The Association Between the Serotonin Receptor Type 1B (*HTR1B*) Gene rs13212041 Polymorphism and Trait Anxiety in China Han College Subjects

Xiaofei Ruan
Shaanxi Normal University
Suwen Fang
Shaanxi Normal University
qi zheng
Shaanxi Normal University
Senqing Qi
Shaanxi Normal University
Yingfang Tian
Shaanxi Normal University
Wei Ren (renwei@snnu.edu.cn)
Shaanxi Normal University https://orcid.org/0000-0003-1862-413X

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Abstract

Trait anxiety is a vulnerable personality factor for anxiety and depression. High levels of trait anxiety confer elevated risk for the development of anxiety and other psychiatric disorders. There is evidence that serotonin receptor type 1B (5-HT1B) gene polymorphisms play an important role in emotional disorders. Genotyping for four single-nucleotide polymorphisms (SNP) (rs11568817, rs130058, rs6297 and rs13212041) was conducted for 388 high trait-anxious (HTA) individuals and 463 low trait-anxious (LTA) individuals in China Han college subjects. The results showed that the frequency of the C-allele and TC+CC genotype in rs13212041 in the LTA individuals was higher than that in the HTA individuals (p = 0.025 and p = 0.014, respectively). Both the C-allele and TC+CC genotype were associated with trait anxiety decreasing (OR = 0.771 and OR = 0.71, respectively). Furthermore, different gene models analysis also showed that the C allele is a protective factor in trait anxiety in Chinese Han college subjects. These findings suggest that the variance in 5-HT1B gene polymorphisms may play a role in trait anxiety in China Han college subjects. The rs13212014 polymorphism may be involved in decreasing the risk of trait anxiety. These results also provide a novel insight into the molecular mechanism about trait anxiety.

Introduction

Trait anxiety is defined as individual's disposition to experience frequent and intense anxiety, and worry in response to various stress situations. Individuals with HTA are considered to be more susceptible to clinical anxiety and depression[1,2], which refers to individuals with HTA as pre-existing forms of anxiety. A large number of behavioral studies have revealed that individuals with HTA exhibit cognitive and emotional disorders similar to anxiety disorders and behavioral biases, such as excessive uneasiness and concern about uncertain events on critical symptoms, threatening information, attention bias, persistent attention, low tolerance for uncertain information, and rejection or dislike of negative results or information[3-5]. The reasons for those results may be related to the information processing efficiency caused by anxious emotion[6]. Evidence from these studies indicates that individuals with high trait anxiety have similar behavioral performance compared to anxiety disorders. Most importantly, there is consistency that excessive concerns about the uncertainty point to the developmental link between high trait anxiety and anxiety disorders.

Serotonin is an important signaling molecule and neurotransmitter, which is widely distributed in the central nervous system and surrounding tissues. In recent years, molecular genetic studies have shown that dysfunction of the 5-HT is closely related to anxiety, depression, loss of appetite, sleeping nap, decreased activity, sexual dysfunction, endocrine function disorder, etc. 5-HT is involved in the regulation of various mental activities and closely related to psychiatric diseases. Some studies have shown that almost all serotonin receptor subtypes are involved in antidepressant or anxiolytic effects[7]. Serotonin receptor type 1B (5-HT1B receptors), an inhibitory G-protein coupled metabotropic receptor that decreases cAMP, is highly expressed in the striatum, pallidum, accumbens nucleus, substantia nigra and ventral tegmental area[8]. 5-HT1B receptor plays an important role in regulating serotonergic neurotransmission, it is reported that the function of 5-HT1B receptors were both presynaptically as inhibitory autoreceptors

located on terminals of serotonin neurons, and postsynaptically as inhibitory heteroreceptors controlling the release of other neurotransmitters[9]. Thus, 5-HT1B receptors are involved in depression, anxiety, migraine, locomotor activity, aggressive behavior, and potentiation of other drug's action[8,10].

Various animal studies have also demonstrated that 5-HT1B receptors play roles in anxiety-like and anxiolytic-like effect. *HTR1B* gene knockout mice exhibited reduced anxiety and hyperactivity [11]. Nautiyal and colleagues showed that the forebrain 5-HT1B heteroreceptors expressed during an early postnatal period contribute to the development of the neural systems underlying adult aggression, and proved that distinct heteroreceptors acting during adulthood were involved in mediating impulsivity[12]. Interestingly, mice lacking 5-HT1B autoreceptors presented decreased anxiety in the open field test [9]. Similarly, studies of Lin and Parsons[13] indicated that stimulation of 5-HT1B receptors increased anxiety-like behavior in the elevated plus maze test in rats, suggesting the role of this receptor subtype in the pathology and treatment of anxiety. Non-selective 5-HT1B/1D receptor agonist GR127935 also showed the anxiolytic-like properties[14]. The observed antianxiety-like effect might be linked to the postsynaptic 5-HT1B receptors or/and 5-HT1B heteroreceptors[15]. However, its exact role is yet unclear.

Previous human studies reported the associations between the different polymorphisms in the gene coding for 5-HT1B receptors and alcohol dependence[16-17], alcohol abuse[18], aggressive behavior [19-20], anger and hostility [21], ADHD[22], substance abuse[23], schizophrenia[24-25], anxiety and depression treatment[26-29]. The most frequently studied HTR1B gene variant is about rs6296, rs13212014, rs6297, rs11568817 and rs130058. Both rs11568817 and rs130058 are significantly associated with substance use disorders, while rs11568817 was associated with nicotine dependence in men with ADHD. Alcohol-dependent individuals with CC genotype were more frequent in the group with early onset of alcohol abuse compared to carriers of T allele[18]. The 3'-untranslated regions (3'-UTR) variant of rs13212041 potential enables the modulation of microRNA-mediated regulation expression of *HTR1B* gene[21]. The variant of rs6296, has been associated with attention-deficit hyperactivity disorder[30], aggressive behavior in children[31], substance misuse disorder and major depression[27].

These allelic associations with trait anxiety are not consistently found. The etiology and pathogenesis of trait anxiety are still largely unclear. It is commonly believed that trait anxiety is the result of different underlying neurobiological mechanisms, such as genetic and environmental influences. Thus, we hypothesized that *HTR1B* gene maybe involved in the development of trait anxiety.

To our knowledge, this is first study to examine the association between *HTR1B* gene polymorphism and trait anxiety in Chinese Han subjects. The results might provide novel insights into the serotonergic regulation mechanisms underlying trait anxiety, it could also help further differentiation of trait anxiety and potential improvement of the prediction for anxiety disorders.

Material And Methods

Participants

All participants provided written informed consent. The study protocol was approved by the ethics committee of Shaanxi Normal University. Participants in the study were China Han subjects, recruited from freshman or senior. Subjects were asked to provide venous blood samples and fill out State-Trait Anxiety Inventory (STAI) (Wenli Li,1995Spielberger et al.,1983). The number of participants that filled out State-Trait Anxiety Inventory (STAI) was 2645, then 2529 valid questionnaires were received. We filtered the top 25% of the participants as the HTA group (case) and last 25% of the participants as the LTA group (control) followed by the score of STAI by SPSS quartile method. At last, 851 participants with valid DNA and valid data were enrolled for our study, including 388 individuals with HTA and 463 individuals with LTA with age and gender matched. The classic case-control research paradigm to conduct in our research. Questionnaire test (post-test) was performed on the subjects before genotyping, according to the self-report of the participants, all the participants had no history of long-term medication, no symptoms of mental or neurological disorders.

Blood collection and DNA isolation

Peripheral blood samples (2ml) were obtained from each participant. Genomic DNA was extracted from peripheral blood of cases and controls using the GoldMag-Mini whole blood Genomic DNA Purification Kit (GoldMag Co.Ltd., Xi'an city, China), as recommended by the manufacturer's instructions. DNA concentration was determined by the NanoDrop Lite spectrophotometer (ThermoFisher Scientfic, Waltham,MA). The concentration of all DNA samples was normalized to 20 ng/ul.

SNP Selection

SNP inclusion and screening criteria are as follows: 1) according to the *HTR1B* polymorphism distribution, we selected SNPs with favorable polymorphisms (MAF≥0.1); 2) as the function of the *HTR1B* coding region has been studied extensively, SNPs located within *HTR1B* regulatory regions were selected 3) based on prior reports, polymorphisms in *HTR1B* gene which have been well studied in other mental disorders including alchol abuse[18], ADHD cormorbidities[22], anger and hostility[21], schizophrenia[25], but current knowledge of the association between trait anxiety and HTR1B polymorphisms in the Chinese Han population have not been well studied.. At last, four polymorphisms of *HTR1B* gene including rs11568817, rs130058, rs6297 and rs13212041 were selected in this study.

Genotyping

These four *HTR1B* gene polymorphisms were genotyped according to the procedure of iPLEX single base extension amplification technology. MassARRAY Nanodispenser (Agena Bioscience, San Diego, CA) was used to design primers for amplification process and single base extension reactions. SNP genotyping was carried out on the MassARRAY iPLEX (Agena Biosience, SanDiego, CA) platform. Agena Bionsience Typer 4.0 software was used to manage and analyze SNP genotypic data. iPLEX primer for *HTR1B* genotyping in this work as listed in the Supplementary file S1.

Data analysis

Quantitative data were shown as median ± standard deviation (SD). Student's t-test was used to compare the differences of quantitative data, and χ^2 test was applied for qualitative data comparison. Deviation from Hardy–Weinberg equilibrium (HWE) of genotypic distribution of each SNP in controls was analyzed using Fisher's exact test. In addition, the Pearson's χ^2 and Fisher's exact tests were used to calculate the allele frequencies of case and control, and MAF in controls was defined as baseline. After adjusting for age and gender, odds ratios (ORs) and 95% confidence interval (95% CI) were calculated using unconditional logistic regression analysis[32]. The relationship between the selected SNPs and trait anxiety was calculated using genotypic model analysis (codominant, dominant, recessive, over-dominant, and log additive) by (SPSS 21, Chicago, IL)[33]. The analyses, which included linkage disequilibrium (LD), haplotype construction, and genetic association at polymorphism loci, were performed using the Haploview software package (Haploview version 4.2). In the LD analysis, pairwise distance among SNPs 500 kb was ignored. D' value was used to evaluate the LD for each pair of SNPs, and variants with the red square indicated that the two related sites were in complete LD (D'=1). D' value equaling to 0.8 indicated that the related SNPs formed one block[34-35]. Haplotypes were constructed using SNPs in the same LD block, and haplotype frequency of > 0.05 was selected. Finally, four SNPs in *HTR1B* gene from each LD block was selected in the current study. In addition to statistical analysis, we also conducted a number of bioinformatics mining for the identified SNPs of *HTR1B* gene, the SNPs information of *HTR1B* gene was retrieved from the National Center for Biotechnology Information (NCBI) database of SNPs (dbSNP (http://www.ncbi.nlm.nih.gov/snp/). The bioinformatics tools including SIFT, PolyPhen, FATHMM, Mutation Accessor, UTRscan Server, MirSNP, PolymiRTS, miRNASNP were used to identify the potential functional SNPs in human *HTR1B* gene. Statistical analyses were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and SPSS (SPSS 21, Chicago, IL)) statistical package. In the study, all the *P*-value were two-sided, and $P < 0.05$ was defined as statistically significant.

Result

Clinical characteristics of samples

The characteristics of the enrolled participants are presented in Table 1. A total of 851 participants were enrolled in this study, including 463 individuals with HTA, and 388 individuals with LTA. The average STAI score was higher in the HTA group than the LTA group (t =-71.076, $P = 0.000$). There is no significant difference between the HTA and LTA group in terms of gender (t= 1.208, $P = 0.227$) or age (t= 0.414, $P= 0.230$).

Basic characteristic of SNP

Four SNPs in *HTR1B* gene including rs11568817, rs130058, rs6297 and rs13212041 (MAF≥0.1) were selected in this study. Basic characteristic of SNPs in the enrolled population are shown in Table 2. All the four SNPs were in HWE in the study ($P>0.05$).

Gene frequency analysis

A summary of allele and genotype frequencies is presented in Table 3. The differences in frequency distributions of alleles between case and control group were compared by Person's χ^2 test, rs13212041 polymorphism of the *HTR1B* gene was significantly different in HTA and LTA group (χ^2 = 6.071, *P* = 0.048), and the other three SNPs including rs130058, rs11568817, rs6297 had no significant associations with trait anxiety (*P* = 0.767, *P* = 0.384, *P* = 0.240 respectively). The allele "C" of rs13212041 from *HTR1B* gene was significantly associated with trait anxiety in the study population (OR = 0.771, 95% CI = 0.6144-0.9686, *P* = 0.025). The individuals carrying the "TC" genotype were significantly more frequent (OR = 0.703, 95%CI = 0.526-0.938, *P* = 0.014) in the subjects with LTA than those with HTA.

Association analysis

The association between SNPs genotypes and trait anxiety under various genetic model is shown in Table 4. Our analysis showed that crude analysis of rs13212041 was significantly associated with the developing of trait anxiety under the over-dominant model (OR = 0.72, 95% CI = 0.54-0.96, *P* = 0.024), log-additive model (OR = 0.77, 95% CI = 0.62-0.97, *P* = 0.025), and the dominant model (OR = 0.71, 95% CI = 0.54-0.93, *P* = 0.014).

LD and haplotype analysis

Via LD analysis, rs13212041 was found to be in strong linkage disequilibrium with rs130058, rs6297 and rs11568817 in Chinese Han students (r^2> 0.8). LD analysis showed that the four SNPs in 3'-UTR region are included in one block using the confidence interval method (Fig. 1). As shown in Table 5, haplotype analysis displayed that the four SNPs constructed five haplotypes ("ATTT", "ATCC", "ATCT", "CATT", and "CTTT"), furthermore, the haplotype "ATCT" in the *HTR1B* gene was significantly more frequent (OR = 0.71, 95% CI = 0.52 - 0.97, *P* = 0.032). According to gene frequency analysis, the C allele is a protective factor, therefore, the haplotype ATCT may be a protective haplotype in trait anxiety.

Bioinformatics analysis

SNP bioinformatics analysis showed that rs11568817 and rs130058 were located in 5'-promoter region, while the rs13212041 was located in the 3'-UTR region of *HTR1B* gene (the TF-CHIP sequence peak region), it may be influenced by some Transcription Factor (TF) of other genes like *FOS* gene which is an important component of AP-1 TF families, as shown by Fig.2. In addition, some research have reported that rs13212041 were located at the binding site of the miR-96 which changes the expression of HTR1B at Post-transcriptional Gene Silencing (PTGS) level[36]. The region of rs6296, rs11568817and rs130058, also have located some miRNA target site. Some studies have shown rs6297 (A1180G) was also located in the proximal region of the *HTR1B* 3'-UTR[37], but the site was not shown in this figure.

Discussion

5-HT1B receptor is a presynaptic and postsynaptic receptor widely distributed in the basal ganglia, hippocampus, and cortex. It plays important roles in multiple behavioral traits, such as locomotion,

feeding, and thermoregulation, and also in arterial contractile regulation mechanisms and has been the focus of much neuropsychiatric and neuropharmacological research[38]. The intronless human *HTR1B* gene, which is located at 6q14.3–q16.3(GDB 132312), encodes a 390-amino-acid polypeptide. Many polymorphisms in the coding sequence and UTRs were screened and multiple correlation studies were carried out in *HTR1B* gene[39]. To be the best of our knowledge, this is the first study reporting the association of SNPs polymorphism located in the *HTR1B* gene and trait anxiety.

In the present study, we investigated four SNPs polymorphism in *HTR1B* gene in 851 individuals of northern Han Chinese students, including 463 HTA individuals and 388 LTA individuals. According to previously reported observations, there is no functional study available for these SNPs including rs11568817, rs130058, rs6297 and rs13212041 with trait anxiety, but few studies have investigated their roles in other mental disorders. Evidence of association was found between the functional SNP (rs130058) and alcohol, cocaine, and heroin dependence[40]. The rs130058 SNP within the *HTR1B* gene were demonstrated to have a differential association with increasing suicidal ideation depending on antidepressant type[41]. Some contribution of the functional promoter combination (rs11568817, rs130058) were found with self-reported anger and hostility among young men[21]. The association between the three SNPs (rs11568817, rs130058, rs6297) and susceptibility to schizophrenia and anxiety disorders have not been reported[25]. According to previous studies only few studies investigated the association of rs13212041 *HTR1B* gene polymorphism with alcohol dependence [18], and Schizophrenia[24]. Our study showed consistent results regarding rs11568817, rs6297and rs130058, but we demonstrated that rs13212041 in the *HTR1B* gene was significantly associated with the personality developing of trait anxiety in the Chinese Han population. The frequency of the TC genotype in LTA individuals was significantly higher than in HTA individuals. Both the C-allele, TC genotype and TC + CC genotype were significantly associated with LTA. Thus, our study provides evidence that rs13212041 was involved in the protective effect of trait anxiety and can decrease the risk of high trait anxiety in China Han college subjects.

Evidence for a second functional regulatory variant was reported by Jensen et al.[42] who characterized the SNP (rs13212041; T1997C) occurring in the distal 3'-UTR of *HTRIB* messenger RNA that disrupts a binding site for the microRNA, miR-96. MicroRNAs are 20–21 nucleotide ribonucleic acids that regulate gene expression by binding to complementary sites on messenger RNA triggering mRNA degradation and/or inhibition of translation[36, 43]. Jensen et al. showed that the rs13212041 polymorphism modulates miR-96 regulation of gene expression. The T-allele mRNA was repressed by miR-96, while the C-allele attenuated this miR-96 regulatory function. Our research is consistent with previous research results. “TC” genotype and C allele of *HTR1B* rs13212041 were significantly associated with low trait anxiety. We presumed “TC” genotype and C-allele polymorphism could disrupt 5-HT1B receptor expression by miR-96, the rs13212041 C-allele attenuated microRNA-mediated downregulation of gene expression relative to the T-allele. This classification, combined with the putative transcriptional and microRNA-mediated mRNA translation/stability effects contributed by the two separate functional polymorphisms. Indeed, the C-allele of rs13212041 appeared to drive the dominant protective effect of

trait anxiety. Maybe this pattern suggests that the microRNA-binding site polymorphism has great behavioral effects.

In addition, 5-HT1B receptors play an important role as inhibitory autoreceptors or heteroreceptors on both serotonergic and non-serotonergic neurons, and modulate serotonergic activity. The role of 5-HT1B receptors might differ depending upon their specific location[44]. In addition, activation of presynaptic 5-HT1B receptors inhibits 5-HT release, and 5-HT1B postsynaptic heteroreceptors are involved in the modulation of addictive behaviors[8]. In agreement with the results of previous studies, 5-HT1B receptors participate in the anxiolytic-like effect, here we provided genetic evidence that 5-HT1B receptor is associated with trait anxiety personality.

From the results of bioinformatics analysis, we also found the rs13212041 maybe changed the TFBS of FOS of downstream gene. FOS genes encode leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1, which can regulate expression of the *HTR1B* gene [43] Although rs13212041 is located in the 5'-untranslated region of the *HTR1B* gene, this SNP could influence TFBS of *FOS* gene, indirectly regulate the downstream gene expression and affect the expression of *HTR1B* gene through gene expression network. The specific mechanism needs to be demonstrated further.

Limitations

One of the study limitations is a lack of careful determination of trait anxiety phenotypes, using Nuclear Magnetic Resonance (NMR), Electroencephalogram (EEG) and other effective anxiety laboratory indicators. However, effective and rigorous scale data and genes type association analysis has shown that rs13212041 is a susceptible site for anxiety, which lays a good working foundation for subsequent in-depth experimental molecular research.

Conclusion

Our study provides a new perspective for understanding the genetic mechanism of trait anxiety personality formation. As far as we know, this is the first study reporting the association of rs13212041 polymorphism located in the *HTR1B* gene coding for 5-HT1B receptor with trait anxiety. Our results suggest that individuals with C-allele are more likely to protect subjects without the risk of high trait anxiety than carriers with T-allele. As rs13212041 polymorphism affects microRNA regulation of *HTR1B* gene expression, these data might suggest the involvement of epigenetic mechanisms in the modulation of serotonergic functions in trait anxiety individuals. Our findings provide novel insights into the serotonergic regulation mechanisms underlying the personality of trait anxiety, which could help in further differentiation of trait anxiety and potential improvement of the therapy for avoiding anxiety disorder.

Declarations

Ethics approval and consent to participate: Approval was obtained from the ethics committee of Shaanxi normal University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Consent for publication: Publication consent was obtained from all individual participants included in the study.

Availability of data and materials: The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors report no conflicts of interest.

Authors' contributions: Methodology and Writing original draft: Xiaofei Ruan; Sample collection: Suwen Fang; Data collection: Qi Zheng; Formal analysis: Senqin Qi; Conceptualization and Supervision: Yingfang Tian; Resources and Supervision: Wei Ren.

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Tables

Table 1 Clinical characteristics of all samples

	LTA	HTA	t	*P*-Value
Gender	463	388	1.208	0.227
Male	86	85		
Female	377	303		
Age (SD)	19.11±2.50	19.04±2.45	0.414	0.230
STAI	31.39±4.11	54.27±5.28	-71.076	0.000

HTA high trait-anxious, *LTA* low trait-anxious

Table 2 Basic characteristic of SNPs in the enrolled population

rs#	Chr.pos	Gene	Region	Minor Allele	(MAF)%		*P*-valuefor HWE test	
					HTA	LTA	HTA	LTA
rs13212041	6:77461407	*HTR1B*	3'-UTR	C	20.88	12.31	0.356	0.666
rs6297	6:77462224	*HTR1B*	3'-UTR	C	11.21	25.49	1.000	0.713
rs130058	6:77463564	*HTR1B*	5'-UTR	A	9.92	8.21	0.781	0.757
rs11568817	6:77463665	*HTR1B*	5'-UTR	C	13.02	10.79	0.501	0.633

MAF minor allele frequency, *HWE* Hardy-Weinberg Equilibrium, *HTA* high trait-anxious group, *LTA* low trait-anxious group

Table 3 Association between SNPs and trait anxiety

SNP	Control n (%)	Case n(%)	χ^2	ORs(95%CI)	*P*-value
rs13212041					
Genotype			6.071	-	*0.048**
TT	255 (55.08)	246 (63.40)		1	-
TC	180 (38.88)	122 (31.44)	5.750	0.703 (0.526-0.938)	*0.019**
CC	28 (6.05)	20 (5.15)	0.970	0.740 (0.406-1.349)	0.366
Allele					
T	690 (74.51)	614 (79.12)		1	-
C	236 (25.49)	162 (20.88)	5.007	0.771 (0.614-0.969)	*0.025**
rs6297					
Genotype			0.530	-	0.767
TT	357 (77.11)	306(78.87)		1	-
TC	98 (21.17)	77 (19.85)	0.259	0.917 (0.656-1.282)	0.670
CC	8 (1.73)	5 (1.29)	0.304	0.729 (0.236-2.252)	0.780
Allele					
T	812 (87.69)	689 (88.79)		1	-
C	114 (12.31)	87 (11.21)	0.490	0.899(0.668-1.21)	0.484
rs130058					
Genotype			1.914	-	0.384
TT	389 (84.02)	315 (81.19)		1	
TA	72 (15.55)	69 (17.78)	0.724	0.479 (0.085-2.701)	0.395
AA	2 (0.43)	4 (1.03)	1.156	0.405 (0.074-2.225)	0.282
Allele					
T	850 (91.79)	699 (90.08)		1	-
A	76 (8.21)	77 (9.92)	1.518	1.232(0.884-1.718)	0.218
rs11568817					
Genotype			2.853	-	0.240
AA	367 (79.27)	295 (76.03)		1	-

Table 3 (continued)

SNP	Control n (%)	Case n(%)	X^2	ORs(95%CI)	*P*-value
AC	92 (19.87)	85 (21.91)	1.563	0.462 (0.134-1.590)	0.245
CC	4 (0.86)	8 (2.06)	2.327	0.402 (0.120-1.348)	0.127
Allele					
A	826 (89.20)	675 (86.98)		1	-
C	100 (10.79)	101 (13.02)	1.991	1.236(0.921-1.659)	0.158

SNP single nucleotide polymorphism, *CI* confidence interval; *OR* odds ratio Notes: *P*-value were calculated by logistic regression adjusted for age and gender. *P<0.05. Case, the high trait anxiety group; Control: the low trait anxiety.

Table 4 Association between genotypes (rs11568817, rs130058, rs6297 and rs13212041) trait anxiety

rs#	Group		χ^2	Adjust analysis	
	Control (%)	Case (%)		ORs(95%CI)	*P*-value
rs13212041					
Codominant model			-		
T/T	255 (55.1)	246 (63.4)		1.00	-
T/C	180 (38.9)	122 (31.4)	5.750	0.70 (0.53-0.94)	*0.019**
C/C	28 (6.0)	20 (5.2)	0.970	0.74 (0.41-1.35)	0.366
Dominant model					
TT	255 (55.1)	246 (63.4)	-	1.00	-
T/C+C/C	208 (44.9)	142 (36.6)	6.044	0.71 (0.54-0.93)	*0.014**
Recessive model					
T/T+T/C	435 (94.0)	368 (94.8)	-	1.00	-
C/C	28 (6.0)	20 (5.2)	0.316	0.84 (0.47-1.52)	0.057
Overdominant model					*0.024**
T/T+C/C	283 (61.1)	266 (68.6)	-	1.00	
T/C	180 (38.9)	122 (31.4)	5.095	0.72 (0.54-0.96)	
log-Additive model					
0,1,2	463 (54.4)	388 (45.6)	5.007	0.77 (0.62-0.97)	*0.025**
rs6297					
Codominant model					0.766

Table 4 (continued)

rs#	Group		χ^2	Adjust analysis	
	Control (%)	Case (%)		ORs(95%CI)	*P*-value
T/T	357 (77.1)	306(78.9)	-	1.00	-
T/C	98 (21.2)	77(19.8)	0.259	0.92 (0.66-1.28)	0.611
C/C	8 (1.7)	5(1.3)	0.304	0.73 (0.24-2.25)	0.582
Dominant model					
TT	357 (77.1)	306(78.9)	-	1.00	-
T/C+C/C	106 (22.9)	82(21.1)	0.380	0.90 (0.65-1.25)	0.537
Recessive model					
T/T+T/C	455 (98.3)	383(98.7)	-	1.00	
C/C	8 (1.7)	5(1.3)	0.742	0.74 (0.24-2.29)	0.600
Overdominant model					
T/T+C/C	365 (78.8)	311 (80.2)	-	1.00	-
T/C	98 (21.2)	77 (19.8)	0.225	0.92 (0.66-1.29)	0.635
log-Additive model					
0,1,2	463 (54.4)	388 (45.6)	0.490	0.90 (0.67-1.21)	0.486
rs130058					
Codominant model			---		
T/T	389(84)	315(81.2)	-	1.00	0.383
A/T	72(15.6)	69(17.8)	0.833	1.18 (0.82-1.7)	0.362
A/A	2(0.4)	4(1)	1.156	2.47 (0.45-13.56)	0.282

Table 4 (continued)

rs#	Group		χ^2	Adjust analysis	
	Control (%)	Case (%)		ORs(95%CI)	*P*-value
Dominant model					
T/T	389(84)	315(81.2)	-	1.00	-
A/T+A/A	74(16)	73(18.8)	1.185	1.22 (0.85-1.74)	0.277
Recessive model					
T/T+A/T	461(99.6)	384(99)	-	1.00	-
A/A	2(0.4)	4(1)	1.082	2.4 (0.44-13.17)	0.297
Overdominant model					
T/T+A/A	391(84.4)	319(82.2)	-	1.00	-
A/T	72(15.6)	69(17.8)	0.761	1.17 (0.82-1.69)	0.384
log-Additive model					
0,1,2	463(54.4)	388(45.6)	1.518	1.24 (0.88-1.73)	0.216
rs11568817					
Codominant model					0.239
A/A	367(79.3)	295(76)	-	1.00	-
C/A	92(19.9)	85(21.9)	0.675	1.15(0.82-1.6)	0.411
C/C	4(0.9)	8(2.1)	0.237	2.49(0.74-8.34)	0.127
Dominant model					
A/A	367(79.3)	295(76)	-	1.00	-
A/C-C/C	96(20.7)	93(24)	1.278	1.21(0.87-1.67)	0.259

Table 4 (continued)

rs#	Group		χ^2	Adjust analysis	
	Control (%)	Case (%)		ORs(95%CI)	*P*-value
Recessive model					
A/A-A/C	459(99.1)	380(97.9)	-	1.00	-
C/C	4(0.9)	8(2.1)	2.719	2.42(0.72-8.08)	0.139
Overdominant model					
A/A-C/C	371(80.1)	303(78.1)	-	1.00	-
A/C	92(19.9)	85(21.9)	0.532	1.13(0.81-1.58)	0.466
log-Additive model					
0,1,2	463(54.4)	388(45.6)	1.991	1.24(0.92-1.66)	0.159

SNP, single nucleotide polymorphism*; CI* confidence interval, *OR* odds ratio; Notes:**P* < 0.05 indicates statistical significance. *P*-values were calculated by two-sided χ^2 tests or Fisher's exact tests for each genotype distribution by unconditional logistic regression adjusted for age, gender. Case, the HTA group; Control: the LTA group.

Table 5 Haplotype association with response

rs11568817	rs130058	rs13212041	rs6297	Freq	OR (95% CI)	*P*-value
A	T	T	T	0.6481	1.00	—
A	T	C	C	0.1181	0.87 (0.64 - 1.18)	0.37
A	T	C	T	0.1157	0.71 (0.52 - 0.97)	*0.032**
C	A	T	T	0.0899	1.15 (0.82 - 1.62)	0.42
C	T	T	T	0.0282	1.14 (0.63 - 2.05)	0.66

**P* < 0.05 indicates statistical significance

Figures

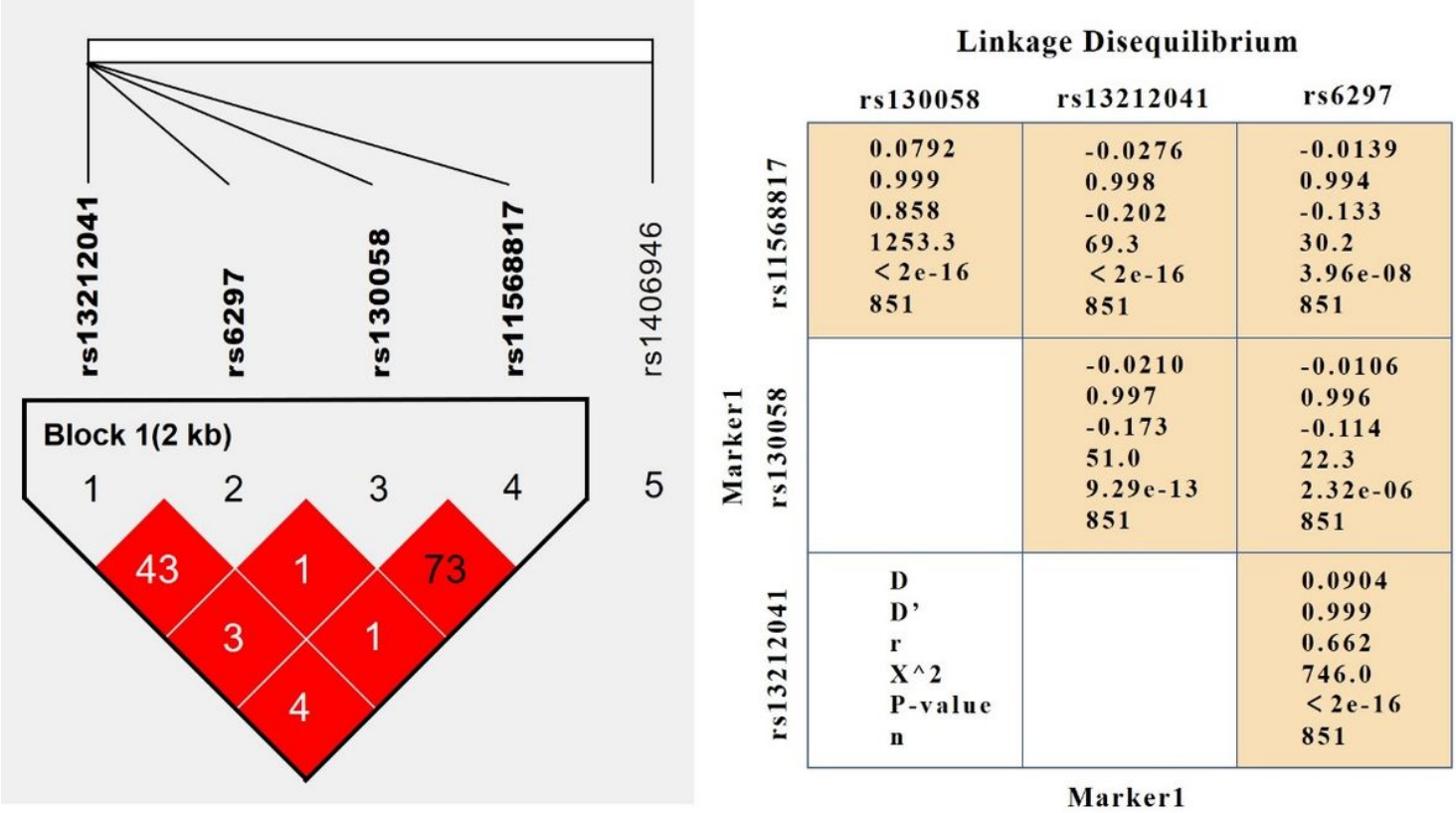

Figure 1

LD plots containing four SNPs from HTR1B gene. LD analysis of HTR1B gene in the Chinese Han students. LD is indicated using standard color schemes with red signifying very strong LD (r2= 1). LD linkage disequilibrium, LOD likelihood of odds, SNP single nucleotide polymorphism

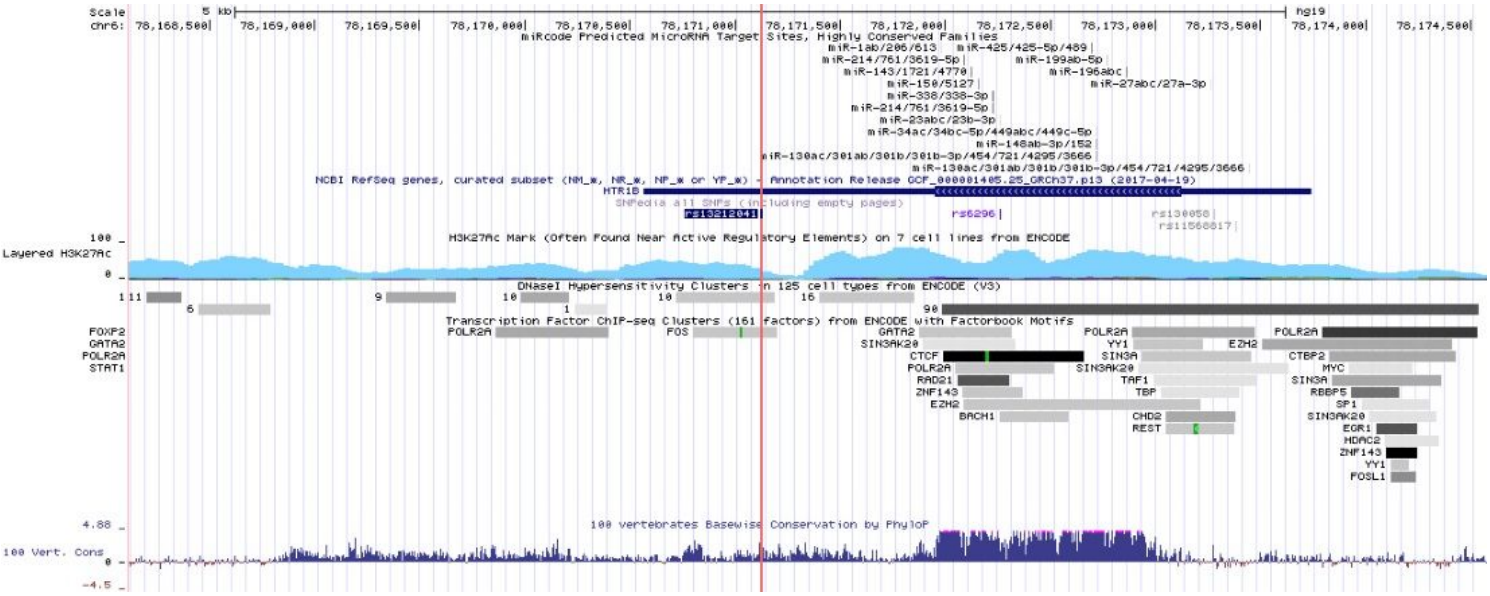

Figure 2

Bioinformatics analysis of HTR1B gene and identified SNPs. The location of SNPs in HTR1B gene are schematically noted.

Supplementary Files

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