Serotonin-related gene pathways associated with undifferentiated somatoform disorder

Kyung Bong Koh [a,*], Eun Hee Choi [b], Young-joon Lee [a], Mooyoung Han [b]

[a] Department of Psychiatry, Yonsei University College of Medicine, Seoul, Korea
[b] Department of Biostatistics, Yonsei University College of Medicine, Seoul, Korea

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ABSTRACT

It has been suggested that serotonergic hypofunction and serotonergic pathway genes underlie the somatic symptoms of somatoform disorders. We examined a variety of serotonin-related gene polymorphisms to determine whether undifferentiated somatoform disorder is associated with specific serotonin-related gene pathways. Serotonin-related polymorphic markers were assessed using single nucleotide polymorphism (SNP) genotyping. One hundred and two patients with undifferentiated somatoform disorder and 133 healthy subjects were enrolled. The genotype and allele frequencies of tryptophan hydroxylase (TPH)1 A218C, TPH2 rs1386494, serotonin receptor 2A-T102C (5-HTR 2A-T102C), 5-HTR 2A-G1438A and serotonin transporter (5HTTLPR) gene were compared between the groups. The Hamilton Rating Scale for Depression and the somatization subscale of the Symptom Checklist-90-Revised (SCL-90-R) were used for psychological assessment. Patients with undifferentiated somatoform disorder had higher frequencies of the TPH1 C allele than healthy controls ($p = 0.02$) but the difference was not significant after Bonferroni correction. The frequency of TPH1 genotype also did not differ significantly between the patients and the healthy controls, nor did TPH2 rs1386494, 5-HTR 2A-T102C, 5-HTR 2A-G1438A or 5HTTLPR allele and genotype frequencies differ significantly between the two groups. These findings suggest that a variety of serotonin-related gene pathways are unlikely to be definite genetic risk factors for undifferentiated somatoform disorder. Therefore, the pathogenesis of the disorder may be related to epigenetic factors, including psychosocial and cultural factors. Nonetheless, future studies need to include a larger sample of subjects and polymorphisms of more serotonin-related gene variants.

1. Introduction

Somatoform disorders tend to be chronic and can cause significant social problems and financial burden. In particular, undifferentiated somatoform disorder, a subtype of somatoform disorders is characterized by one or more unexplained physical complaints lasting for at least six months but below the threshold for diagnosing somatization disorder (Guggenheim, 2000). The prevalence of undifferentiated somatoform disorder is known to be quite high, ranging from 10.2% to 30.6% (De Waal et al., 2004; Chang et al., 2005; LeiKeiknes et al., 2007). Treating somatoform disorders is challenging because they cannot be treated according to the existing biomedical model (Koh, 2002). However, antidepressants are commonly used in the treatment of somatoform disorders because these disorders often coexist with depressive symptoms (Belous et al., 2001).

Serotonin is believed to play an important role in depression, and a number of serotonergic pathway genes have been examined (Owens and Nemeroff, 1994) including the tryptophan hydroxylase (TPH) gene, which is involved in catalysis of the rate-limiting step for serotonin biosynthesis (Peters et al., 2004) and regulates levels of serotonin (Mann, 1999). Therefore, variations in the TPH gene could contribute to the predisposition to low serotonergic neurotransmission (Rujescu et al., 2002). TPH1 is regarded as a candidate gene for major depressive disorder and is believed to influence antidepressant response (Pickar and Rubinow, 2001).

Recently, genetic variants of the TPH2 gene were also reported to be involved in the pathogensis of major depressive disorder (Zill et al., 2004a). In humans TPH1 and TPH2 are expressed in nearly equal amounts in several brain regions (the frontal cortex, thalamus, hippocampus, hypothalamus and amygdala). However, TPH2 is predominantly expressed in the brain stem, the major locus of serotonin-producing neurons (Zill et al., 2004b).

The serotonin receptor 2A (5-HTR 2A) gene is also believed to be a candidate gene for depression, because 5-HTR 2A binding potential is associated with major depressive disorder and suicidal behavior (Oquendo and Mann, 2001). Du et al. (2000) reported that the C allele of the 5-HTR 2A T102C gene polymorphism is related to depression and suicidal behavior However, Eley et al. (2004) suggested that the T allele is a more likely risk factor for depression. Other serotonin-related gene polymorphism candidates for depression include the A1438G polymorphism in the promoter region of the 5-HTR 2A gene, which is significantly

* Corresponding author. Tel.: +82 2 2228 1624; fax: +82 2 313 0891.
E-mail address: kbkoh@yuhs.ac (K.B. Koh).

associated with major depressive disorder (Choi et al., 2004) and seasonal affective disorder (Enoch et al., 1999). In addition, a functional polymorphism located in the promoter region of the serotonin transporter gene (5-HTTLPR), has been shown to be associated with seasonal affective disorder (Rosenthal et al., 1998). However, the 5-HTTLPR s allele was reported to be linked to suicidal behavior (Anguelova et al., 2003a; Lin and Tsai, 2004; Li and He, 2007) but not to unipolar depression (Anguelova et al., 2003b; Lasky-Su et al., 2005).

In addition, somatic symptoms of somatoform disorders are thought to be connected to serotonergic neurotransmission because serotonin is known to regulate the functions relevant to one of these disorders, such as pain disorder (Guggenheim, 2000; Hennings et al., 2009). There is also some evidence suggesting that an abnormality in processing immuno-reactive serotonin transporter protein (IR-STP) is involved in somatoform disorders (Belous et al., 2001).

In contrast to the large body of research examining genetic risk factors for depressive disorders, research into the genetic etiology of somatoform disorders is in its infancy. Genetic data indicate that somatization disorder has genetic components because it tends to run in families (Sadock and Sadock, 2003). In addition, another study found that somatic symptoms of somatoform disorder patients might be associated with functional polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) (Hennings et al., 2009). Therefore, it was suggested that serotonergic hypofunction and serotonergic pathway genes are likely to underlie the somatic symptoms of somatoform disorders. However, few studies have investigated the relationship between the variety of serotonin-related gene polymorphisms and somatoform disorders.

In the present study, we examined TPH1 A218C, TPH2 rs1386494, 5-HTR 2A-T102C, 5-HTR 2A-A1438G and 5-HTTLPR gene polymorphisms in patients with undifferentiated somatoform disorder and healthy subjects to determine if the disorder is associated with specific serotonin-related gene pathways.

2. Methods

2.1. Subjects

This study was reviewed and approved by the Institutional Review Board of Yonsei University College of Medicine at Severance Hospital. The purpose and procedures of the study were explained to all potential subjects from the same geographic and ethnic origin, and informed consent was obtained from those who decided to participate. The subjects' depression levels were assessed using the Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1960) by one experienced psychiatrist (K.B.K.).

One hundred and thirty-three healthy subjects (53 men, 80 women) were recruited from unrelated Korean volunteers for hospital guide service and hospital employees such as secretaries, janitors, clerical workers, aid-nurses, nurses, medical residents and medical students. The mean age ($\pm$ SD) of the healthy subjects was 32.2 ($\pm$ 7.6) years, with a range of 25 to 53 years. The subjects were selected after completing a self-report questionnaire and an interview to confirm the absence of physical and psychiatric disorders as well as family history of psychiatric disorders. None of the volunteers reported being treated for or having symptoms of physical or psychiatric disorders and family history of psychiatric disorders in the self-report questionnaires. Specifically, volunteers were included in the group after psychiatrists confirmed that they had no abnormalities at their most recent regular physical check-ups and were taking no medication.

In addition, unrelated Korean outpatients with diagnoses of undifferentiated somatoform disorder from the Department of Psychiatry at Severance Hospital (Seoul, Korea) were enrolled in this study. A semi-structured interview was conducted and diagnoses were made by one experienced psychiatrist (K.B.K.) using the Korean version (Hahn et al., 2000; Hahn and Hong, 2000) of the Structured Clinical Interview Schedule for the Diagnostic and Statistical Manual-4th edition (DSM-IV) (First et al., 1996) upon the first visit to the outpatient department.

Physical examinations and a number of laboratory examinations were conducted. These tests included a complete blood count (CBC), urinalysis, serum electrolytes, liver function tests, chest x-ray, thyroid function test, and electrocardiogram. Other tests, such as electromyography, electroencephalography, brain magnetic resonance imaging (MRI), liver ultrasonogram, gastroscopy, and colonoscopy were conducted if necessary in either the Department of Psychiatry or other departments.

Patients with physical diseases or abnormal laboratory findings were excluded from the study. Patients who developed additional medical or psychiatric disorders (e.g., major depressive disorder or dysthymic disorder) during the course of the study were also excluded. The final sample included 102 patients with undifferentiated somatoform disorder (47 men, 55 women). The mean age of the patients with somatoform disorder was 41.9 ($\pm$ 11.0) years, with a range of 20 to 59 years. The mean duration of

their illness was 12.3 ($\pm$ 20.0) months. The patients were subdivided into two groups by cut-off score (18 points) of the HRSD: patients with higher levels of depression (total score of the HRSD: 18 and more) and lower levels of depression (total score of the HRSD: less than 18).

Most of the subjects were unmedicated, but some patients who were within the first few days of starting medication were included in this study. They received antidepressants (selective serotonin reuptake inhibitors: SSRIs) such as fluoxetine or paroxetine. However, those who showed changes in emotional and behavioral symptoms after initiation of the medication were excluded.

Among the patients (n = 113) who completed the entire testing process, 11 subjects were excluded from the data analysis because they had either comorbid mental disorders or physical diseases: major depressive disorder (3 subjects), dysthymic disorder (6 subjects) and diabetes mellitus (2 subjects). Among the healthy subjects (n = 136) who completed the entire testing process, three were excluded from the data analysis because they had dysthymic disorder (1 subject), adjustment disorder (1 subject) and diabetes mellitus (1 subject).

2.2. Psychological measures

The psychological measures included the Hamilton Rating Scale for Depression (HRSD) and the somatization subscale of the Korean version (Kim et al., 1984) of the Symptom Checklist-90-Revised (SCL-90-R) (Derogatis et al., 1976). The SCL-90-R is a 90-item self-rating instrument that was developed to assess the psychopathology of the previous week, and it includes nine subscales. The somatization subscale has 12 items.

2.3. Single nucleotide polymorphisms genotyping

Genomic DNA was extracted from the peripheral blood using a Puregene® DNA purification kit (Gentra, Minneapolis, MN, USA). Tryptophan hydroxylase (TPH1 A218C and TPH2 rs1386494) and serotonin receptor (5-HTR 2A-T102C and 5-HTR 2A-A1438G) polymorphisms were genotyped using a TaqMan fluorogenic 5` nuclease assay (Applied Biosystems, Foster City, CA, USA). A polymerase chain reaction (PCR) was conducted with 5 µl containing 10 ng of genomic DNA, 2.5 µl of TaqMan Universal PCR Master Mix, and 0.13 µl of 40X assay mix. Thermal cycle conditions were as follows: 50 °C for 2 minutes to activate uracil N-glycosylase and to prevent carry-over contamination, 95 °C for 10 minutes to activate DNA polymerase, followed by 45 cycles at 95 °C for 15 seconds and 60 °C for 1 minute. All PCRs were performed using 384-well plates in a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA), and allelic discrimination analysis was performed with an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

For genotyping of 5HTTLPR, PCR was carried out in a total volume of 10 µl containing 10 ng genomic DNA, 0.5 µM of each of the sense (5'-GGCGTTGCCGCTCTGAATGC-3') and antisense (5'-GAGGGACTGAGCTGGACAACCAC-3') primers, 0.5 mM of each of four deoxynucleotide phosphates (dATP, dCTP, dGTP, dTTP), 0.25 unit i-StarTaq DNA Polymerase (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea). To amplify the fragment, 500 mM of betaine was added to the PCR system. Amplification conditions consisted of an initial denaturing step at 95 °C followed by 35cycles at 95 °C for 30 seconds, 65 °C for 1 minute and 72 °C for 1 minute. This was followed by a final extension step at 72 °C for 10 minutes. To detect the amplified DNA fragment, we analyzed 2 µl of the reaction mixtures on a 3% agarose gel (ReadyAgarose 96 plus 3% TBE Gel, Bio-Rad).

2.4. Statistical analyses

Either a chi-square test or t-test was performed to compare demographic variables and the levels of psychological measures between the disorder group and the healthy group. The Hardy-Weinberg equilibrium (HWE) for all genotyping results was tested in both patients and healthy subjects using the chi-square test. Comparisons of genotype and allele frequencies between the two groups (patients vs. healthy subjects; higher levels of depression vs. lower levels of depression) were also made using the chi-square test. Bonferroni correction was applied to data for multiple tests.

3. Results

3.1. Demographic data

Patients with undifferentiated somatoform disorder were significantly older than the healthy control subjects (Mean $\pm$ SD, 41.9 $\pm$ 11.0 vs. 32.2 $\pm$ 7.6; t = 6.89, df = 152, p < 0.0001) but there was no significant difference in sex between the two groups ($\chi 2$ = 0.92, df = 1, p = 0.34).

3.2. Levels of depression and somatic symptoms in undifferentiated somatoform disorder

The somatoform disorder patients scored significantly higher on the HRSD and on the somatization subscale of the SCL-90-R than the control subjects (Table 1). Significant correlation was found between