Int J Clin Exp Med 2015;8(10):18837-18842
www.ijcem.com /ISSN:1940-5901/IJCEM0013121

Original Article

Association between polymorphism of the norepinephrine transporter gene rs2242446 and rs5669 loci and depression disorders

Yu Pan[1,2*], Qi Cheng[3*], Mo-Shui Shan[4*], Jin Yan[1]

*[1]Department of Psychology and Mental Health, Second Military Medical University, Shanghai, China; [2]Department of Medical Psychology, General Hospital of PLA, Beijing, China; [3]Department of Juvenile and Children's Behavioral Medicine, 102 Hospital of PLA, Changzhou, China; [4]Department of 215 Clinical, Sleeping Center, Dalian Nursing Home, Dalian, China. *Equal contributors.*

Received July 19, 2015; Accepted September 3, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: Objective: To explore the association between polymorphism of the norepinephrine transporter (NET) gene rs2242446 and rs5669 loci and depression in Chinese Han population. Methods: A case-control study was carried out, the gene types and allele distributions of NFT gene rs2242446 and rs5569 loci in 302 depression patients and 302 healthy controls were detected by Taqman SNP genotyping technology. Results: The gene types and allele frequency distributions of NFT gene rs2242446 and rs5569 loci had significant differences between case group and control group (rs2242446, $x^2=26.045$, $P<0.05$, $x^2=8.827$, $P<0.05$, rs5569, $x^2=42.47$, $P<0.05$, $x^2=20.9$, $P<0.05$). The CC genotype of NET gene rs2242446 locus and rs5569 loci was a protective factor of depression compared with the CT and TT genotypes. Conclusion: The NET genepoly morphism of rs2242446 and rs5569 loci was a ssociated with depression in Chinese Han population, in which the CC genotype of rs2242446 and rs5569 loci was a protective factor of depression.

Keywords: Depression, norepinephrine transporter gene

Introduction

Depression is a kind of common mental disorders, and its mainly clinical symptom is lasting mood depression, which may develop from unhappiness, grief to inferiority and even suicide attempt or suicidal behavior. According to WHO statistics, the disease burden of depression will be ranked second and account for 5.7% of the total disease burden in 2020 [2]. Because of the high prevalence, high suicidal rate and high disease burden of depression, it has become a globally concerned socio-economic issue [1]. The annual incidence of depression is about 3%-5% in China, of which 10%-15% patients have suicidal tendency, and the recurrence rate rises with the increased prevalence of illness [2]. Experts predict that depression is likely to become the second major disease with huge disease burden after cardiovascular disease.

The prevention and treatment of depression have become one of the hot topics in the society, of which the etiology mechanism is the key of the hot researches. Studies show that there is obvious genetic predisposition to depression, involving three major gene system, and the candidate genes mainly include serotonin (5-HT), dopamine (DA) and norepinephrine (NE) [3-7]. Norepinephrine transporter (NET) has been widely concerned due to its role in the treatment of antidepressant drugs [11-14]. However, with regard to the NET gene, whether it serves as the pathogenic gene for depression has been controversial. The study of Zill P, Owen D, et al. shows that the NET gene has no correlation with depression, but the result of Sun et al. is opposite [8-10]. This study was a case control study, which included Chinese Han population as the subjects and compared the NET gene polymorphism of rs5569 and rs2242446 loci between case group and control group, aiming to explore association between the NET gene

Table 1. The age and sex distributions of patients in case group and control group

Groups	N	Age (χ±s)	Sex	
			Males	Females
Case group	302	32.2±10.4	148	154
Control group	302	30.5±10.7	150	152
t/X^2		-1.9	0.026	
P		*P*>0.05	P>0.05	

polymorphism of rs2242446 and rs5669 loci with depression.

Subjects and methods

Subjects

The patient group: Patients who visited outpatient department in the Second Military Medical University from April 2013 to April 2015 were recruited. The inclusion criteria of depression were based on the Fourth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). All the enrolled patients were Chinese Han population and the scores of Hamilton Depression Scale (HAMD) (17 item version) were greater than 17 points. The exclusion criteria were as follows: genetic diseases; mental disorders due to severe physical disease; secondary mental disorders caused by other various causes. After oral informed consent, all the patients were voluntary to participate in this study.

The control group: We recruited health control subjects who underwent health examination in Physical Examination Centerin Ahospital from April 2013 to April 2015. All the subjects were Chinese Han in nationality, without history of mental disorders and family history of mental disorders. They were all voluntary to participate in this study.

Methods

DNA extraction: 2 ml venous blood of subjects were extracted with EDTA anticoagulant tube. Qiagen reagent kit (Germany) was used to extract the DNA according to the instructions.

The detection of NET gene polymorphism of rs5569 and rs2242446 loci: The allele genes of rs5569 and rs2242446 were detected by TaqMan SNP gene typing technique, reacted by standard gene SNP typing analysis reagent (Taqman^R SNP genotyping Assays, ID: 43513-79). The PCR reaction system was 5 ul, which contained 5 ng DNA template, and it was placed in a 384-well plate for reaction. The reaction conditions of PCR were as follows: pre degenerationat 95°C for 10 min, denaturation at 92°C for 15 s, annealing at 60°C for 1 min, 45 cycles in total.

Statistical analysis

All data were analyzed with SPSS16.0 software. The differences of genotype and allele frequency among the groups were compared by X^2 test.

Results

Comparison of the demographic data and gene typing of the depression case group and the control group

This study enrolled 302 cases, including 148 males and 154 females, who were aged from 18 to 65 years. A total number of 302 health controls were recruited, including 150 males and 152 females, who were aged from 18 to 63 years. T-test and X^2 test were employed to compare the age and sex distributions between case group and control group, and it was found that the differences were not statistically significant (**Table 1**).

Taqman SNP genotyping results indicated that the NET gene polymorphism of the rs2242446 and the rs5569 lociwere shown in **Figures 1** and **2**. In case group, CC genotype, TT genotype, and TT genotype of rs5569 accounted for 30%, 50%, and 20%, respectively, while in control group, CC genotype, TT genotype, and TT genotype of rs5569 accounted for 56%, 34% and 10%, respectively. In control group, CC genotype, TT genotype, and TT genotype of rs2242446 accounted for 6%, 40% and 54%, respectively, while in control group, CC genotype, TT genotype, and TT genotype of rs2242446 accounted for 20%, 35% and 45%, respectively.

Hardy-Weinberg genetic equilibrium test

Hardy-Weinberg genetic equilibrium was tested by X^2 test, and the results showed that the gene distributions of rs5569 and rs2242446 in case group had no significant differences with the theoretical distributions of Hardy-Weinberg

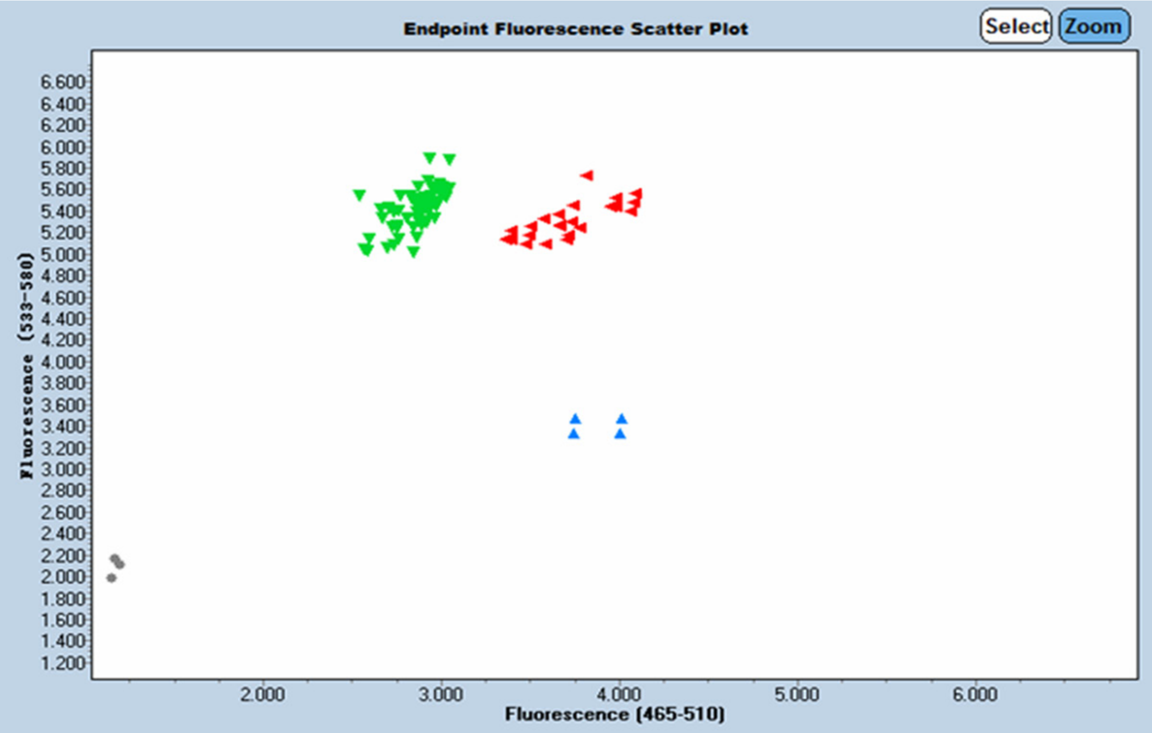

Figure 1. The genotyping map of NET gene rs2242446 locus (Blue: CC genotype; Red: CT genotype; Green: TT genotype).

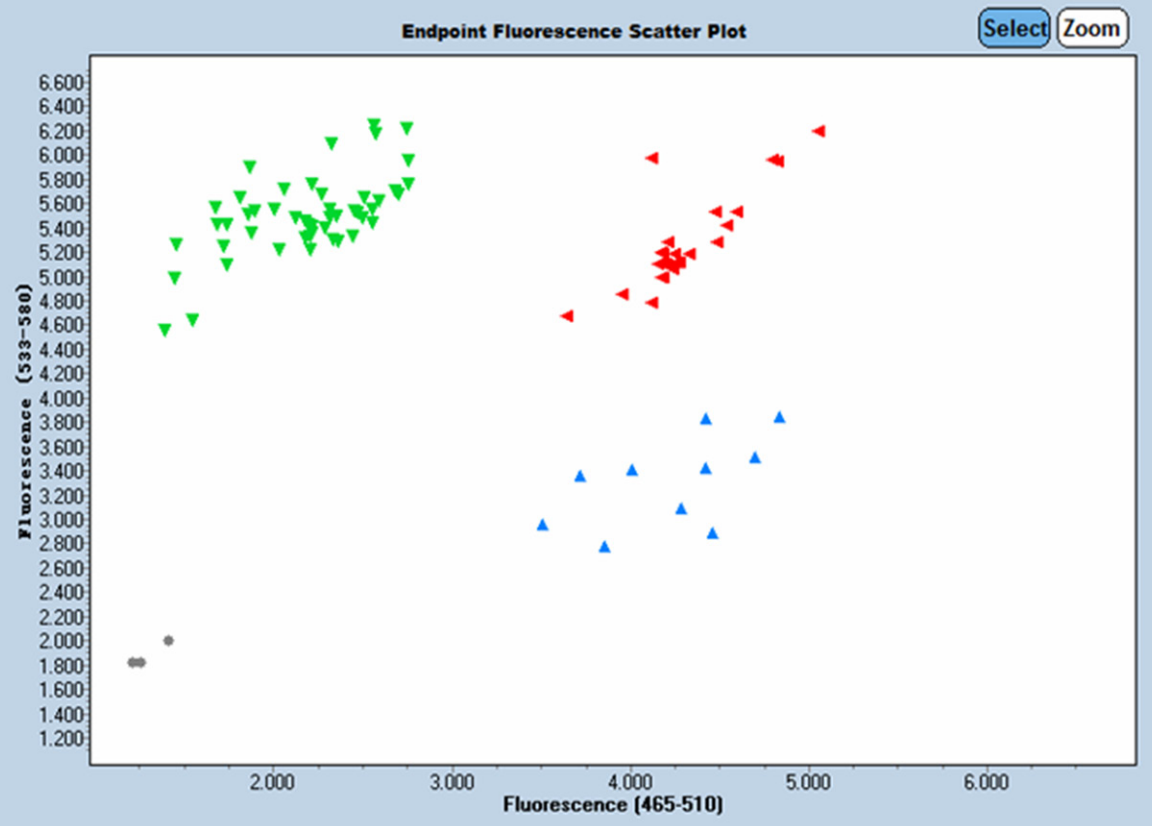

Figure 2. The genotyping map of NET gene rs5569 locus (Blue: TT genotype; Red: CT genotype; Green: CC genotype).

Table 2. The genotype and allele frequency distribution of rs2242446 in case group and control group

Groups	N	Allele (%)		Genotype (%)		
		C	T	CC	CT	TT
Case group	302	79 (26.0)	223 (74.0)	18 (6.0)	121 (40.0)	163 (54.0)
Control group	302	113 (37.5)	189 (62.5)	60 (19.9)	106 (35.1)	136 (45.0)

Note: Take CC as reference, the OR values of TC and TT were separately 0.26 (95% CI 0.15-0.47, $X^2=-0.27$, $P<0.05$) and 0.25 (95% CI 0.14-0.44, $X^2=-0.26$, $P<0.05$).

Table 3. The genotype and allele frequency distribution of rs5569 in case group and control group

Groups	N	Allele (%)		Genotype (%)		
		C	T	CC	CT	TT
Case group	302	166 (55.0)	136 (45.0)	91 (30.1)	151 (50)	60 (19.9)
Control group	302	220 (72.8)	82 (27.2)	169 (56.0)	103 (34.1)	30 (9.9)

Note: Take CC as reference, the OR values of TC and TT were separately 0.37 (95% CI 0.26-0.53, $X^2=-0.25$, $P<0.05$) and 0.27 (95% CI 0.16-0.45, $X^2=-0.28$, $P<0.05$).

genetic equilibrium (rs5569, $X^2=0.043$, $P>0.05$; rs2242446, $X^2=0.59$, $P>0.05$), and the observed values of rs5569 and rs2242446 gene in control group were consistent with the expected values (rs5569, $X^2=5.5$, $P>0.05$; rs2242446, $X^2=19.8$, $P<0.05$). In addition to rs2242446 gene of control group, the subjects of other groups were representative of the population.

Distribution of rs2242446 gene polymorphism in the case group and the control group

The rs5569 gene polymorphism among subjects in the study was as follows. In case group, the gene frequency of CC genotype, CT genotype and TT genotype accounted for 6%, 40%, and 54%, respectively; While in the control group, the CC genotype, CT genotype and TT genotype accounted for 20%, 35%, and 45%, respectively. There were significant differences of the gene frequency and allele frequency distribution between case group and control group ($X^2=26.045$, $P<0.05$, $X^2=8.827$, $P<0.05$), as shown in **Table 2**.

The distribution of rs5569 gene polymorphism in case group and control group

The rs5569 gene polymorphism of subjects in this study was as follows: The gene frequency of the CC genotype, CT genotype and TT genotype accounted among case group accounted for 30%, 50%, and 20%, respectively. Whilst in control group, the CC genotype, CT genotype and TT genotype accounted for 56%, 34% and 10%, respectively. There were significant differences of the gene frequency and allele frequency distribution between case group and control group ($X^2=42.47$, $P<0.05$; $X^2=20.9$, $P<0.05$) (**Table 3**).

Association between rs2242446 and rs5569 gene polymorphism and depression

The study found that the CC genotype of rs2242446 and rs5569 gene was a protective factor for depression. By setting CC genotype as the reference gene and calculating the risk degree of CT and TT genotype, the OR values of rs2242446 were 0.26 (95% CI 0.15-0.47, $X^2=-0.27$, $P<0.05$) and 0.25 (95% CI 0.14-0.44, $X^2=-0.26$, $P<0.05$), respectively. The OR values of rs5569 were 0.37 (95% CI 0.26-0.53, $X^2=-0.25$, $P<0.05$) and 0.27 (95% CI 0.16-0.45, $X^2=-0.28$, $P<0.05$), respectively.

Discussion

Depression is a disease with complex etiology and higher genetic predisposing, which severely reduces the people's quality of life and makes them have suicidal tendency. It has a large proportion of disease burden and becomes a common social and economic problem in China. The possible pathogenic factors of depression include genetics, social psychology and personality, but the exact cause is not clear [15]. A large number of studies have demonstrated that NEdys function is closely related to severe depression, and NET can reabsorb synaptic norepinephrine to maintain the body noradrenaline level. Therefore, the imbalance of norepinephrine caused by NET level changes in brain may be associated with the occurrence of depression. As a result, NET gene is one of the three major genes in the genetic etiology of depression, which is quite important in the etiology study of depression.

NET gene contains 14 exons, about 45 kb [16]. Rs5569 and rs2242446 gene are located at exon 9 1287G/A of NET gene, which is a silent mutation. The significance of this locus to depression has been controversial, which was a hot topic in the world during the recent years. We performed a case-control study and aimed to investigate the variation of rs5569 and rs2242446 gene polymorphism distribution between case group and control group, and find the potential association between rs5569 and rs2242446 gene polymorphism and depression.

The results showed that the genotype distribution and allele frequency of NET gene rs5569 and rs2242446 loci had significant differences between case group and control group, suggesting that both genes were indeed correlated with depression. This study also found that CC genotype of rs5569 and rs2242446 gene was a protective factor for depressive episodes, consistent with the conclusion drawn from Yu Yan et al.'s study that was performed on Han population of south China, but contrary to the results of RyuSH et al.'s study conducted in Korea [17, 18]. Therefore, more systematic studies with larger sample size are still required to determine the correlation between the NET gene and depression.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jin Yan, Department of Psychology and Mental Health, Second Military Medical University. No. 800, Xiangyin Road, Shanghai 200433, China. Tel: +8602125070351; Fax: +8602125070351; E-mail: yanjiun975@126.com

References

[1] Spiessl H, Hubner-Liebermann B, Hajak G. Depression, a widespread disease: epidemiology, care situation, diagnosis, therapy and prevention. Dtsch Med Wochenschr 2006; 131: 35-40

[2] Kautto M, Kampman O, Mononen N, Lehtimäki T, Haraldsson S, Koivisto PA, Leinonen E. Serotonin transporter (5-HTTLPR) and norepinephrine transporter (NET) gene polymorphisms: susceptibility and treatment response of electroconvulsive therapy in treatment resistant depression. NeurosciLett 2015; 590: 116-20.

[3] Nobile M, Cataldo Mq, Gioula R, Battaglia M, Baschirotto C, Bellina M, Marino C, Molteni M. A case-control and family-based association study of the 5-HTTLPR in pediatric-onset depressive disorders. Biol Psychiatry 2004; 56: 292-295.

[4] Hamnlen C, Shih JH, Brennan PA. Intergenerational transmission of depression: test of an interpersonal stress model in a community sample. J Consult Clin Psyehol 2004; 72: 511-522.

[5] Dindo L, Coryel IW. Comorbid major depression and panic disorder: significance of temporal sequencing to familial transmission. J Affeet Disord 2004; 82: 119-123.

[6] Liu X, Zheng P, Zhao X, Zhang Y, Hu C, Li J, Zhao J, Zhou J, Xie P, Xu G. Discovery and validation of plasma biomarkers for major depressive disorder classification based on liquid chromatography-mass spectrometry. J Proteome Res 2015; 14: 2322-30.

[7] Telch MJ, Beevers CG, Rosenfield D, Lee HJ, Reijntjes A, Ferrell RE, Hariri AR. 5-HTTLPR genotype potentiates the effects of war zone stressors on the emergence of PTSD, depressive and anxiety symptoms in soldiers deployed to iraq. World Psychiatry 2015; 14: 198-206.

[8] Zill P, Engel R, Baghai TC, Juckel G, Frodl T, Müller-Siecheneder F, Zwanzger P, Schüle C, Minov C, Behrens S, Rupprecht R, Hegerl U, Möller HJ, Bondy B. Identification of a naturally occurring polymorphism in the promoter region of the norepinephrine transporter and analysis in major depression. Neuropsychopharmacology 2002; 26: 489-493

[9] Sun N, Xu Y, Wang Y, Duan H, Wang S, Ren Y, Peng J, Du Q, Shen Y, Xu Q, Zhang K. The combined effect of norepinephrine transporter gene and negative life events in major depression of Chinese Han population. J Neural Transm 2008; 115: 1681-168

[10] Owen D, Du L, Bakish D, Lapierre YD, Hrdina PD. Norepinephrine transporter gene polymorphism is not associated with susceptibility to major depression. Psychiatry Res 1999; 87: 1-5.

[11] Dencker SJ. The need for new and better antideressants: Reboxetine a new option. A cta Psychiatr Scand Suppl 2000; 402: 6.

[12] Yoshida K, Takahashi H, Higuchi H, Kamata M, Ito K, Sato K, Naito S, Shimizu T, Itoh K, Inoue K, Suzuki T, Nemeroff CB. Prediction of Antidepressant Response to Milnacipran by Norepinephrine Transporter Gene Polymorphisms. Am J Psychiatry 2004; 161: 1575.

[13] Charles U, Nnadi Joseph F, Malhotra AK. Pharmacogenetics in mood disorder. Curr Opin Psychiatry 2005; 18: 33.

[14] Dziedzicka-Wasylewska M, FaronGorecka A, Kusmider M, Drozdowska E, Rogóz Z, Siwanowicz J, Caron MG, Bönisch H. Effect of Antidepressant Drugs in Mice Lacking the

Norepinephrine Transporter. Neuropsychopharmacology 2006; 31: 2424.

[15] Sun N, Xu Y, Sun JW. Norepinephrine transporter gene associated with Chinese Han population and analysis of major depression. ZhongGuo Shen Jing Ji Bing ZaZhi 2007; 33: 416-419.

[16] Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001; 68: 978.

[17] Yu Y, Zhao JP, Yang D, et al. Association studies norepinephrine transporter gene polymorphism and Chinese Han population in southern major depressive disorder. Chinese Journal of Clinical Psychology 2012; 20: 29-31.

[18] Ryu SH, Lees H, Lee HJ, Cha JH, Ham BJ, Han CS, Choi MJ, Lee MS. Association between Norepinephne Transporter Gene Polylnorphism and Major Depression. Neuropsyehobiology 2004; 49: 174-177.