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Research Report

Effect of tryptophan hydroxylase-2 gene variants on amygdalar and hippocampal volumes

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ABSTRACT

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the synthesis of serotonin (5-HT). Genetic variations in human TPH2, a newly identified isoform of TPH, have been shown to impact on enzymatic activity of TPH and to be associated with emotion-related personality traits and mood/anxiety disorders. Identification of an intermediate phenotype that bridges the relationship between genes and behavior may be of great importance in the further clarification of how *hTPH2* contributes to emotional regulation. Previous studies have shown that a polymorphism in the upstream regulatory region of hTPH2 (SNP G-703T, rs4570625) correlates functional MRI response of the amygdala. In this study, we examined the effect of this genotype on amygdalar and hippocampal volumes in 208 mentally healthy individuals. To measure volumes of amygdala and hippocampus, gray matter regions of interest were outlined manually on three-dimensional MRI data obtained using a 1.5-T scanner. Additionally, personality traits were evaluated using the Temperament and Character Inventory (TCI). Those subjects with T allele carriers were associated with significantly smaller volumes in bilateral amygdala and hippocampus and higher reward dependence than those with G allele homozygotes. These results suggest that amygdalar and hippocampal volumes assessed using MRI may be a useful intermediate phenotype that will uncover the biological pathway linking 5-HT synthesis and emotional behaviors and affective disorders.

1. Introduction

Serotonin (5-hydroxytryptamine or 5-HT) is a widespread neurotransmitter in the central nervous system. 5-HT neurons are mainly found in different raphe nuclei, from which they project to numerous brain regions regulating mood and affect such as cortical areas, limbic system including amygdala and hippocampus, and basal ganglia (Freedman and Shi, 2001; Parent et al., 1981). 5-HT signaling is an important regulator of early central nervous system development (Lauder, 1993) and

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of adult neurogenesis (Gould, 1999). 5-HT is involved in many brain functions and neuropsychiatric disorders, and pharmaceuticals targeting the 5-HT system are widely used for the treatment of various psychiatric disorders.

The first and rate-limiting enzyme in 5-HT biosynthesis is tryptophan hydroxylase (TPH). It had been thought that TPH was derived from a single gene (now referred to as *TPH1*) until a second TPH isoform (*TPH2*) was recently described (Cote et al., 2003; Walther and Bader, 2003; Walther et al., 2003). While *TPH1* is primarily expressed in the periphery, *TPH2* is predominantly expressed in the brain and exclusively maintains brain 5-HT synthesis across the lifespan (Cote et al., 2003; Gutknecht et al., 2009; Walther et al., 2003; Zhang et al., 2004; Zill et al., 2004a). Furthermore, a relatively rare single nucleotide polymorphism in human *TPH2* (*hTPH2*) has been shown to alter TPH enzymatic efficiency in vitro (Zhang et al., 2005).

The association of *hTPH2* genetic variation with behavioral phenotypes including neuropsychiatric disorders and personality traits has been investigated. Specific polymorphisms in *hTPH2* have been reported to have an association with major depression (Van Den Bogaert et al., 2006; Zhang et al., 2005; Zhou et al., 2005; Zill et al., 2004b), affective disorders (Harvey et al., 2004; Lopez et al., 2007), suicidality (Jollant et al., 2007; Ke et al., 2006; Lopez de Lara et al., 2007; Lopez et al., 2007; Van Den Bogaert et al., 2006; Yoon and Kim, 2009; Zill et al., 2004b), autism (Coon et al., 2005), early onset obsessive-compulsive disorder (Mossner et al., 2006), attention deficit hyperactivity disorder (Sheehan et al., 2005; Walitza et al., 2005), panic disorder (Maron et al., 2007), chronic fatigue syndrome (Goertzel et al., 2006; Smith et al., 2006), and Tourette's syndrome (Mossner et al., 2007). Additionally, previous studies have suggested that *TPH2* is associated with personality traits related to emotional instability (Gutknecht et al., 2007; Lopez de Lara et al., 2007) and with risk for cluster B and cluster C personality disorders (Gutknecht et al., 2007).

To further clarify the biological pathways underpinning individual differences in emotional behaviors and risk for psychiatric conditions, including anxiety and depression, the identification of an intermediate phenotype for *hTPH2* variations is of great importance. In terms of a functional intermediate phenotype, Brown and colleagues reported that T allele carriers of the *hTPH2* rs4570625 variant were associated with greater functional magnetic resonance imaging (fMRI) amygdala response while viewing angry or fearful faces than were G/G individuals (Brown et al., 2005). Canli and colleagues replicated the findings by Brown et al. (2005); they reported that T carriers had greater fMRI signal increase in response to faces with both positive or negative valence than G/G individuals (Canli et al., 2005).

To our knowledge, however, no studies have investigated an association between hTPH2 variation and brain *structural* intermediate phenotype. Accordingly, the aim of this study was to investigate the effect of *hTPH2* rs4570625 polymorphism on regional brain volume as assessed by structural MRI and personality traits in mentally healthy individuals. Since reduced volume of amygdala and hippocampus has been reported in emotion-related psychiatric disorders including major depression, posttraumatic stress disorder, and suicidality (Geuze et al., 2005; Honea et al., 2005; Kasai et al., 2008; Monkul et al., 2007; Rogers et al., 2009; Strakowski et al., 2002), we predicted that T allele carriers would be associated with smaller volumes in these regions than G/G homozygotes. We also predicted that this polymorphism would affect personality traits that were evaluated using the Temperament and Character Inventory (TCI) (Cloninger, 1987; Cloninger et al., 1993), especially in harm avoidance (HA) and reward dependence (RD) as HA temperament dimension is frequently and positively associated with mood and anxiety disorders (Ampollini et al., 1999; Bayon et al., 1996; Brown et al., 1992; Cowley et al., 1993; Farmer et al., 2003; Farmer and Seeley, 2009; Hansenne et al., 1998, 1999; Mulder et al., 1994; Naito et al., 2000; Ongur et al., 2005; Richman and Frueh, 1997; Richter et al., 2000; Ruchkin et al., 1998; Saviotti et al., 1991) and with small hippocampal volume (Yamasue et al., 2008a), and RD is also associated with mood and anxiety disorders (Ampollini et al., 1999; Farmer and Seeley, 2009; Ongur et al., 2005; Richman and Frueh, 1997; Ruchkin et al., 1998).

2. Results

The genotypic distributions of the three genotypes in the *TPH2* genes are as follows: G/G 53 (25.5%), G/T 106 (51.0%), T/T 49 (23.6%). The distributions of the three TPH2 genotypes were not significantly different from those expected according to the Hardy–Weinberg equilibrium. The three genotypes in the TPH2 gene were classified into two genotype subgroups according to the previous study (Brown et al., 2005): the genotypes with the T allele (here termed 'T carriers') versus the G/G genotype (here termed 'G/G individuals'). No significant difference was observed in gender, age, handedness, self-socioeconomic status (SES), or parental SES between the two genotype groups (Table 1). Total gray matter, total white matter, cerebrospinal fluid (CSF), and intracranial volume (ICV) volume also did not differ significantly between the two genotype groups ($p>0.29$).

For the genotype effects on amygdalar and hippocampal volumes, the repeated-measures ANOVA showed that there was a significant main effect of the *hTPH2* genotype ($F[1,206]=5.17$ $p=0.024$). There was no significant interaction between genotype and region ($F[1,206]=0.026$, $p=0.87$), genotype and hemisphere ($F[1,206]=0.70$, $p=0.41$), or genotype and region and hemisphere ($F[1,206]=1.67$, $p=0.20$). The statistical conclusion from the main ANOVA is that subjects with T allele carriers have significantly smaller ROI volumes without either significant laterality or regional specificity among bilateral amygdala and hippocampus than those with homozygous G alleles (Fig. 1). There were no significant interactions between gender and genotype ($F[1, 204]=0.94$, $p=0.33$), gender and genotype and region ($F[1, 204]=0.27$, $p=0.60$), gender and genotype and hemisphere ($F[1, 204]=0.46$, $p=0.50$), or gender and genotype and region and hemisphere ($F[1, 204]=0.18$, $p=0.67$). Percentage changes and effect sizes of the difference in brain volume between genotypes are described in Table 2. Furthermore, subjects with T allele carriers showed significantly higher scores on reward dependence than those with G/G homozygotes ($t[190]=-2.39$, $p=0.018$) (Table 1). Additionally, genotype effects were re-investigated after excluding outlying data (a single subject with outlying bilateral hippocampal volumes) and the main effect of the *hTPH2* genotype did not alter ($F[1,205]=4.91$, $p=0.028$).