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Catechol-O-methyltransferase gene val158met polymorphism and depressive symptoms during early childhood

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RESEARCH ARTICLE

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Catechol-*O*-Methyltransferase Gene *val*158*met* Polymorphism and Depressive Symptoms During Early Childhood

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Catechol-*O*-Methyltransferase (COMT) is a critical regulator of catecholamine levels in the brain. A functional polymorphism of the *COMT* gene, *val*158*met*, has been linked to internalizing symptoms (i.e., depression and anxiety) in adolescents and adults. We extended this research by investigating whether the *val*158met polymorphism was associated with childhood symptoms of depression and anxiety in two independent samples of young children (Ns = 476 and 409). In both samples, preschool-aged children were genotyped for the *COMT val*158-met polymorphism. Symptoms of psychopathology were assessed via parent interviews and primary caregiver reports. In both samples, children homozygous for the *val* allele had higher levels of depressive symptoms compared to children with at least one copy of the *met* allele. Our findings extend previous research in older participants by showing links between the *COMT val*158*met* polymorphism and internalizing symptoms in early childhood. © 2013 Wiley Periodicals, Inc.

Key words: catechol-*O*-methyltransferase; *val*158*met*; anxiety; depression; internalizing; replication

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INTRODUCTION

Depression and anxiety (i.e., internalizing disorders) are two of the most common forms of psychiatric disorder and constitute a bulk of the burden related to psychiatric disease [Reiger et al., 1993; Kessler et al., 1994; Olfson et al., 2002]. The etiology of these disorders is unclear but heritability estimates from twin cohorts suggest a substantial genetic component [Bierut et al., 1999; Eley and Stevenson, 1999; Kendler and Prescott, 1999; Kendler et al., 2006]. With respect to specific genes, the extant psychiatric genetics research implicates gene polymorphisms that regulate neurotransmitter release, transport and degradation [Krishnan and Nestler, 2010] in internalizing disorders. One such class of neurotransmitters is catecholamines, which include dopamine and epinephrine; optimal levels of catecholamines are critical in modulating sensory and motor responses and executive functions [Willner, 1995; Goldman-Rakic, 1998; Arnsten and Li, 2005; Brocki et al., 2009]. Animal models and studies of adult humans have consistently pointed to alterations in mesocorticolimbic catecholamine levels, and dopamine levels in particular, in depressive and anxious phenotypes [Pogorelov et al., 2005; Nestler and Carlezon, 2006; Ruhe et al., 2007; Dremencov et al., 2009]. For example, functional imaging research from human adults indicates that diminished cortical dopamine levels may play a role in behaviors related to some internalizing disorders, such as memory deficits, sleep and behavioral disturbances, and psychomotor retardation [Martinot et al., 2001; Fox et al., 2005; Golimbet et al., 2007; Demetrovics et al., 2010; Meyers et al., 2011; Meyer, 2012].

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Catechol-*O*-methyltransferase (COMT), a catabolic enzyme that degrades cortical catecholamines, including dopamine and epinephrine, plays a vital role in regulating mesocorticolimbic catecholamine levels [Meyer-Lindenberg and Weinberger, 2006]. The gene encoding *COMT* (Gene ID: 1312) is mapped to chromosome 22p11, and contains four exons [Brahe et al., 1986]. Further, a non-synonymous G → A single nucleotide polymorphism (rs4680) in exon four leads to a valine (*val*) to methionine (*met*) peptide change in the mature protein, and is called the *val*158*met* polymorphism (GenBank accession no. Z26491). This substitution impacts the thermostability of the COMT protein and reduces the enzyme catabolic function, therefore reducing dopamine degradation in carriers with at least one copy of the *met* allele by more than one-third compared to carriers homozygous for the *val* allele [Lotta et al., 1995; Lachman et al., 1996; Chen et al., 2004].

Due to the functional nature of the *COMT val*158*met* polymorphism on cortical dopamine levels, links between *COMT* and internalizing disorders have been extensively explored in adult psychiatric populations. For example, Hamilton et al. [2002] reported a strong association between the *COMT val*158*met* polymorphism and panic disorder in a family-based sample of 70 panic disorder pedigrees and 83 parent-offspring triads. According to Massat et al. [2005], the *val* allele is associated with early-onset major depression, and others have shown that this variant may increase risk for panic disorders and anxiety [Rothe et al., 2006; Domschke et al., 2007; Hosák, 2007]. Finally, Hettema et al. [2008] showed that the *COMT val*158*met* polymorphism is part of a genetic risk haplotype shared across a range of internalizing disorders, including major depression, anxiety and panic disorder. Even though multiple studies have reported on links between the *COMT* gene locus and internalizing phenotypes, there is disagreement in the adult literature on which *COMT val*158*met* allele confers risk. There are a few reports where the link between this polymorphism has either failed to replicate [Middeldorp et al., 2010; Opmeer et al., 2010] or have identified the *met* allele as the risk allele for internalizing disorders [Baekken et al., 2008; Wray et al., 2008; Aberg et al., 2011].

Researchers have posited that the inconsistencies in this literature stem from a number of factors, including lack of statistical power to detect genetic associations, as most studies have used small samples (i.e., fewer than 200 subjects) [Chen et al., 2011]. There is strong agreement in the literature on the need for larger samples, and, more importantly, replication of gene-phenotype associations in independent samples [Ioannidis et al., 2001; Zintzaras and Lau, 2008; Bosker et al., 2011]. Furthermore, to better understand the etiology of internalizing disorders, theorists emphasize the need for study designs that investigate the early developmental origins of these conditions [Zahn-Waxler et al., 2000]. Early childhood is a period of critical brain development when cortical and limbic regions, and the dopaminergic neurotransmitter system in particular, are undergoing maturation through processes such as neuronal synaptogenesis, myelination, and synaptic pruning [Sowell et al., 2003]. This period of increased brain plasticity is thought to influence sensory and perceptual functions that may have long-term consequences for shaping adaptive and maladaptive behavior [Nestler et al., 2002; Sowell et al., 2003; Krishnan and Nestler, 2010]. It is important to note that longitudinal studies have shown continuities between internalizing symptoms from childhood to adolescence and recurrence of depression in adult life [Bardone et al., 1996; Lavigne et al., 1998; Hofstra et al., 2000; Mesman and Koot, 2001; Mesman et al., 2001; Woodward and Fergusson, 2001; Fergusson and Woodward, 2002], suggesting that research identifying early-emerging risk markers for depressive symptoms may have important preventative implications. However, little work has been done on appropriate candidate genes, such as the *COMT val*158*met*, and emerging internalizing symptoms in early-life.

The aim of this study is to examine links between the *COMT val*158*met* polymorphism and early-emerging internalizing symptoms. Due to the important role of the COMT enzyme in the brain's catecholamine metabolism, we hypothesized that functional polymorphisms of this gene would be associated with symptoms of anxiety and depression in preschoolers. Furthermore, based on findings from adult samples, we tentatively hypothesized that the *val* allele of the *COMT* functional polymorphism would be associated with higher levels of internalizing symptoms. To increase confidence in our findings, we tested these hypotheses in two independent samples of preschoolers.

METHODS

Participants

Table I lists the demographic information for the study samples. The primary sample (hereafter referred to as Study 1) consisted of 559 children and their parents residing in Long Island, NY, USA. Of these, 476 children (254 males) contributed DNA and were therefore available for genetic analysis. Children's mean age was 42.2 months (SD = 3.1). Most participants came from middle-class families (M = 44.8; SD = 10.9), as measured by Hollingshead's Four Factor Index of Social Status [Hollingshead, 1975]. Children were of average cognitive ability (M = 103.1, SD = 13.7), indexed by the Peabody Picture Vocabulary Test [PPVT-4, Dunn and Dunn, 1997].

Replication Sample

In this sample (hereafter referred to as Study 2), participants were an unselected community sample of 409 (201 males) children and their primary caregivers (93% were the child's mother) from southwestern Ontario, recruited for a larger study of genetic and other biological and contextual influences on child temperament and psychopathology risk. Children's mean age was 36.2 months (SD = 0.16). Over half of participants (50.4%) in this sample also came from middle-income families with family income ranging from $40,000 to $100,000 CAD (see Table I for further details). The family demographic data for this sample closely resembles the most recent London, Ontario census data available [Statistics Canada, 2006]. Children were administered the PPVT-4 to screen for gross cognitive impairment and English proficiency (M = 112.4, SD = 14.8).

Informed consent was obtained from the parent prior to participation. Both studies were approved by the respective university's human subjects ethics review committees.

TABLE I. Demographic Characteristics of Study Samples

	Study 1 (Stony Brook, N = 476)	Study 2 (UWO, N = 409)
Child age (mean months [SD])	42.24 (3.14)	43.20 (3.60)
Child sex, male (% [N])	252 (55.0)	201 (49.1)
Child race, Caucasian (% [N])	487 (87.1)	371 (90.5)
Maternal age (mean years [SD])	36.04 (4.44)	35.30 (4.97)
Paternal age (mean years [SD])	38.30 (5.39)	37.22 (4.15)
Parent marital status (married, % [N])	93.7 (524)	80.5 (330)
Maternal employment (% [N])	51.2 (286)	52.4 (215)
Family income (% [N])		
<$50,000	9.2 (50)	55.6 (228)
$50,000–$100,000	51.3 (287)	26.6 (109)
>$101,000	39.7 (222)	17.8 (73)
Report/interview		
Completion (% [N])	85.1 (476)	99.2 (406)

Stony Brook = Stony Brook University, Long Island, NY, USA; UWO = The University of Western Ontario, London, ON, Canada.

Genotyping

Genomic DNA was purified from buccal swab cellular extracts and stored according to manufacturer instructions (Qiagen, Valencia, CA). Following Ruiz-Sanz et al. [2007], an allele-specific PCR detection method was used to determine the *COMT val*158*met* genotype. Briefly, allele specific primers containing specificity enhancing mismatches, 5′-CGGATGGTGGATTTCGCTGaCG-3′ (G-allele specific) and TCAGGCATGCACACCTTGTCCTTtAT (A-allele specific), were used in the presence of control primers to amplify allele specific amplicons. The PCR conditions used were: 5 min of initial denaturation at 94°C followed by 30 cycles of 30 sec of denaturation at 94°C, 30 sec annealing at 62°C and 20 sec of extension at 72°C, followed by a final extension of 5 min at 72°C. To improve reaction fidelity, we used the Invitrogen PCRx Enhancer (Invitrogen, Carlsbad, CA) as an adjuvant in our PCR amplifications. Amplicons were separated on 6% polyacrylamide gels, visualized using ethidium bromide and documented using the Bio-Rad 2000 gel documentation system (Bio-Rad Laboratories, Mississauga, ON, Canada). All genotyping was performed by technicians unaware of other study data.

In Study 1, 141 children (34.1%) were valine homozygous, 199 (48.2%) were heterozygous, and 73 (17.7%) children were homozygous for the methionine substitution. These genotypic frequencies are in Hardy–Weinberg equilibrium ($\chi^2 = 0.04$; $P = 0.85$). In Study 2, 118 children (29.4%) were valine homozygous, 190 (46.3%) were heterozygous, and 93 (23.2%) children were homozygous for the methionine substitution, also in Hardy–Weinberg equilibrium ($\chi^2 = 0.95$; $P = 0.33$).

Behavior measures. In Study 1, the Preschool Age Psychiatric Assessment [PAPA; Egger et al., 2006] Version 1.4 was used to assess symptoms of psychopathology as defined by Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria [American Psychiatric Association, 2000] relevant to children in this age group. The PAPA is the first published diagnostic interview to assess parent-reported psychopathology in children between the ages of 2 and 5 years. It uses a structured format and an interviewer-based approach. Symptoms occurring 3 months prior to the interview are rated to enhance accurate recall. Adequate test–retest liability has been reported (range of intra-class correlations [ICC] for dimensional symptom scores = 0.56–0.80, median = 0.66, Egger et al. [2006]). For this study, we used PAPA indices of depressive and anxious symptoms. We also used symptoms of oppositional defiant disorder (ODD) and attention deficit/hyperactivity disorder (ADHD) to determine whether the *COMT* genotypes were related specifically to depressive and anxious symptoms or general manifestations of psychopathology.

The PAPA depression scale included ratings for any depressive disorder, that is, major depressive disorder, dysthymia, or depression not otherwise specified. Similarly, the anxiety disorder scale was created summing ratings from specific phobia, separation anxiety, social phobia, GAD, agoraphobia and panic disorder items. Dimensional scores were created by summing the ratings of all items included in the algorithms created by Egger et al. [2006] to derive child's depressive and anxiety symptoms and symptoms of ODD and ADHD.

Interviews were conducted by advanced graduate students in clinical psychology who received training on the administration of the PAPA from a member of the PAPA group. To examine inter-rater reliability, a second rater independently rated audiotapes of 21 PAPA interviews. ICCs for the symptom scales used in this study were as follows: depression (0.85), anxiety (1.00), ODD (0.99) and ADHD (0.99). Internal consistency (α) was calculated for each symptom scale and indicated good reliability for depression ($\alpha = 0.75$), anxiety ($\alpha = 0.83$), ODD ($\alpha = 0.84$), and ADHD ($\alpha = 0.89$). According to the PAPA, the number of children meeting the DSM–IV diagnosis threshold for each disorder were 23 (4.9%) for MDD, 3 (0.7%) for GAD, 50 (12%) for ODD and 13 (3.1%) for ADHD.

In Study 2, symptom data were obtained by having the child's primary caregiver complete the Early Childhood Inventory-4 [ECI-4; Gadow and Sprafkin, 1997]. The ECI-4 is a 108-item behavior rating scale that assesses symptoms of 15 DSM–IV childhood

TABLE II. Study Variables by Child *COMT* Genotype

	Child *COMT* val158*met* Genotype			
	Study 1 (Stony Brook University)[a]		Study 2 (University of Western Ontario)[b]	
Variable	val/val (N = 141) M (SD)	val/met + met/met (N = 272) M (SD)	val/val (N = 102) M (SD)	val/met + met/met (N = 260) M (SD)
Anxiety	11.09 (7.23)**	9.19 (8.39)**	16.84 (7.56)*	15.58 (5.62)*
Depression	5.12 (4.19)***	3.89 (4.37)***	5.11 (0.74)**	4.87 (0.87)**
ODD	5.36 (7.83)	4.81 (6.74)	5.43 (3.24)	4.97 (2.81)
ADHD	2.60 (6.19)	2.75 (6.50)	16.11 (7.50)	15.19 (6.64)

N, sample size; SD, standard deviation; PPVT, Peabody Picture Vocabulary Test; SES, socioeconomic status as indexed by Hollingshead's Four Factor Index of Social Status [Hollingshead, 1975]; PAPA, Preschool Age Psychiatric Assessment; ECI-4, Early Childhood Inventory-4 [Gadow and Sprafkin, 1997]; COMT, catechol-*O*-methyltransferase; val, valine; met, methionine.
Mean ranks were tested using Wilcoxon rank sum test, as symptom scores were not normally distributed due to absence of internalizing symptoms in some children. These children received a score of "0" on the relevant symptom sub-scale on the PAPA and ECI-4.
[a]Symptom data based on maternal interview reports from the PAPA.
[b]Primary caregiver reports from the ECI-4.
*$P < 0.10$.
**$P < 0.05$.
***$P < 0.01$.

emotional and behavioral disorders. The ECI-4 has shown satisfactory test-retest reliability, as well as concurrent and predictive validity [Sprafkin et al., 2002]. For the present study, symptom severity scores for depression, anxiety, ODD, and ADHD were created. Symptom severity scores were created by summing all symptoms rated as occurring "never" (coded as 0) "sometimes" (coded as 1) "often" (coded as 2) or "very often" (coded as 3) [Gadow and Sprafkin, 1997]. The depression scale was created using items similar to the PAPA, that is, symptoms of major depressive disorder, dysthymia, or depression not otherwise specified were considered (M = 5.14, SD = 2.97; range of number of symptoms = 0–8). Similarly, the anxiety disorder scale was created by summing the scores of all anxiety disorders measured (separation anxiety disorder, specific phobia, obsessions, compulsions, generalized anxiety disorder, social phobia, and PTSD (M = 16.08, SD = 6.42; range of number of symptoms = 4–34). The total number of possible symptoms was 11 for the ECI-4 depression scale and 44 for the anxiety scale.

Data Analyses

First, associations were examined between *COMT* genotypes and child psychopathology symptoms on the PAPA (Study 1) and ECI-4 (Study 2). These associations were tested under dominant, recessive and co-dominant models for the ancestral allele (*val* allele). To address potential population stratification, we conducted our analyses on the Caucasian participants based on parent-reported ethnicity in both samples. After excluding non-whites, for final analysis, we had 413 and 362 (of the 371 children in Study 2, genotype data was missing for nine children) from Study 1 and Study 2, respectively.[1] As some children had no parent-reported symptoms, we used non-parametric tests (Mann–Whitney *U*-test) for some analyses as needed. All tests were conducted using PASW 18.

[1]Demographic information for the Caucasian sub-set of the study sample is provided as supplementary table.

RESULTS

Symptoms were unassociated with demographic variables such as child gender and socio-economic status in either sample (all ps > 0.56). No associations between *COMT* genotypes and child gender (all ps > 0.10) or socioeconomic status were found in either sample (all ps > 0.55). In Study 1, we found significant associations between *COMT* genotypes and PAPA symptoms of depression ($Z = -2.82$, $P = 0.01$) and anxiety ($Z = -2.27$, $P = 0.02$) under a valine recessive model (Table II). The *COMT* *val*158*met* polymorphism was associated with child internalizing symptoms assessed by the PAPA, such that children with at homozygous for the *val* allele had significantly higher symptoms of anxiety and depression (all ps < 0.05). In contrast, the two *COMT* *val*158*met* allelic groups did not differ significantly on ODD ($Z = -0.79$, $P = 0.42$) and ADHD symptoms ($Z = -0.004$, $P = 0.99$).

Table II also shows associations between *COMT* *val*158*met* genotype and primary caregiver reported emotional and behavioral symptoms on the ECI-4. Consistent with the results from Study 1, we observed positive associations between child depressive symptoms and the *COMT* *val*158*met* genotype under a *val* recessive model ($Z = -2.19$, $P = 0.03$). According to primary caregiver reports, *val* homozygotes exhibited higher symptoms of depression than children with at least one copy of the *met* allele. The relationship between *val*158*met* genotypes and anxiety symptom reports on the ECI-4 reached only a trend level ($Z = -1.66$, $P = 0.10$). No associations were found between *COMT* genotype and externalizing symptoms such as ODD ($Z = -1.02$, $P = 0.22$) and ADHD ($Z = -0.75$, $P = 0.45$).

DISCUSSION

We examined whether the *COMT* *val*158*met* gene polymorphism was associated with psychopathological symptoms in preschoolers

in two separate samples. Findings from both samples indicate that the *COMT val*158*met* functional polymorphism is associated with symptoms of depression (and anxiety in one of the samples) in young children but not with child externalizing symptoms. More specifically, our analysis shows that children homozygous for the *val* allele have more depressive symptoms than children with at least one copy of the *met* allele. These findings are consistent with recent association studies in samples of young children [Drury et al., 2010], adolescents [Wahlstrom et al., 2007; Nobile et al., 2010] and adults [Enoch et al., 2003; McGrath et al., 2004; Massat et al., 2011], in which the *val* allele was associated with significantly higher anxious and depressive symptoms.

The associations we found may be mediated by the influence of the *COMT* genotype on dopamine function in the brain, which has been implicated in neurobiological models of depression. Dopamine plays a critical role in synaptic plasticity through multiple mechanisms. For example, it controls the activity of AMPA and NMDA receptors through phosphorylation, and it also regulates voltage-gated ion channels such as sodium and calcium channels by affecting their phosphorylation state as well [Girault and Greengard, 2004]. The increased clearing of dopamine in *val* homozygotes may affect synaptic plasticity through secondary mechanisms involving AMPA and NMDA receptor activity, which may lead to cognitive and other deficits, and thus increased depressive and anxious symptoms. While speculative, some observations support this hypothesis; for example, in a recent study of a community sample of pre-adolescent children, *met* carriers performed better on prefrontal-dependent tests of cognition such as working memory tasks [Barnett et al., 2007]. Data from functional neuroimaging studies also show decreased prefrontal activation in the *val* homozygotes compared with the *met* carriers while completing tests of executive function [Mier et al., 2010]. In light of the fact that both decreased prefrontal activation and early cognitive deficits, such as poor performance on working memory tasks, have been consistently associated with increased risk for both anxiety and depression later in life [see Drevets et al., 2008; Gotlib and Joormann, 2010; Marazziti et al., 2010, for reviews], genetic influences on cognition may shape one early-emerging pathway to internalizing disorder. The relationship between the genotypes and anxiety symptoms was found only at the level of a trend in Study 2. This could be related to differences in assessment approaches between the two studies. More specifically, interview-based measures, such as the PAPA, are conducted by trained interviewers and may therefore afford greater statistical power to detect genotype-phenotype associations by decreasing measurement error with respect to the phenotype.

Our findings are contrary to some reports in the adult literature indicating that the *met* allele increases risk for internalizing disorders [Baekken et al., 2008; Wray et al., 2008; Aberg et al., 2011], and studies that show no effect for this gene polymorphism at all [Middeldorp et al., 2010]. Some have posited that the failures of replication and inconsistent nature of findings regarding the *COMT val*158*met* polymorphism in adults could be due to confounding influences such as sex, ethnicity, and current or previous history of mental disorders. First, with regard to sex, COMT enzyme activity differs greatly in adult males and females, with females having significantly lower prefrontal cortex COMT activity [Chen et al., 2004]; furthermore, some data indicate that estradiol acts as a regulator of COMT expression in adult females [Karayiorgou et al., 1999; Worda et al., 2003]. Therefore, the influence of estradiol may be an important confound in studies exploring links between the *COMT* polymorphism and internalizing symptoms in adults that has not been consistently or effectively addressed in study designs. Second, in a recent meta-analysis, Domschke et al. [2007] found ethnicity-specific associations between *COMT* gene and internalizing disorders, with Caucasian adults homozygous for the *val* allele exhibiting greater internalizing problems; in contrast, the *met* allele carriers of Asian descent reported higher internalizing problems. Taken together, the use of an ethnically homogenous sample of preschoolers may have helped us minimize these possible confounds.

A major strength of our study is the use of two independent samples to investigate links between the *COMT val*158*met* polymorphism and depressive symptoms, but our study also had a few limitations. Although we conducted our associations in an ethnically homogenous sample, population stratification could still be a factor, although experts differ in opinion regarding the impact of population stratification [Hutchison et al., 2004]. It is also plausible that other functional polymorphisms flanking the *COMT* SNP could be in linkage-disequilibrium with these loci and may lead to type I errors, but the functional nature of this polymorphism, and the replication of our results, makes this an unlikely possibility. Furthermore, complex phenotypes such as depression and anxiety are influenced by the interplay of many different genes [Reif and Lesch, 2003]. Therefore, the role of the dopamine system in the development of internalizing disorders is likely regulated by the interaction between multiple genes [see Opmeer et al., 2010 for an overview]. Other genetic variation such as epigenetic regulation of genes involved in catecholamine metabolism has also been hypothesized as a possible contributor to the development of complex traits such as depression. Recent evidence indicates that epigenetic variation such as promoter CpG methylation of the *COMT* gene effects COMT mRNA status and translation of both soluble and membrane bound COMT enzyme [Mill et al., 2006]. It is therefore plausible that interindividual differences in methylation status of the *COMT* gene promoter region may be linked to emerging symptoms of psychopathology. Finally, we did not explore the interactions between the *COMT* val158met polymorphism and early childhood environment. It is likely that some of the emerging clinical symptoms we report are an outcome of interactions between this polymorphism and early childhood environmental contexts. In a recent study in preschoolers exposed to early social deprivation, Drury et al. [2010] reported fewer symptoms of depression in *met* allele carriers as compared to their homozygous *val* allele counterparts. Our data complements the work of Drury et al. [2010] by showing that the importance of *COMT* functional variation and its links to emerging internalizing phenotypes. The relationship between this polymorphism and early life environment seems to be context specific as Evans et al. [2009] found no evidence of interaction between this locus and maternal depression as predictor of childhood internalizing symptoms. Indeed, future research designs should explore interactions between this polymorphisms and additional early life contexts such as parenting behaviors as predictors of emerging psychopathology. In spite of these limitations, we believe that the current study makes an

important addition to the literature on molecular genetics of internalizing phenotypes. To our knowledge, this is the first study of its kind to report and replicate associations between *COMT val*158*met* polymorphism and early-emerging depressive symptoms.

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