Quervain DJF: The risk of posttraumatic stress disorder after trauma depends on traumatic load and the Catechol-O-methyltransferase Val158Met polymorphism. Biol Psychiatry 67:304–308, 2010.

Scheeringa MS, Myers L, Putnam FW, Zeanah CH: Diagnosing PTSD in early childhood: An empirical assessment of four approaches. J Trauma Stress 25:359–367, 2012.

Scheeringa MS, Zeanah CH, Myers L, Putnam FW: New findings on alternative criteria for PTSD in preschool children. J Am Acad Child Adolesc Psychiatry 42:561–570, 2003.

Segman RH, Cooper-Kazaz R, Macciardi F, Goltser T, Halfon Y, Dobroborski T, Shalev AY: Association between the dopamine transporter gene and posttraumatic stress disorder. Mol Psychiatry 7:903–907, 2002.

Sesack SR, Hawrylak VA, Matus C, Guido MA, Levey AI: Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. J Neurosci 18:2697–2708, 1998.

Stam R: PTSD and stress sensitisation: A tale of brain and body. Part 1: Human studies. Neurosci Biobehav Rev 31:530–557, 2007.

Tang H, Quertermous T, Rodriguez B, Kardia SLR, Zhu X, Brown A, Pankow JS, Province MA, Hunt SC, Boerwinkle E, Schork NJ, Risch NJ: Genetic structure, self-identified race/ethnicity, and confounding in case-control association studies. Am J Hum Genet 76:268–275, 2005.

van IJzendoorn MH, Belsky J, Bakermans-Kranenburg MJ: Serotonin transporter genotype 5HTTLPR as a marker of differential susceptibility? A meta-analysis of child and adolescent gene-by-environment studies. Transl Psychiatry 2:e147, 2012.

Address correspondence to:
Stacy S. Drury, MD, PhD
Tulane University Health Sciences Center
1440 Canal Street, TB52
New Orleans, LA 70112

E-mail: sdrury@tulane.edu