A Genetic Susceptibility Mechanism for Major Depression
Combinations of polymorphisms Defined the Risk of Major Depression and Subpopulations

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INTRODUCTION

Major depression (MD) is a highly inherited psychiatric disorder. At present, the pathogenesis of MD has remained unclear. Family, twin, and adoption studies suggested that genetic contribution to the disease is one of the main etiological factors. The heritability of MD is about 60%.1–3 In the prevailing pathogenic model, MD is a disorder with abnormal synaptic connectivity in which Monoamine neurotransmission systems are involved. Some studies also showed that the dysfunction of norepinephrine (NE) neurotransmission is an important hypothesis for the pathogenesis of MD.4 Studies of NE metabolites showed decreased urinary levels of 3-methoxy-4-hydroxyphenylglycol, the major metabolite of NE in depressive states of unipolar patients, and antidepressant treatment could cause decreased NE turnover.5–9

The norepinephrine transporter (NET) is a major target for antidepressant drugs such as serotonin noradrenalin reuptake inhibitors (SNRI), and selective NE reuptake inhibitor (NRI). According to the clinic therapeutic effects of antidepressant drugs, NET might play important roles in pathophysiology and pharmacological treatment of MD, and has become one of the attractive candidate genes in MD research.9–14 As a Na+/Cl− -dependent substrate-specific transporter, NET is a 617-amino acid protein and contains 12 cross membrane sectors. NET gene (SLC6A2) is located on chromosome 16q12.2, and it spans approximately 45 kb and consists of 14 exons (protein coding regins).15 Till now, studies of NET mainly focused on the 5′ flanking promoter region T-182C polymorphism16 and the silent polymorphism G1287A, located in exon 9,17 but the relationship between polymorphisms of NET gene and MD remained unclear. The current findings as mentioned above, the present study attempts to examine the relationship between polymorphisms of NET gene and MD in northern Han Chinese population.

MATERIAL AND METHODS

Subjects

The sample consisted of 388 unrelated patients with MD (185 males and 203 females; average age, 30.90 ± 9.76 years, range 16–63 years) who were recruited from the Shanxi Medical University Institute of Mental Health and 388 matched normal controls (176 males and 212 females, average age 29.49 ± 10.63 years, range 16–64 years). All patients and Control volunteers were interviewed by the consensus of at least 2 experienced psychiatrists and diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria.24 Detailed information of a history of the illness, hospitalization, and medication was noted, and patients with mental and organic diseases, history of drug dependence, major neurological disorder, and substance dependence were excluded.
Single-Nucleotide Polymorphism Identification

Following the standard procedures, genomic DNA extrac-
tion was prepared from elbow vein whole blood samples. Two single-nucleotide polymorphisms (SNPs) of the NET gene, T-182C and G1287A, were examined in this study. The primer analysis software primer 5.0 was used to design primer pairs, and each primer was checked against BLAST to ensure the specificity.

Polymerase chain reaction (PCR) was used to amplify 2 polymorphisms of NET gene for T-182C and G1287A. For genotyping the T-182C polymorphism, forward primer 5'-CTG TGG TGC TGT TGT ATT GAC G-3' and reverse primer 5'-GGG TTT TGG TGT TTT ACT GCT T-3' were used. The PCR reaction mixtures contained 60 ng of genomic DNA, 200 μmol/L dNTPs, 0.2 μmol/L each primer, 2.5 μL 10 × PCR buffer, and 1 unit of Taq DNA polymerase with a total volume of 25 μL. PCR amplification was performed using the following cycling profile: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 63°C annealing for 30 seconds, 72°C for 30 seconds, and final elongation at 72°C for 10 minutes. For genotyping the G1287A polymorphism, forward primer 5'-GGC TCT GCT TGG ATA AAG GGA AA-3' and reverse primer 5'-GTC TGG CGC TTT GCG TTT ACT GCT G-3' were used. The PCR reaction mixtures contained 60 ng of genomic DNA, 200 μmol/L dNTPs, 0.2 μmol/L each primer, 2.5 μL 10 × PCR buffer, and 1 unit of Taq DNA polymerase with a total volume of 25 μL. PCR amplification was performed using the following cycling profile: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 59°C annealing for 30 seconds, 72°C for 30 seconds, and final elongation at 72°C for 10 minutes. NET gene mutations were confirmed by bidirectional direct sequencing analysis with a model 3700 DNA analyzer (Applied Biosystems, Foster City, CA).

TABLE 1. Genotype Distributions and Allele Frequencies of T-182C Polymorphism in the NET Gene Between Patients With Major Depression or its Clinical Subtypes and Control Subjects

| Group            | n   | Genotype, n (%) | Allele, n (%) |
|------------------|-----|----------------|---------------|
|                  |     | T/T T/C T/C    | T/C           |               |
|                  |     |                | χ²  df  P      | T/C           | χ²  df  P  |
| Major depression | 388 | 173 (46.4)     | 172 (44.3)    | 43 (11.1)     | 6.865 2 0.034 518 (66.8) 258 (33.2) 6.458 1 0.011 |
| MD, positive FH  | 80  | 31 (38.8)      | 40 (50.0)     | 9 (11.3)      | 5.388 2 0.068 102 (63.8) 58 (36.3) 5.154 1 0.023 |
| MD, negative FH | 308 | 142 (46.1)     | 132 (42.9)    | 34 (11.0)     | 5.089 2 0.079 416 (67.5) 200 (32.5) 4.367 1 0.037 |
| MD, early-onset  | 200 | 87 (43.5)      | 89 (44.5)     | 24 (12.0)     | 6.658 2 0.035 263 (65.8) 137 (34.2) 6.075 1 0.014 |
| MD, late-onset   | 188 | 86 (45.7)      | 83 (44.1)     | 19 (10.1)     | 3.145 2 0.028 225 (67.8) 121 (32.2) 2.913 1 0.088 |
| MD, suicide      | 272 | 121 (44.5)     | 117 (43.0)    | 34 (12.5)     | 7.911 2 0.019 359 (66.0) 185 (34.0) 6.801 1 0.009 |
| MD, no suicide   | 116 | 52 (44.8)      | 55 (47.4)     | 9 (7.8)       | 1.870 2 0.393 159 (68.5) 73 (31.5) 1.514 1 0.219 |
| Control          | 388 | 102 (52.1)     | 160 (41.2)    | 26 (6.7)      | 564 (72.7) 212 (27.3) * |
analyses with these 2 polymorphisms were not applicable. To evaluate the genotype–genotype interaction between the 2 loci of T-182C and G1287A in the NET gene for risk of MD and its subclinical phenotypes, 8 combinatorial genotypes of the 2 loci were analyzed by logistic regression (Table 4). Compared with those having both -182T/T and 1287G/G genotype, we found that in patients with MD, early-onset MD, MD with suicide concept group, the -182C/C and 1287G/A combinatorial genotype has significant risk (OR = 2.468, \( P = 0.040 \); OR = 4.050, \( P = 0.003 \); OR = 3.130, \( P = 0.010 \)). In patients with FH group, the -182C/C and 1287A/A combinatorial genotype has significant risk (OR = 8.100; \( P = 0.010 \)).

### DISCUSSION

Recently, researches of NET gene polymorphism start to focus on the 5’ flanking promoter region T-182C polymorphism and the silent polymorphism G1287A located in exon 9. The T-182C polymorphism is 182 upstream of the first codon in the 5’ flanking promoter region of the NET gene, where several potential transcriptional elements are located, and seem to have an important meaning as enhancer of transcription and correct splicing. The T/C point mutation lies in this intron may lead to an altered transcriptional activity by changes in the DNA structure.16,26 The G1287A polymorphism located in exon9 of the NET gene, and the G→A change is a silent mutation. It caused amino acid sequence change without protein structural effect, then possibly affects protein function. Among potential genetic markers of MD, it is a particularly interesting candidate because of its higher heterozygosity than the others.17,27 Therefore, attempting to explore the relationship between T-182C and G1287A polymorphisms of the norepinephrine transporter gene and MD, we performed a case–control association study in northern Han Chinese population.

In this study, significant differences were found in genotypic and allelic frequencies of T-182C polymorphism between the patient and control group. The CC genotype portion (11.1%) and the C allele frequency (33.2%) of MD patients are both higher than the control group (6.7% and 27.3%, respectively), indicating that the NET gene is possibly a susceptible gene for MD. Furthermore, using TT and GG genotype as reference, respectively, we observed the relative risk factor change tendency of various genotypes. The result showed that when referenced by TT genotype, the OR value increased gradually from TC to CC genotype and had remarkable difference; when referenced by T allele, the OR value of C allele also significantly increased. These results suggested that the T-182C polymorphism of NET gene may be a risk factor for MD, which is consistent with previous findings in Asian population.18,19 Ryu et al performed a case–control association study with 112 South Korea MD patients and 136 healthy controls, and found that the TT genotype frequency in the case group was significantly lower than that in the control group, showing there was a positive relationship between the T-182C gene polymorphism and MD. However, there were also some inconsistent results in Han Chinese and white populations. Chang et al found no relationship between the T-182C polymorphism of NET gene and MD.20,21,23 The contradictory findings are possibility due to race, analysis method, and sample size differences and clinical heterogeneity of illness. In addition, we could not detect an association between G1287A polymorphism and MD, which is consistent with previous findings.20,21,23 But using antidepressant drug such as methylphenidate, Yang et al12 observed the association between NET gene and NRI antidepressant, and further discovered that the G1287A gene polymorphism has significant efficacy in response to NRI antidepressant, indicating a positive relationship between G1287A polymorphism and MD. Larger replication studies with different ethnic samples for these markers are needed in future study.

Several studies have reported that FH and the morbidity age are associated with depressive patients,28–31 and norepinephrine may be associated with the suicide concept of MD. In

### TABLE 2. Genotype Distributions and Allele Frequencies of G1287A Polymorphism in the NET Gene Between Patients with Major Depression or its Clinical Subtypes and Control Subjects

| Genotype, n (%) | Allele, n (%) |
|----------------|--------------|
| Group          | n          | G/G | G/A | A/A | \( \chi^2 \) | df | \( P \) | G | A | \( \chi^2 \) | df | \( P \) |
| Major depression | 388       | 199 (51.3) | 159 (41.0) | 30 (7.7) | 0.316 | 2 | 0.856 | 557 (71.8) | 219 (28.2) | 0.080 | 1 | 0.777 |
| MD, positive FH | 80        | 40 (50.6)  | 31 (39.2)  | 8 (10.1)  | 1.169 | 2 | 0.577 | 111 (76.3) | 47 (23.7)  | 0.307 | 1 | 0.580 |
| MD, negative FH | 308       | 159 (51.5) | 128 (41.4) | 12 (7.1)  | 0.049 | 2 | 0.976 | 446 (72.2) | 172 (27.8) | 0.011 | 1 | 0.916 |
| MD, early-onset | 200       | 97 (48.5)  | 91 (45.5)  | 12 (6.0)  | 0.774 | 2 | 0.686 | 285 (71.3) | 115 (28.8) | 0.180 | 1 | 0.671 |
| MD, late-onset  | 188       | 102 (54.3) | 68 (36.2)  | 18 (9.6)  | 2.534 | 2 | 0.282 | 272 (72.3) | 104 (27.7) | 0.001 | 1 | 0.977 |
| MD, suicide     | 272       | 138 (50.7) | 114 (41.9) | 20 (7.4)  | 0.119 | 2 | 0.946 | 390 (71.7) | 157 (28.3) | 0.085 | 1 | 0.770 |
| MD, no suicide  | 116       | 61 (52.6)  | 45 (38.8)  | 10 (8.6)  | 0.670 | 2 | 0.715 | 167 (72.0) | 65 (28.0)  | 0.017 | 1 | 0.895 |
| Control         | 388       | 200 (51.5) | 162 (41.8) | 26 (6.7)  | \n
**Significant level calculated through a 1000-fold permutation method. FH = family history, MD = major depression.**
Logistic Regression Analysis of Combinatory 2 Loci of T-182C and G1287A in NET Gene for Risk of Major Depression and its Clinical Subtypes

| Group                  | OR 95% CI | P-value |
|------------------------|-----------|---------|
| Major depression       |           |         |
| MD, positive FH        | 1.286/0.762–2.169/0.346 | 1.504/0.882–2.566/0.133 |
| MD, negative FH        | 1.567/0.807–3.043/0.183 | 1.051/0.277–3.983/0.942 |
| MD, suicide            | 3.130/1.270–7.706/0.390 | 2.452/0.212–28.303/0.438 |
| MD, no suicide         | 1.285/0.661–2.495/0.459 | 1.471/0.373–5.807/0.579 |

| MD, early-onset         | 1.286/0.762–2.169/0.346 | 1.504/0.882–2.566/0.133 |
| MD, suicide            | 3.130/1.270–7.706/0.390 | 2.452/0.212–28.303/0.438 |
| MD, no suicide         | 1.285/0.661–2.495/0.459 | 1.471/0.373–5.807/0.579 |

| MD, suicide            | 3.130/1.270–7.706/0.390 | 2.452/0.212–28.303/0.438 |
| MD, no suicide         | 1.285/0.661–2.495/0.459 | 1.471/0.373–5.807/0.579 |

In conclusion, we investigated 2 main polymorphisms within the 5' promoter and coding region of the NET gene in this study and found possible genetic combinatorial risk factors for MD and MD sub-populations. The pathogenesis of MD is still unclear at present, and it is possible that other sequence variations are also important in determining susceptibility to MD. As a multifactorial complex disease, MD probably occurs by various genetic and environmental influences. Therefore, further studies with larger size and more complicated factors are needed to replicate and extend the initial finding.

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