Genetic Vulnerability to Affective Psychopathology in Childhood: A Combined Voxel-Based Morphometry and Functional Magnetic Resonance Imaging Study

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Background: The majority of affective psychopathology is rooted early in life and first emerges during childhood and adolescence. However, little is known about how genetic vulnerability affects brain structure and function in childhood since the vast majority of studies published so far have been conducted on adult participants. The present investigation examined for the first time the effects of catechol-*O*-methyltransferase (COMT) valine (val) 158 methionine (met) (val158met) polymorphism, which has been shown to moderate predisposition to negative mood and affective disorders, on brain structure and function in children.

Methods: Voxel-based morphometry and functional magnetic resonance imaging were used to measure gray matter volume and emotional reactivity in 50 children aged between 10 and 12 years. We tested the hypothesis that met158 allele affects structural brain development and confers heightened reactivity within the affective frontolimbic circuit in children.

Results: The met158 allele was positively associated with gray matter volume in the left hippocampal head where genotype accounted for 59% of interindividual variance. In addition, the met158 allele was positively associated with neuronal responses to fearful relative to neutral facial expressions in the right parahippocampal gyrus where genotype accounted for 14% of the interindividual variance.

Conclusions: These results indicate that the met158 allele is associated with increased gray matter volume and heightened reactivity during emotional processing within the limbic system in children as young as 10 to 12 years of age. These findings are consistent with the notion that genetic factors affect brain function to moderate vulnerability to affective psychopathology from childhood.

Key Words: Affective psychopathology, childhood, COMT, emotional processing, hippocampus

Susceptibility for affective psychopathology depends on the dynamic interplay between genetic and environmental risk factors (1,2). The catechol-*O*-methyltransferase (COMT) valine (val) 158 methionine (met) (val158met) polymorphism has been shown to moderate predisposition to negative mood and affective disorders. In recent years, several imaging genetic studies have demonstrated the effects of this and other risk genes on brain structure and function in healthy adult participants (3). Given that very few psychiatric illnesses arise de novo in adulthood (4), it is important to extend the current imaging genetic work to include child samples. In the present investigation, we used voxel-based morphometry (VBM) and functional magnetic resonance imaging (fMRI) to examine the effects of COMT val158met polymorphism on brain structure and emotional processing in children aged between 10 and 12 years.

Catechol-*O*-methyltransferase is an enzyme that catalyzes the O-methylation of extracellular dopamine in the brain (5) and is mainly found in its membrane-bound (MB-COMT) form in postsynaptic neurons (6,7). The most abundant expression of COMT, both in terms of messenger RNA density (7,8) and enzyme activity (8,9), is found in the prefrontal cortex and the parahippocampal gyrus. The enzymatic activity of COMT is modulated by a guanine (G) to adenine (A) single nucleotide polymorphism (SNP) change (known as val158met or rs4680) in the COMT gene. This translates into a valine to methionine amino acid change in codon 158 that causes a threefold to fourfold decrease in its molecular thermostability. The alleles have been shown to be codominant with the met158 allele associated with decreased COMT activity, resulting in higher synaptic dopamine levels; the val158 allele associated with increased COMT activity, resulting in lower synaptic levels; and the heterozygote genotype (val158/met158) associated with an intermediate level of COMT activity (8).

Several studies suggest that the met158 allele is advantageous for cognitive performance (10–15) and prefrontal function (11,15–18) not only in adults but also in children (19,20). However, a series of recent studies have also implicated the met158 allele in negative mood and affective disorders, including increased levels of anxiety in women, obsessive-compulsive disorder in men, panic disorder, alcoholism, aggressiveness, bipolar affective disorder, major depression, and higher sensitivity to pain (as reviewed by Drabant *et al.* [21]). The met158 allele has also been associated with a high level of anxiety (22) and early-onset antisocial behavior (23) in children and adolescents, although a recent investigation of emotional symptoms in children 6 to 7 years old did not find an association (24).

While the impact of the val158met polymorphism on prefrontal function has been characterized extensively in recent years, only a few functional imaging studies have explored the relationship between this polymorphism and brain activation during emotional processing (21,25–27). Smolka *et al.* (26) reported a

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dose-dependent increase in limbic and prefrontal activation associated with met158 during the processing of unpleasant but not pleasant visual stimuli in a small group of healthy volunteers ($n = 35$). More recently, Drabant *et al.* (21), using 101 healthy volunteers exposed to fearful and angry facial expressions, found met158 to be associated with a dose-dependent increase in activation within a frontolimbic circuit including the hippocampus and the ventrolateral prefrontal cortex; furthermore, in met158 homozygotes, there was increased functional coupling between limbic and prefrontal regions. These studies suggest that met158 increases reactivity within a frontolimbic circuit that is critical for emotional regulation, thereby providing support to the implication of this allele in negative mood and affective disorders. The same frontolimbic circuit appears to show COMT-related differences in gray matter volume as revealed by recent structural neuroimaging studies (28–30).

It is currently unclear, however, whether the effects of the val158met polymorphism on brain responses to emotional stimuli that have been reported in adult participants are also evident in children. This is an important question since there is increasing evidence that the majority of affective psychopathology is rooted early in life and first emerges during childhood and adolescence (4). If gene-related differences in adult participants reflect alterations that occurred during childhood and adolescence, then a better characterization of these alterations during childhood is critical for understanding how genes affect brain structure and function to mediate vulnerability to affective psychopathology (31).

It is also unclear whether variation in the val158met polymorphism is associated with differences in brain morphology from early age. Dopaminergic innervation increases during brain maturation and decreases during late adolescence and early adulthood (32). Thus, it has been proposed that differences in synaptic dopamine levels associated with the val158met polymorphism may be associated with different trajectories of brain maturation (30). A recent investigation demonstrated that the impact of COMT genotype on gray and white matter density in young adults is dependent on the age of the participants in female subjects but not in male subjects (30). However, no previous studies have examined whether the val158met polymorphism affects structural brain development in childhood.

We therefore used VBM and fMRI to examine for the first time the impact of the functional val158met polymorphism in the COMT gene on brain structure and emotional processing in 50 children aged between 10 and 12 years. Participants were presented with pictures of fearful and neutral faces on a screen and were required to detect the gender of each face. We tested the hypothesis that met158 allele affects structural brain development and confers increased sensitivity to emotional stimuli within the affective frontolimbic circuit in children.

Methods and Materials

Subjects

A total of 50 boys aged 10 to 12 years old participated in the present study. Participants were recruited from the longitudinal Twins Early Development Study (TEDS) database as part of an ongoing twin neuroimaging project that included mostly typically developing children, as well as an oversample of children in the top 10% of the United Kingdom population for conduct problems (Supplement 1). The short version of the Wechsler Abbreviated Scales of Intelligence (WASI) was used to assess IQ (33). In addition, the Strengths and Difficulties Questionnaire (SDQ) (34) was used to measure emotional problems, conduct problems, hyperactivity, peer problems, prosociality, and total behavioral difficulties in all participants as rated by both parents and teachers. All subjects were genotyped for the val158met in the COMT gene (see below). Our sample of 50 volunteers comprised 14 met158/met158, 22 val158/met158, and 14 val158/val158 individuals. A one-way analysis of variance (ANOVA) revealed that the three genotype groups did not differ in age, IQ, or any of the SDQ indicators ($p > .05$) (Table 1).

Experimental Task

The experimental paradigm involved presenting emotional and neutral faces taken from the *Pictures of Facial Affect* (35) but cropped to remove hair. For each face, subjects had to make a gender classification (male or female) by pressing left or right response buttons; no explicit recognition or categorization of the emotional expression was required. Each stimulus was presented for 3000 msec and successive stimuli were separated by an interstimulus interval of 750 msec, resulting in a stimulus onset asynchrony of 3750 msec. A total of 80 stimuli were presented on a computer screen in a single scanning session that lasted 6 minutes and 24 seconds; the stimuli were arranged in 10 blocks, each comprising eight fearful or eight neutral faces. The experimental paradigm also comprised two "rest" blocks in which no faces were presented but a fixation cross remained on the screen for 32 seconds. The order of presentation of fearful and neutral faces was counterbalanced across subjects.

Genotyping

DNA was extracted from blood or cheek swabs using standard methods (36). Genotyping of the rs4680, which encodes the val158met polymorphism, was performed by KBioscience (http://www.kbioscience.co.uk; Hertz, United Kingdom) using a competitive allele-specific polymerase chain reaction (PCR) system (CASP). The region amplified was atcacccagcggatggtggatttcgctggc[A/G]tgaaggacaaggtcacccttgtggttggag. The genotyping results of a sample of 130 subjects, which included our 50 participants, were under Hardy-Weinberg equilibrium.

Image Acquisition

Structural brain images were acquired using a General Electric Signa 3.0 Telsa Excite II magnetic resonance imaging (MRI) scanner (General Electric Medical Systems, Milwaukee, Wisconsin) at the Institute of Psychiatry. Structural scanning consisted of an isotropic resolution three-dimensional (3-D) inversion recovery prepared spoiled gradient echo. Two hundred through-plane partitions (each 1.1 mm thick) were collected, with two partitions being discarded at each end of the imaging volume to minimize wrap-round artefacts.

In addition, functional image volumes (192 scans for each subject) were collected using T2*-weighted gradient echo-planar imaging (EPI) sequence with 28 slices (slice thickness 3.5 mm, gap = .3 mm) covering the whole brain (repetition time [TR] = 2 sec, echo time [TE] = 25 msec, field of view = 220 × 220, matrix size 64 × 64). Stimuli were projected onto a high-resolution screen located in front of the participant's head and were viewed via a mirror attached to the head coil.

Data Analysis

Behavioral Data. Analysis of response accuracy and reaction times was performed using the Statistical Package for Social Science (SPSS), version 15.0 (SPSS Inc., Chicago, Illinois). The three genotype groups were compared using a 2 × 3 ANOVA with facial expression as repeated measures. Inferences were made using a statistical threshold of $p < .05$.