Journal of Psychiatric Research xxx (2014) 1–7

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

SLC6A2 variants may predict remission from major depression after venlafaxine treatment in Han Chinese population

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ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form
6 November 2014
Accepted 28 November 2014

Keywords:
Major depressive disorder
Norepinephrine transporter
SLC6A2 gene
Venlafaxine
Remission

ABSTRACT

Objective: Venlafaxine, an antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI) type, is used to treat patients with major depressive disorder (MDD). Much evidence suggests that genetic polymorphisms may modulate serotonergic and noradrenergic function, thereby affecting the treatment efficacy of venlafaxine. The aim of this study was to examine whether polymorphisms in the norepinephrine transporter gene (*SLC6A2*) associate with remission after venlafaxine treatment for MDD.
Method: An 8-week naturalistic treatment study with venlafaxine was carried out in 243 Han Chinese patients with MDD. The patients were screened for seven single-nucleotide polymorphisms of the *SLC6A2* gene. Of the enrolled patients, 161 completed the 8-week treatment. The 21-item Hamilton Depression Rating Scale (HDRS) was used to assess the improvement of depressive symptoms in each subject from baseline to the endpoint. For better presentation of time-course change of remission status, a Cox regression analysis for remission incidence during the 8-week treatment was conducted.
Results: Between remitters and non-remitters, significant differences in genotype frequencies were observed in five of the investigated *SLC6A2* variants (rs28386840, rs1532701, rs40434, rs13333066, rs187714). GCG haplotype (rs40434 - rs13333066 - rs187714) in the *SLC6A2* gene showed a association with non-remission. A Cox regression analysis for remission incidence during the 8-week treatment course significantly depends on *SLC6A2* variants (rs28386840, rs40434, and rs187714).
Conclusion: Our results suggest that the variation of the *SLC6A2* gene is associated with treatment remission after venlafaxine in patients with MDD.

1. Introduction

Major depressive disorder (MDD) is a highly prevalent psychiatric disorder with a high risk of disability and mortality. Antidepressants are the first-line treatment for MDD; however, the treatment outcome is unpredictable in a given patient and response rate is variable, ranging from 50 to 60%. In general, only one third of patients with MDD patients achieve remission after an antidepressant monotherapy trial (Rush et al., 2006). Therefore, there is an urgent need to identify biomarkers that predict treatment response before administering antidepressants. Many studies have focused on relating genetic polymorphisms, which are not affected by demographic data with clinical variables, on the assumption that variability in mechanism-associated genes may contribute to the treatment response (Dong et al., 2009; Uher et al., 2009; Cattaneo et al., 2013; Niitsu et al., 2013).

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http://dx.doi.org/10.1016/j.jpsychires.2014.11.017
0022-3956/

Among various antidepressants, venlafaxine is a common serotonin-norepinephrine reuptake inhibitor (SNRI), which targets not only the serotonin transporter, but also the norepinephrine transporter (NET). The norepinephrine transporter gene (solute carrier family 6, member 2, *SLC6A2* gene), located on chromosome 16q12.2 spanning approximately 45–47 kb and including 14 exons (Bruss et al., 1993), may be a candidate gene for response to venlafaxine. A previous study has shown that the rs5569 single nucleotide polymorphism (SNP) of *SLC6A2* may be associated with nortriptyline (a norepinephrine reuptake inhibitor, NRI) treatment response ($n = 105$) (Kim et al., 2006). However, the association between rs5569 polymorphism and response to nortriptyline was not replicated in a later, larger study ($n = 330$) (Uher et al., 2009). In addition, an intronic SNP of *SLC6A2*, rs1532701, was observed to predict response to nortriptyline, although the significance did not survive in multiple testing (Uher et al., 2009). These studies suggest that *SLC6A2* polymorphisms may be associated with NRI response, but not with selective serotonin reuptake inhibitor (SSRI) response. Therefore, *SLC6A2* polymorphisms may also be associated with response to venlafaxine, which being an SNRI, incorporates NRI function.

To the best of our knowledge, previous studies on the association between *SLC6A2* gene polymorphism and treatment efficacy of SNRI monotherapy, are limited (Yoshida et al., 2004; Min et al., 2009; Houston et al., 2012b). Moreover, these studies mostly investigate two common *SLC6A2* SNPs: rs2242446 (T-182C) and rs5569 (G1287A), and may not consider the entire variability of expression of the *SLC6A2* gene. Therefore, these studies cannot rule out an association between *SLC6A2* variation and SNRI response. Recently, the intronic *SLC6A2* SNP rs36024 was found to be associated with treatment response in treatment-resistant MDD using fluoxetine–olanzapine combination therapy (Houston et al., 2012a). Moreover, a novel polymorphism, rs28386840 (T-3081A), is located upstream of the transcription initiation site of the *SLC6A2* gene, and has been proven to be a functional polymorphism because it alters the promoter function of the *SLC6A2* gene (Kim et al., 2006). Nevertheless, the connection of this SNP to antidepressant response is poorly explored in the literature. Although one such study has been done to analyze rs28386840 polymorphism in 252 Caucasians with MDD and showing no association between treatment response and rs28386840 (Baffa et al., 2010), the treatment in that study was complex, comprising various antidepressants and concurrent use of mood stabilizers and antipsychotics, thereby weakening the study conclusions. Therefore, we decided to examine *SLC6A2* variation in association with response to SNRI treatment monotherapy.

The aim of this study was to examine whether *SLC6A2* polymorphisms influence venlafaxine monotherapy outcome in patients with MDD. A promoter and some intronic variants of the *SLC6A2* would be tested, and two common SNPs, rs2242446 and rs5569, would be replicated in an 8-week naturalistic treatment cohort study with venlafaxine.

2. Materials and methods

2.1. Participants

The study design was approved by the Institutional Review Board for the Protection of Human Subjects at the Tri-Service General Hospital (TSGH), a medical teaching hospital belonging to the National Defense Medical Center in Taipei, Taiwan. Participants recruited from outpatient and inpatient population, and all given written informed consent. To minimize the effect of ethnic differences on gene frequencies, all participants were unrelated Han Chinese in Taiwan. Each participant was interviewed by a psychiatrist for initial evaluation and was subsequently interviewed by a well-trained psychologist using a Chinese version of the modified Schedule of Affective Disorder and Schizophrenia-Lifetime (SADS-L) (Endicott and Spitzer, 1978; Merikangas et al., 1998). The inter-rater reliability κ values of the SADS-L were good to excellent for major depression, bipolar disorder, anxiety disorder, schizophrenia, and substance abuse/dependence (Huang et al., 2004).

The inclusion criteria were as follows: 1) age 20–65 years; 2) diagnosis of MDD according to DSM-IV-TR (American Psychiatric Association, 2000); 3) no other psychiatric axis I disorder present; 4) the 21-item Hamilton Depression Rating Scale (HDRS) score ≥ 18 (Hamilton, 1960); and 5) screening period ≥ 4 weeks for washout of prior antidepressant or other psychotropic medications. The exclusion criteria included: 1) current or past bipolar disorder, anxiety disorder, schizophrenia, or substance abuse/dependence (except nicotine dependence); 2) significant active physical illness; 3) women with pregnancy or lactation; 4) previous head trauma with loss of consciousness; 5) epilepsy; or 6) thyroid disease. An 8-week, open-label, flexible-dose venlafaxine regimen with initial dose 150 mg/day was begun and dosage was adjusted according to the patient's symptoms at week 2. Patients with MDD who started to use other antidepressants to the exclusion of venlafaxine, or to use mood stabilizers or antipsychotics, were dropped from the naturalistic follow-up study.

2.2. Selection of genetic variants

We selected total of 7 SNPs with minor allele frequencies (MAFs) of more than 0.1 to cover the *SLC6A2* gene on the basis of a review of the literature, the human *SLC6A2* polymorphisms listed in the NCBI SNP database (www.ncbi.nlm.nih.gov/projects/SNP/), and the Human HapMap Project database (www.hapmap.org). Two SNPs have been widely investigated in the literature: rs2242446 in the promoter region and rs5569 in exon 9 (Uher et al., 2009; Niitsu et al., 2013); these were used for validating this study by replication of previous studies. A functional polymorphism, rs28386840, located upstream of the promoter region of *SLC6A2*, was included (Kim et al., 2006). Some intronic SNPs have been suggested to be associated with treatment response in subjects with treatment-resistant depression (Houston et al., 2012a). Therefore, we selected for this study 4 intronic SNPs with minor allele frequencies of more than 0.1: rs1532701, rs40434, rs13333066 in intron 1 (Houston et al., 2012a); and rs187714 in intron 3.

2.3. Genotyping methods for the SLC6A2 gene

Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit (DNAzol®; Invitrogen, Carlsbad, California, USA). We used PCR-restriction fragment length polymorphism (RFLP) strategies for rapid genotyping of rs28386840. A 148-bp region of *SLC6A2* was amplified by PCR with forward primers *NET137S* (5′-CTGTAGTTTTCTTGCCCCTCAAG-3′) and reverse primer *NET38A* (5′-GAGACAGCAAAGGGAAGGAAACCA-3′) (Kim et al., 2006). The PCR product was then digested with 5 units of BsrI at 65 °C for 2 h for the rs28386840 A/T polymorphism. Digestion products were run on 7% polyacrylamide gels. The other studied SNPs were genotyped by using TaqMan assays with VIC® dye and FAM™ dye (Applied Biosystems, Foster City, CA). We used the Applied Biosystems StepOne™ software and StepOnePlus™ real-time PCR systems for thermocycling and data collection. For genotyping accuracy and quality control, we blinded duplicates selected from 50 random samples using two methods: an RFLP method and bidirectionally direct sequencing method with a model

Please cite this article in press as: Yeh Y-W, et al., *SLC6A2* variants may predict remission from major depression after venlafaxine treatment in Han Chinese population, Journal of Psychiatric Research (2014), http://dx.doi.org/10.1016/j.jpsychires.2014.11.017