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Gender-specific association between serotonin transporter polymorphisms (*5-HTTLPR* and *rs25531*) and neuroticism, anxiety and depression in well-defined healthy Han Chinese

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ABSTRACT

Background: A tri-allelic serotonin transporter promoter polymorphism (*5-HTTLPR/rs25531*) more effectively determines the levels of transcriptional efficacy than that with the bi-allelic *5-HTTLPR* polymorphism in vitro. Both are reportedly associated with personality traits of negative emotionality, but with conflicting findings. One explanation for this is that a gender difference may play a role in genetic contribution. Here, we hypothesized that the tri-allelic genotype of the serotonin transporter is more closely linked to neuroticism, an anxiety- and depression-related trait, than the bi-allelic variation, particularly in a gender-dependent way.

Methods: The genotypes of the *5-HTTLPR* and *rs25531* loci were determined in 1139 well-defined physically and mentally healthy Han Chinese (550 men, 589 women; mean age 38.3 ± 10.3 years). All participants completed the neuroticism measure of the short-form Maudsley Personality Inventory (MPI). The levels of anxiety and depression were assessed by the Beck Anxiety Inventory (BAI) and the Beck Depression Inventory (BDI), respectively.

Results: A significant tri-allelic genotype-by-gender interaction effect was found in the MPI-neuroticism measure. *S′S′* homozygotes were associated with higher neuroticism than *L′* allele carriers in men. Also, both the BAI and BDI scores were higher in the *S′S′* homozygotic men. In the bi-allelic analyses, however, there was only an association between *SS* genotype and MPI-neuroticism in men.

Limitations: Sub-analyses by gender-stratification may reduce the statistical power.

Conclusions: Our findings confirm that gender differences exist in the genetic contributions of the serotonin transporter in human neuroticism, and anxiety/depression. Our data provide further support for *rs25531,* strengthening the effects of *5-HTTLPR*.

1. Introduction

Neuroticism, a fundamental personality trait, is characterized by worry, emotional instability, over-reactiveness or nervousness (Eysenck and Eysenck, 1985). It is a well-known risk factor for various psychiatric disorders, particularly for depression and anxiety disorders (Aldinger et al., 2014; Jylha and Isometsa, 2006; Uliaszek et al., 2009). Heritability studies have documented that genetic determinants contribute substantially to variance in the trait of neuroticism (Bouchard and Loehlin, 2001; Iliadis et al., 2015; Jang et al., 1996). This trait has been proposed to have a higher heritability than associated disease statuses, and may be more closely related to the underlying genetic liability than the etiologically heterogeneous mental disorders (Almasy and Blangero, 2001; Goldstein and Klein, 2014; Klein, 1998). Much interest has therefore been focused on the anxiety- and depression-related trait of neuroticism in genetic research.

The most frequently studied candidate gene for traits of negative emotionality has been the serotonin transporter (5-HTT)-linked polymorphic region (*5-HTTLPR*) polymorphism (Lesch et al., 1996) in the promoter of the *SLC6A4* gene that encodes 5-HTT, which plays an important role in serotonergic neurotransmission by facilitating reuptake of serotonin from the synaptic cleft. The *5-HTTLPR* is a 43 bp

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insertion/deletion polymorphism that has two allelic forms, the long variant (*L*) and the short variant (*S*), with the *L*-allele exhibiting a 3-fold higher mRNA expression than the *S*-allele in vitro (Heils et al., 1996; Lesch et al., 1996). The *S* allele of the *5-HTTLPR* polymorphism has been associated with personality traits like harm avoidance (an anxiety-related trait) and neuroticism, but the findings reported have been inconsistent (Munafo et al., 2005, 2009; Terracciano et al., 2009). One explanation for the inconsistent results is that a gender difference may exist in genetic contribution. Indeed, this association tended to be stronger in male subgroups and studies including primarily men (Du et al., 2000; Gelernter et al., 1998; Lesch et al., 1996; Vormfelde et al., 2006; Zhang et al., 2015).

In addition to the *S* and *L* alleles, an important variation of the *L* allele has been reported (Hu et al., 2006). The *L* variant, with an adenosine at the single nucleotide polymorphism (SNP) *rs25531* (L_A), located in the proximity of the *5-HTTLPR*, expresses higher levels of 5-HTT than does the *L* variant with a guanine at *rs25531* (L_G), which, like the *S* allele, expresses lower levels of 5-HTT in vitro. Therefore, there has been critical discussion regarding the inadequacy of the traditional dichotomous analysis of *5-HTTLPR*, due to its inability to distinguish between the L_A and L_G alleles (Murphy et al., 2013), which may reduce statistical power. So far, only a few studies have attempted to demonstrate a role for the newer tri-allelic (*S*, L_G, L_A) polymorphism in traits of negative emotionality.

Minelli et al. (2011) initially identified the association of the tri-allelic *5-HTTLPR* polymorphism with harm avoidance, but in a relatively small clinical subsample, who had depression or anxiety disorders (n=55). However, two successive studies using healthy subjects (determined only by self-reporting) or an admixture of healthy and diseased subjects, with a greater proportion of females, did not find an association between harm avoidance or neuroticism and the tri-allelic locus (Odgerel et al., 2013; Plieger et al., 2014). Since past studies using a traditional bi-allelic *5-HTTLPR* classification have shown a gender-specific tendency, analyzing the newer tri-allelic *5-HTTLPR* polymorphism in a large sample and considering the effect of gender difference on neuroticism measures may give us more detailed and convincing genetic association data. Furthermore, subjects with mental disorders (e.g., affective disorders and drug and alcohol abuse) or various physical diseases (e.g., diabetes and cardiovascular diseases) may rate neuroticism differently from normal subjects (Goodwin et al., 2006; Maier et al., 1995; Phillips et al., 2010; Quirk and McCormick, 1998). Studying drug-free, physically and mentally healthy subjects can minimize these confounding factors to reveal more precisely the *5-HTT* genetic effects on the anxiety- and depression-related personality trait of neuroticism.

The present study, using a large, drug-free Han Chinese sample of well-defined healthy subjects, determined by structured psychiatric assessment and medical health examinations, was designed to explore the impact of gender difference on the association between the serotonin transporter polymorphisms and neuroticism, anxiety and depression measures, and whether tri-allelic reclassification yields clearer results than the bi-allelic approach in a gender-stratified analysis.

2. Methods

2.1. Participants

The study cohort was composed of volunteers who underwent annual health examinations at Tri-Service General Hospital, a medical teaching hospital of the National Defense Medical Center in Taipei, Taiwan. All participants were unrelated ethnic Han Chinese. Using a screening questionnaire, subjects taking any medication for at least one month prior to the start of the study or those with a personal history of medical diseases, psychiatric illnesses, or pregnancy were excluded. After initial screening, 1340 adult Han Chinese were recruited. All provided informed consent before participating in the study. The hospital's institutional review board approved the study protocol, which adhered to the guidelines of the Declaration of Helsinki. Demographic data including age and sex were collected.

2.2. Assessment of psychiatric morbidity

Each enrolled subject was further evaluated by a trained research assistant using the Chinese version of the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998), a structured diagnostic instrument designed to yield psychiatric diagnoses according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria (APA, 1994). Thirty-four subjects were excluded because they had a diagnosed mental illness, mainly depression or anxiety disorders. Included subjects were evaluated for their anxiety/mood status and neurotic personality trait.

2.3. Assessment of anxiety and depression levels

The Chinese version of the Beck Anxiety Inventory (BAI), a self-rated 21-item scale, was used to measure the intensity of anxiety experienced in the past week (Lin, 2000). Each item was rated on a four-point scale, ranging from “not at all” (0) to “severely” (3). The total scores ranged from 0 to 63, with higher scores indicating higher anxiety. Mood status was assessed with the Chinese version of the Beck Depression Inventory-II (BDI), a 21-item questionnaire assessing self-reported levels of depression during the preceding two weeks (Chen, 2000). Each question was assigned a score of 0–3, with 3 indicating the most severe depressive features (total score range: 0–63). Higher total scores correlated with more severe depression. The Chinese BAI and BDI were both found to be highly reliable and valid (Chen, 2000; Lin, 2000).

2.4. Assessment of the personality trait of neuroticism

The Chinese version of the short-form Maudsley Personality Inventory (MPI) neuroticism scale was used to measure the propensity for neuroticism (Lee et al., 1990). The scale includes 13 self-reported items, and each item was rated as 0=no, 1=uncertain, or 2=yes. Total scores ranged from 0 to 26, with higher scores indicating a higher tendency toward neuroticism. The Chinese MPI-neuroticism scale has been demonstrated with good test-retest reliability (correlation coefficient, 0.90) (Lee et al., 1990), and used widely in both community and medical settings in Taiwan (Lee et al., 2006; Liao et al., 2002). In our sample, the internal consistency for these 13 scale items was high (Cronbach's α, 0.82).

2.5. Assessment of medical conditions

All of the study participants then received health check-ups, which included a physical examination, biochemical analysis (blood, urine, and stool specimens), chest X-ray, and electrocardiogram. Heart rate and blood pressure (systolic and diastolic) were measured. Hepatic and renal function was assessed using standard procedures. Fasting plasma glucose was determined by the glucose oxidase method, while triglyceride and total cholesterol levels were measured using the dry, multilayer analytical slide method (Chang et al., 2014a, 2014b).

In this stage, one hundred and sixty-seven participants with physiological conditions such as cardiovascular diseases (e.g., hypertension, arrhythmia), metabolic disorders (e.g., hypercholesterolemia, hypertriglyceridemia, and diabetes mellitus), liver or kidney diseases, malignancy, neuropathy, or obesity (body mass index: ≥30 kg/m^2) were further excluded.