Influence of TPH2 variants on diagnosis and response to treatment in patients with major depression, bipolar disorder and schizophrenia

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ABSTRACT

The present study is aimed at exploring whether some single nucleotide polymorphisms (SNPs) within the tryptophan hydroxylase 2 gene (TPH2) could be associated with major depression (MD), bipolar disorder (BD) and schizophrenia and whether they could predict clinical outcomes in Korean in-patients treated with antidepressants, mood stabilizers and antipsychotics, respectively. One hundred forty-five patients with MD, 132 patients with BD, 221 patients with schizophrenia and 170 psychiatrically healthy controls were genotyped for six TPH2 SNPs (rs4570625, rs10748185, rs11179027, rs1386498, rs4469933, and rs17110747). Baseline and final clinical measures, including the Montgomery-Åsberg Depression Rating Scale (MADRS), Young Mania Rating Scale and Positive and Negative Syndrome Scale, for patients with MD, BD and schizophrenia, respectively were recorded. None of the SNPs under investigation were associated with MD, BD and schizophrenia. However, in patients with MD, the rs4570625-rs10748185 G-A haplotype was significantly associated with higher endpoint MADRS severity, though not with response. Our results suggest that TPH2 variants neither have a major role in MD, BD and schizophrenia nor in response to treatments.

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1. Introduction

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the biosynthesis of serotonin. It converts the amino acid tryptophan to 5-hydroxytryptophan, which is then decarboxylated into serotonin (Walther and Bader, 2003). Two isoforms, TPH1 and TPH2, are known. While TPH1 is mostly expressed in the periphery and only partially in the brain, TPH2 is exclusively expressed in neuronal cell types (Invernizzi, 2007), particularly in the raphe nuclei where the vast majority of serotonergic cell bodies are located (Walther and Bader, 2003; Patel et al., 2004; Zill et al., 2007). Following the discovery of the TPH2 in TPH1-homozygous knockout mice that continued to produce 5-HT in the brain but not in other tissues (Walther and Bader, 2003), increasing attention has been given to the role of the human homologue TPH2 located on chromosome 12q21 in mental diseases, as this is a positional candidate region for several psychiatric disorders such as major depression (MD) and bipolar disorder (BD) (Morissette

et al., 1999; Abkevich et al., 2003; Curtis et al., 2003). In addition, taking into account the significant role of serotonin in mood control as well as in a wide variety of functions, including, among the others, regulation of sleep, pain perception, hormonal activity, cognition, aggression, sexual drive, appetite and energy level (Azmitia, 2007), it is not surprising that consistent efforts have been recently paid to uncover whether genetic variants within TPH2 could play a major role into the development and response to treatment of several psychiatric disorders. Indeed, serotonin is largely involved in both mood disorders and schizophrenia, as demonstrated also by the therapeutic effect of serotonin modulators.

The findings suggest a possible involvement of TPH2 in the etiology and response to drugs in MD. First of all, in drug-free depressed patients who committed suicide, the levels of TPH protein and TPH2 mRNA in the dorsal raphe nucleus have been found to be higher as compared with matched healthy controls (Boldrini et al., 2005; Bach-Mizrachi et al., 2006). Also, such patients had more TPH2 grain density per neuron in the dorsal raphe nucleus than controls (Bach-Mizrachi et al., 2006). In addition, in an animal model expressing a TPH2 variant similar to a rare human variant (R441H) previously associated with MD, the expression of such mutant TPH2 resulted in markedly decreased brain 5-HT production and led to behavioral abnormalities related to depression and anxiety (Beaulieu

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et al., 2008). Of further interest, several case-control studies have suggested that several TPH2 genetic variants could be associated with MD in both Caucasian (Zill et al., 2004; Zhang et al., 2005; Zhou et al., 2005; Van Den Bogaert et al., 2006; Haghighi et al., 2008) and Chinese (Tsai et al., 2009) patients suffering from MD, even though contrasting results have been reported as well (Garriock et al., 2005; Gizatullin et al., 2008; Mann et al., 2008; Illi et al., 2009). Additionally, several variants in TPH2 have been linked to response to antidepressants (Peters et al., 2004; Zhang et al., 2005; Tzvetkov et al., 2008; Tsai et al., 2009) as well as to electroconvulsive therapy (Anttila et al., 2009) in many independent samples including different ethnicities, even though results were not always replicated (Illi et al., 2009; Peters et al., 2009).

TPH2 variants have also been suggested to play a role in BD. First of all, higher levels of TPH2 expression have been found in the dorsolateral prefrontal cortex of patients with BD as compared with matched controls (De Luca et al., 2005a). Also, several case control studies have found significant associations between specific single nucleotide polymorphisms (SNPs) and haplotypes within TPH2 and BD in samples including mainly Caucasian subjects (Harvey et al., 2004; Van Den Bogaert et al., 2006; Harvey et al., 2007; Lin et al., 2007; Cichon et al., 2008; Roche and McKeon, 2009), even though such findings were not consistently replicated in other samples of Caucasian (Campos et al., 2010; Grigoroiu-Serbanescu et al., 2008) as well as Korean (Choi et al., 2010) subjects with BD. It is worth mentioning, however, that, even though a set of SNPs located in the 5' region of TPH2 previously associated with BD in a German sample was not replicated in a Romanian population (Grigoroiu-Serbanescu et al., 2008), the association became significant in a subgroup of patients with paternal transmission of the disease, raising the question as to whether at least some TPH2 variants could be specific for some subpopulations of patients but not for others. Additionally, recent findings (Lin et al., 2007) suggesting the existence of epistatic interactions between TPH2 and TPH1 underscore the importance of considering how complex genetic interactions could differently modulate the risk for a given disorder as compared to single genes separately analyzed.

Following such findings a number of studies began focusing on a possible role of TPH2 in schizophrenia as well. However, even though limited to a few investigations, the experimental research on this topic has not found evidence for a possible involvement of several TPH2 variants in the etiology of schizophrenia so far (De Luca et al., 2005b; Higashi et al., 2007; Shiroiwa et al., 2010; Tee et al., 2010) and for an alteration in the expression of TPH2 in the dorsolateral prefrontal cortex of schizophrenia patients as compared with patients suffering from BD and healthy controls (De Luca et al., 2005a). On the other hand, it is noteworthy that a number of studies mainly focused on the rs4570625, an SNP located in the putative promoter region of TPH2 (Kennedy et al., 2003), found an association with several further psychiatric disorders including panic disorder (Kim et al., 2009), obsessive compulsive disorder (Mossner et al., 2006) and attention deficit/hyperactive disorder (Walitza et al., 2005), even though, taking into account the lack of replications, such results should be considered with caution and deserve further investigations.

Overall, such findings suggest that several genetic variants in TPH2 could play an important role in the etiology of several psychiatric disorders as well as in the response to some of their treatments, even though there is no complete consensus yet with regard to the specific variants involved. Note, however, that significant differences across the studies in terms of psychiatric disorders, pharmacological treatments, psychometric assessments and ethnicities under investigation as well as different criteria for defining remission and response could partially explain why discrepant findings have sometimes been observed.

Accordingly, the aim of the present study was to replicate and extend such findings in a Korean population of patients suffering from different psychiatric disorders. In detail, the first aim of the present article is to investigate whether specific SNPs within TPH2 including rs4570625 located in the promoter region and further whether five SNPs which have received little or no attention so far (rs10748185, rs11179027, rs1386498, rs4469933, and rs17110747) could be associated with MD, BD and schizophrenia. The second aim of the present article is to explore whether such variants could predict clinical outcome in patients suffering from MD, BD and schizophrenia naturalistically treated with antidepressants, mood stabilizers and antipsychotics, respectively.

2. Methods

2.1. Subjects

The sample under investigation in the present study comprised 145 in-patients suffering from MD, 132 in-patients with BD and 221 in-patients with schizophrenia who were consecutively recruited at the Department of Psychiatry of the Catholic University of Korea College of Medicine, Seoul, Korea. Patients were eligible for inclusion if they had a documented clinical diagnosis of MD, BD or schizophrenia according to the DSM-IV criteria, as assessed by the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998).

There was not any particular restriction with respect to treatments, concomitant comorbidities and first vs. following episodes of disease. However, patients were excluded if they had current severe or unstable medical and neurological conditions, current treatment with a long-acting antipsychotic, concomitant alcohol and substance abuse disorders and if they were not of Korean ethnicity. The choice of not using excessively tight inclusion and exclusion criteria was motivated by the decision to include a sample of subjects that could be representative of usual psychiatric in-patients in Korea. A further sample of 170 Korean psychiatrically healthy subjects, who underwent the same assessment of psychiatric patients to exclude possible psychiatric disorders, deriving from the same location of the psychiatric patients included in the present study and including both community volunteers and medical staff, was also included to compare genotype and allelic frequencies between the four populations of subjects under investigation.

All patients admitted to the hospital were assessed for the severity of illness at baseline and at discharge by means of psychometric questionnaires specific for each disorder under investigation. More in detail, MD severity was assessed by means of the Montgromery-Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979), mania severity in patients with BD with current manic or mixed state was assessed by means of the Young Mania Rating Scale (YMRS) (Young et al., 1978) and familiar history of schizophrenia severity was assessed by means of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Scorers were trained with the specific instruments with good inter-rater reliability ($\kappa > 0.8$). Additionally, the following clinical and demographic variables were recorded: gender, age, clinical subtypes, age at onset, familiar history of psychiatric disorders (based on patients' reports following direct questioning by clinicians), lifetime suicide attempts, duration of admission, drugs at discharge and concomitant anxiolytics. The study protocol was approved by the institutional review board (approval number HC10TISI0031). All patients (18-65 years old) provided written informed consent before participating into the study.

2.2. Outcome measures

The main outcome measures of the present study were: 1) differences between genetic and allelic frequencies in patients with MD, BD and schizophrenia as well as healthy control subjects and 2) possible influence of the six SNPs within TPH2 under investigation on clinical improvement as well as on response and remission rates in the three groups of psychiatric patients mentioned above separately analyzed. Both continuous and categorical analyses were performed. Regarding categorical ones, in accordance with previous studies, response was a priori defined as a $\geq 50\%$ symptoms' reduction from baseline to discharge (e.g. (Hirschfeld et al., 2004; Leucht et al., 2007; Riedel et al., 2010)). Remission was defined as a MADRS score ≤ 7 at discharge for patients with MD (Riedel et al., 2010) and as a YMRS score ≤ 12 for patients with BD (Perlis et al., 2006). Unfortunately it was not possible to determine remission rates for patients with schizophrenia, as current consensus-based operational criteria require data from eight single items of the PANSS (Andreasen et al., 2005) (or the Brief Psychiatric Rating Scale (Flemenbaum and Zimmermann, 1973)) that was not recorded in the present study.

2.3. DNA analysis

Genomic DNA was extracted from blood by standard methods and quantified. The high-throughput genotyping method using pyrosequencer (Biotage AB, Sweden) was used for genotyping six SNPs (rs4570625, rs10748185, rs11179027, rs1386498, rs4469933 and rs17110747) of TPH2 under investigation (Table 1). PCR primers (Bioneer, Daejeon, Korea) and sequencing primers (Bioneer, Daejeon, Korea) used for the pyrosequencing assay were designed by using the Pyrosequencing Assay Design Software v1 (Biotage AB, Sweden) and one primer of each primer set was biotinylated.