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RESEARCH ARTICLE

The 5-HTTLPR Confers Susceptibility to Anorexia Nervosa in Han Chinese: Evidence from a Case-Control and Family-Based Study

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Abstract

Accumulating evidence has implied that serotonin system dysfunction may be involved in the etiology of anorexia nervosa (AN). Serotonin-transporter-linked promoter region (5-HTTLPR) polymorphism is the genetic variant coding for the serotonin transporter and has a modulatory effect on its expression. This study aimed to investigate the possible association between the 5-HTTLPR and the susceptibility and severity of AN in Han Chinese using a case-control (255 patients and 351 controls) and family based study (198 trios). Eating disorder examination was used to measure the severity of AN behavioral symptoms. For the case-control study, the 5-HTTLPR showed significant association with AN in our sample (genotypic $P = 0.03$). The frequency of S allele was significantly higher in patients than that in controls (OR = 1.38, 95%CI: 1.06–1.79, $P = 0.017$). For the family-based study, the S allele of 5-HTTLPR was preferentially transmitted rather than non-transmitted from the parents to affected offspring (P = 0.013). The results of ANCOVA test revealed no significant association between the 5-HTTLPR polymorphism and severity of AN. Our findings suggested that 5-HTTLPR is able to confer susceptibility to AN in Han Chinese.

Introduction

Anorexia nervosa (AN) is an eating disorder, characterized by excessive food restriction, typically arising from a morbid fear of weight gain that motivates patients to avoid eating. Although the pathogenesis of AN remains unknown, several lines of evidence supported the idea that AN is frequently associated with symptoms of anxiety, obsessive-compulsiveness and depression, and same pathophysiological mechanisms may underlie these symptoms [1].

Serotonin (5-HT) is a major neurotransmitter in the mammalian central nervous system (CNS). 5-HT modulates various CNS physiological activities including food intake, the sleep-wake cycle, cognition, and a wide repertoire of emotional behaviors [2]. Current studies have indicated that 5-HT system is involved in various psychiatric disorders and in the regulation of the feeling of satiety [3]. Consequently, it has been implied that 5-HT activity may be important in the physiopathology of AN [4].

Genetic epidemiological studies have assembled convincing evidence that AN is substantially influenced by genetic factors, and that the genetic component contributing to 50% ~ 70% of the variance in AN [5]. Therefore, several studies have attempted to search for genes in the 5-HT system that may be involved in the susceptibility to AN. One of the candidate genes is the serotonin transporter (5-HTT) gene, which encodes the human 5-HTT protein. A functional polymorphism in its 5' regulatory promoter region (termed 5-HTTLPR: the 5-HTT gene-linked polymorphic region), consisting of two common alleles that correspond to a 44-base pair insertion (L allele) or deletion (S allele), regulates transcription of the 5-HTT gene [6]. The S allele of 5-HTTLPR polymorphism was found to reduce transcription efficiency for the 5-HTT gene, resulting in decreased 5-HTT expression and associated with a number of serotonin-related psychiatric disorders, such as bipolar disorder, depression, violent suicide.

So far, case-control studies have examined the association between 5-HTTLPR polymorphism and AN. Some preliminary findings have indicated a significant association of the S allele with AN [7,8], but such positive results were not replicated in other studies [9,10,11,12,13]. This fact may be due to population stratification, a limitation inherent to the population case-control study design. Therefore, the use of nuclear families is a critical feature being against population stratification [14]. In this study, we first carried out a two-phase study including both case-control and family-based analyses to examine the association between 5-HTTLPR polymorphism and AN in Han Chinese. In addition, it is well-known that AN is a complex and polygenic disorder, and specific genetic loci may contribute to the pathophysiology and phenotypic variability seen in AN [15]. Previous studies have indicated an influence of 5-HTTLPR on the severity of symptoms in psychiatric disorders, such as major depressive disorder [16], panic disorder [17], obsessive compulsive disorder [18], attention-deficit/hyperactivity disorder [19] and autism [20]. To the best of our knowledge, there is no report about the role of 5-HTTLPR in the severity of symptoms in AN. Therefore, the second aim of this study was to examine whether 5-HTTLPR was associated with specific symptom clusters in a sample of Han Chinese patients with AN.

Methods

Ethics statement

All participants provided written informed consent prior to inclusion in this project, and were treated in accordance with the Declaration of Helsinki. The study protocol and process was assessed and approved by the ethics committee at the Shanghai Mental Health Center. The Ethics Committee of the Shanghai Mental Health Center provided ethics approval for adolescent participants to provide their own consent rather than requiring consent for next of kin, caretakers or guardians.

Subjects

We performed both case-control and family-based analyses in this study. For the family-based study, 198 trios consisting of 594 subjects were recruited. All probands from the family-based study were also part of the case-control study, which is detailed as follows. Their parents self-reported no history of AN. For the case-control study, a total of 255 patients with AN were recruited from the Department of Clinical Psychology, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine. Each patient was assessed and diagnosed by at least two senior psychiatrists according to DSM- IV (American Psychiatry Association). The sample consisted of 13 male and 242 female patients with a mean age at entry into the study of 19.4±4.7 years.

An additional 351 healthy control subjects (12 males and 339 females, mean age: 20.0±1.5 years) were recruited from hospital staff and students of School of Medicine in Shanghai, and then interviewed by a research psychiatrist according to the SCID-P [21]. Control subjects who reported a history of any psychiatric disorder or a metabolic, endocrine or gastrointestinal illness that could affect body weight were excluded from this study. All of the participants for each portion of the study were of Han Chinese origin.

Clinical Measures

All measures were administrated at prior to enrollment. (1) Physical assessment: to calculate body mass index (BMI = kg/m^2), participants' weight and height were evaluated by a trained research assistant using a stadiometer. All patients were weighted in light indoor clothing, without shoes. (2) Eating disorder examination: eating attitudes and behaviors were specifically investigated by means of the Eating Disorder Examination Questionnaire (EDE-Q). The EDE-Q is a semi-structured investigator-based interview specifically devoted to the assessment of ED psychopathology, providing different levels of descriptive data concerning current ED psychopathology. The EDE-Q global scale was used to assess the severity of ED behavioral symptoms. The EDE-Q has previously demonstrated good reliability and validity [22,23].

Genotyping

Blood samples were obtained from the participants and then Genomic DNA was isolated from whole blood using a Tiangen DNA isolation kit (Tiangen Biotech, Beijing, China). The analysis of the 5-HTTLPR variant was done using PCR as the method described by Xu et al [24]. The following primer pair was designed to span the variant region in the *5-HTT* promoter: 5′-GGCGTTGCCGCTCTGAATGC-3′ (forward) and 5′-GAGGGACTGAGCTGGACAACCAC-3′ (reverse). PCR was performed with the annealing temperature at 62°C for 33 cycles. The PCR products were subjected to electrophoresis in 2% agarose gels, stained with ethidium bromide and visualized under UV light.

Statistical analysis

We used SPSS 17.0 (SPSS Inc., Chicago, IL, USA) to perform either the t-test or the chi-square test to compare demographic characteristics between the AN and control groups. For the case-control analysis, the Hardy-Weinberg equilibrium, allele and genotype frequencies of 5-HTTLPR polymorphism were determined using SHEsis (http://analysis.bio-x.cn) [25]. The family-based association test was performed on transmission disequilibrium test (TDT) program of Haploview 4.1. The possible effect of the 5-HTTLPR genotype on symptom severity was performed with ANCOVA by comparing the mean EDE-Q scores of each genotype. Variables that affected EDE-Q scores (i.e., sex, age, BMI, education and age at onset) were included as covariates. The statistical power of our sample size was calculated on Quanto program (Version 1.2.3, available at http://hydra.usc.edu/GxE). All tests were two-tailed and a significance level was set at $P<0.05$.

Results

Demographic and clinical features

Social-demographic and clinical characteristics of our sample are reported in Table 1. There were no significant differences between the patients with AN and control subjects with respect to age and sex. The patients with AN have significantly lower BMI than control subjects (16.2±2.7 vs 20.6±2.6, $P\prec0.01$).

Table 1. Demographic features in AN patients and controls.

	Patients (n = 255)	Controls (n = 351)	χ^2/t	*P*
Gender (M/F)	13/242	12/339	1.05	0.31
Age (years)	19.4±4.7	20.0±1.5	−1.43	0.15
BMI (kg/m2)	16.2±2.7	20.6±2.6	−14.35	<0.01
Education (years)	12.1±2.8	NA		
Age at onset (years)	16.7±3.5	NA		
	Patients (n = 177)			
EDE-Q total score	2.3±1.3	NA		
EDE-Q restraint	1.8±1.6	NA		
EDE-Q eating concern	2.5±1.5	NA		
EDE-Q weight concern	2.3±1.5	NA		
EDE-Q shape concern	2.8±1.6	NA		

BMI, body mass index; EDE-Q, Eating Disorder Examination Questionnaire.

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5-HTTLPR and susceptibility to AN

5-HTTLPR has two common forms that are distinguished by a long and short allele depending on the insertion or deletion of 44bp. For the case-control study, the genotype distribution of the 5-HTTLPR polymorphism in control group was in accordance with Hardy-Weinberg equilibrium ($P = 0.38$). Comparisons of genotype and allele frequencies for the polymorphism between patients with AN and control subjects are presented in Table 2. The 5-HTTLPR showed significant association with AN in our sample (genotypic $P = 0.03$). The frequency of S allele was significantly higher in patients than that in controls (OR = 1.38, 95%CI: 1.06–1.79, $P = 0.017$). Family-based test showed that genotype distribution of 5-HTTLPR conformed to Hardy-Weinberg equilibrium in the parents and no Mendelian inheritance error was found. Allele frequencies analysis is shown in Table 3. The S allele of 5-HTTLPR was preferentially transmitted rather non-transmitted from the parents to affective offspring ($P = 0.013$). Under the assumption of a modest effect size and log additive model, the statistical power of our sample was more than 90% and 76% for the case-control and family-based analyses, respectively.

5-HTTLPR and severity of AN

A total of 177 unmedicated patients examined with the EDE-Q to evaluate pathological symptoms of AN. Then the scores of five factors of the EDE-Q were compared based on the genotypes of 5-HTTLPR. The results of ANCOVA test revealed no significant association between the 5-HTTLPR polymorphism and severity of AN (Table 4).

Table 2. Distribution of genotypes and alleles for the 5-HTTLPR polymorphism in AN patients and controls.

		Genotype N (%)					Allele N (%)				
5-HTTLPR	**N**	**S/S**	**S/L**	**L/L**	χ^2	***P***	**S**	**L**	χ^2	***P***	**OR (95%CI)**
Patients	255	158 (62.0)	77 (30.2)	20 (7.8)	6.89	0.03	393 (77.1)	117 (22.9)	5.68	0.017	1.38 (1.06–1.79)
Controls	351	180 (51.3)	138 (39.3)	33 (9.4)			498 (70.9)	204 (29.1)			

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Table 3. Results of TDT analysis for 5-HTTLPR polymorphism in AN families.

	Allele			
Trios (N = 198)	S	L	χ^2	*P*
Transmitted	72	45	6.23	0.013
Non-transmitted	45	72		

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Discussion

In the present study, we found significant difference in allele and genotype frequencies of 5-HTTLPR between the patient and control groups. Since the false-positive results may emerge due to the effects of population stratification in case-control investigation design, family-based study was carried out as an effective approach to follow up the findings from the case-control investigation [26]. In the family samples, the S allele of 5-HTTLPR was observed to be over-transmitted from heterozygous parents to probands. This confirmed the association of 5-HTTLPR and AN. Recently, analysis of the pooled results from independent studies by meta-analytic methods provided significant evidence for the association of S allele of 5-HTTLPR polymorphism with AN [27]. Our findings provided supportive evidence for this association in Han Chinese for the first time.

It is known that AN is a syndrome with a multifactorial etiopathogenesis involving psychological and biological factors [28]. Epidemiological data has pointed out that stressful experiences have important consequences for the development of AN [29,30]. At the molecular level, a number of experimental studies supported the idea about the 5-HTTLPR stress-sensitivity hypothesis [31] that 5-HTTLPR and stress jointly convey stable changes in serotonin transporter (SERT) expression [32]. The S carriers tend to exhibit lower expression of 5-HTT coupled with reduced reuptake of 5-HT from the synapse, and this may result in stronger psychopathological reactions to stressful experiences than those with L allele [33]. Brain imaging studies provide the potential for understanding neurotransmitter function, such as 5-HTT, and structural changes in relevant to human behaviors. Bailer et al. [34] used positron emission tomography (PET) imaging with McN5652 to assess the 5-HTT activity and found that women covered from restricting-type AN had elevated binding potential of 5-HTT in the dorsal raphe and anteroventral striatum in comparison to women who recovered from bulimia-type AN. These results implied that AN has neurobiological characteristics due to persistent disturbance of 5-HTT function. On the other side, a recent magnetic resonance imaging study

Table 4. Distribution of EDE-Q scores in the AN patients with the three 5-HTTLPR genotypes.

	5-HTTLPR				
EDE-Q	L/L (n = 13)	L/S (n = 60)	S/S (n = 104)	*F*	*P*
Restraint	2.65±1.74	1.89±1.76	1.57±1.48	2.45	0.09
Eating	2.42±1.62	2.81±1.57	2.28±1.40	1.89	0.15
Shape	2.91±1.89	3.00±1.63	2.58±1.48	0.98	0.38
Weight	2.48±1.68	2.24±1.47	2.23±1.47	0.15	0.86
Global	2.61±1.58	2.48±1.39	2.17±1.28	0.93	0.40

The *P*-values were adjusted for sex, age, BMI, education and age at onset.
EDE-Q, Eating Disorder Examination Questionnaire.

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has showed that in young women (mean age = 25.6 years), the effect of stress on structural connectivity between the left hippocampus and both the amygdala and the putamen may be regulated by a 5-HTTLPR genotype-mediated mechanism of implicit learning after negative experiences [35]. Meanwhile, neuroimaging studies have shown that structural abnormalities in these brain regions are commonly seen in patients with AN [36,37,38]. Given the regulatory effects of 5-HTTLPR on life stress, the aforementioned results suggested that changes in brain structure among patients with AN may be influenced by stressful experiences and 5-HTTLPR is likely to play an important role in this process.

As a secondary aim, this study examined whether 5-HTTLPR was associated with specific symptom clusters. Our sample was divided into three groups on the basis of subject genotype and EDE-Q score differences between subjects with the different genotypes were analyzed. Data obtained showed that the 5-HTTLPR polymorphism is not associated with the severity of AN. This seems to suggest 5-HTTLPR is not likely to facilitate the possibility of developing these psychopathological traits. AN is one of the most heritable complex disorders. Previous studies have illustrated the influence of dopaminergic and serotonergic system on the psychopathological features displayed by patients with AN [11,39,40,41]. With regard to serotonin genes, Gervasini et al. [42] proposed that polymorphisms in serotonin gene system and especially their epistatic interactions leading to low neurotransmitter activity may be associated with a higher severity of psychopathological traits in eating disorder patients. Several lines of evidence suggested that interaction of 5-HTTLPR and serotonin genes exert important biological effects in multiple psychiatric disorders [43,44,45,46]. Therefore, further studies are needed that include wider arrays of serotonin genes to fully ascertain the influence of 5-HTTLPR on the psychopathological traits of AN.

When interpreting the results of this study, we would be remiss in not noting some limitations. Aside from the small sample size used in this study, there are two potential limitations that are worth noting. First, while subjects were all of Chinese origin, we could not completely exclude the possibility of a population structure effect in our sample [47]. Second, the occurrence of AN—being a polygenic disorder—is widely known to depend on the interaction of multiple factors [48]. Usually no single gene is responsible for this disorder, and the methods used in individual studies may have limited power to detect what may be a potentially small effect. Third, the case-control portion and the family-based one were not independent. All probands from the family-based were also part of the case-control study. Last, the control subjects were recruited from hospital staff and students of the School of Medicine in Shanghai, even who were psychiatrically screened for mental disorder, and the samples were not representative of the general population.

In conclusion, our results presented herein indicated that 5-HTTLPR is able to confer susceptibility to AN in Han Chinese. For validation, further independent studies in larger samples are needed to confirm these preliminary observations and to establish other possible implications, such as the psychopathological traits of this disorder.

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Author Contributions

Conceived and designed the experiments: JC. Performed the experiments: JC. Analyzed the data: CZ. Contributed reagents/materials/analysis tools: SY. Wrote the paper: CZ. Contributed to sample collection: QK WJ JF MZ.

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