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A Genetic Susceptibility Mechanism for Major Depression

Combinations of polymorphisms Defined the Risk of Major Depression and Subpopulations

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Abstract: Major Depression (MD) is a highly inherited psychiatric disorder. The norepinephrine transporter (*NET*) gene plays important role in pathophysiology of MD. This study attempted to examine the relationship between polymorphisms of *NET* gene and MD.

Patients with MD and healthy controls were recruited and subgrouped. The T-182C and G1287A polymorphisms of *NET* gene were genotyped by direct sequencing. The genotypic and allelic frequencies were compared using the Pearson $\chi 2$ analysis. The linkage disequilibrium was analyzed using the UNPHASED program.

Significant differences in genotypic and allelic frequencies of T-182C polymorphism were observed between MD subgroups and controls. When referenced by TT genotype, the OR value increased gradient from TC to CC genotype; when referenced by T allele, the odds ratio value of C allele also increased. Compared with those having both −182 T/T and 1287 G/G genotypes, in patients with MD, early-onset MD, and MD with suicide concept group, the −182 C/C and 1287 G/A combinatorial genotype has significant risk; yet in patients with MD family history, the −182 C/C and 1287 A/A combinatorial genotype has significant risk.

Different combinations of T-182C and the G1287A polymorphisms of *NET* gene might increase morbidity risk of MD subpopulations.

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Abbreviations: FH = family history, MD = major depression, MHPG = 3-methoxy-4-hydroxyphenylglycol, NE = norepinephrine, NET = norepinephrine transporter, NRI = norepinephrine reuptake inhibitor, OR = odd ratio, PCR = polymerase chain reaction, SNRI = serotonin noradrenalin reuptake inhibitors.

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.[5–8]

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] Til now, studies of NET mainly focused on the 5′ flanking promoter region T-182C polymorphism[16] and the silent polymorphism G1287A, located in exon 9,[17] but the findings are inconsistent. Ryu et al demonstrated a positive association between the *NET* gene and MD,[18,19] whereas Owen et al found no association.[20–23]

Based on the initial 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

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dependence were excluded. Further, patients were classified into 6 homogeneous clinical subgroups: MD with family history (MD, positive FH), MD without family history (MD, negative FH), early-onset MD (MD, early-onset), late-onset MD (MD, late-onset), MD with suicide concept (MD, suicide), and MD without suicide concept (MD, no suicide). All healthy controls were interviewed to exclude any current or previous psychiatric disorders. All the subjects were Han Chinese living in the North of China, and were given written informed consent. Ethics approval for the study was granted by the Ethical Committee of the First hospital of Shanxi Medicine University, Shanxi.

Single-Nucleotide Polymorphism Identification

Following the standard procedures, genomic DNA extraction 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 CTG TTG AAG TGT CGC-3′ and reverse primer 5′-GGC TCT GCT TGG ATA AAG GGA AA-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 unite 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'-GGG TTT TGG TGT TTT ACT GCT T-3' and reverse primer 5'-CTG TGG TGC TGT TGT ATT GAC 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 unite 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).

Statistical Analysis

The χ^2 goodness-of-fit test was applied to test Hardy–Weinberg equilibrium for the genotypic distribution of SNPs. The Pearson χ^2 analysis was performed to compare the genotypic and allelic frequencies between the patient and control groups using SPSS software (SPSS for Windows 11.5, SPSS Inc, Chicago, IL). When sample sizes were smaller than expected (<5 subjects), Fisher exact test was substituted for the Pearson χ^2. The significance level was set at a P value of 0.05 (2-tailed) corrected by the Monte Carlo correction. The UNPHASED program was used to analyze the linkage disequilibrium between the patient and control groups.[25]

RESULTS

No significant differences were found between the patient and control group in average age and sex. Genotypic distributions of each SNP in MD patients and healthy control subjects did not significantly deviate from the Hardy–Weinberg equilibrium.

The results of the genotype distributions and allele frequencies for T-182C and G1287A polymorphisms of the *NET* gene in patients and control subjects were shown in Tables 1 and 2, respectively. There were significant differences in genotypic and allelic frequencies of T-182C polymorphism observed between the patients with MD, early-onset MD, MD with suicide concept, and controls after Monte Carlo correction ($\chi^2 = 6.865$, $P = 0.034$; $\chi^2 = 6.458$, $P = 0.011$. $\chi^2 = 6.658$, $P = 0.035$; $\chi^2 = 6.075$, $P = 0.014$. $\chi^2 = 7.911$, $P = 0.019$; $\chi^2 = 6.801$, $P = 0.009$; Table 1). And There were also significant differences in allelic frequencies of T-182C polymorphism observed between the patients with FH, without FH and controls after Monte Carlo correction ($\chi^2 = 5.154$, $P = 0.023$; $\chi^2 = 4.367$, $P = 0.037$; Table 1). No significant differences in genotypic or allelic frequencies of G1287A polymorphism were observed between the patients and controls (Table 2).

To further define the contribution of T-182C polymorphism to MD susceptibility, T-182C and G1287A polymorphisms in the NET gene were analyzed for the risk of MD (Table 3). When T allele group was taken as a reference, the odd ratio (OR) of C allele group is 1.325 (95% confidence interval [CI]: 1.066–1.647); when TT genotype group was taken as a reference, the OR of TC genotype group is 1.255 (95% CI: 0.934–1.687), the OR of CC genotype group is 1.931 (95% CI: 1.139–3.273).

In addition, the linkage disequilibrium between the 2 investigated polymorphisms was calculated using the UNPHASED program. It was found that the 2 polymorphism sites were not in linkage disequilibrium with each other. Accordingly, haplotype

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

		Genotype, *n* (%)						Allele, *n* (%)				
Group	n	T/T	T/C	C/C	χ^2	df	*P*	T	C	χ^2	df	*P*
Major depression	388	173 (44.6)	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 (34.3)	6.075	1	0.014*
MD, late-onset	188	86 (45.7)	83 (44.1)	19 (10.1)	3.145	2	0.208	255 (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	202 (52.1)	160 (41.2)	26 (6.7)				564 (72.7)	212 (27.3)			

Significant level calculated through a 1000-fold permutation method. FH = family history, MD = major depression.
* $P < 0.05$, vs. control group.

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	χ^2	df	*P*	G	A	χ^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	0.557	111 (70.3)	47 (29.7)	0.307	1	0.580
MD, negative FH	308	159 (51.5)	128 (41.4)	22 (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)				562 (72.4)	214 (27.6)			

Significant level calculated through a 1000-fold permutation method. FH = family history, MD = major depression.

analyzes 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 -182 C/C and 1287 A/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.

TABLE 3. The Analysis of the *NET* Gene (T-182C and G1287A) for Risk of Major Depression

	Case	Control	OR (95% CI)	*P*
T-182C				
T	518	564	1	
C	258	212	1.325 (1.066, 1.647)	0.011*
TT	173	202	1	
TC	172	160	1.255 (0.934, 1.687)	0.132
CC	43	26	1.931 (1.139, 3.273)	0.013*
G1287A				
G	557	562	1	
A	219	214	1.033 (0.807, 1.254)	0.777
GG	199	200	1	
GA	159	162	0.986 (0.735, 1.323)	0.927
AA	30	26	1.160 (0.662, 2.031)	0.604

CI = confidence interval, OR = odd ratio.
* $P < 0.05$, vs T allele group.

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 gradiently 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 al[13] 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 4. Logistic Regression Analysis of Combinatory 2 Loci of T-182C and G1287A in NET Gene for Risk of Major Depression and its Clinical Subtypes

	-182T/T-1287G/A	-182T/T-1287A/A	-182T/C-1287G/G	-182T/C-1287G/A	-182T/C-1287A/A	-182C/C-1287G/G	-182C/C-1287G/A	1287A/A-182C/C-
Group	OR/95% CI/*P*	OR/95% CI/*P*	OR/95% CI/*P*	OR/95% CI/*P*	OR/95% CI/*P*	OR/95% CI/*P*	OR/95% CI/*P*	OR/95% CI/*P*
Major depression	0.926/0.606–1.416/0.723	1.510/0.633–3.603/0.351	1.314/0.868–1.990/0.197	1.244/0.811–1.908/0.316	0.912/0.395–2.107/0.830	1.452/0.711–2.963/0.304	2.468/1.019–5.979/0.040*	3.484/0.687–17.677/0.110
MD, positive FH	0.514/0.216–1.225/0.128	1.620/0.409–6.411/0.488	1.208/0.594–2.457/0.602	1.543/0.775–3.073/0.215	1.157/0.304–4.398/0.830	1.013/0.270–3.799/0.985	2.025/0.494–8.295/0.318	8.100/1.271–51.603/0.010*
MD, negative FH	0.990/0.638–1.537/0.966	1.056/0.398–2.801/0.912	1.114/0.722–1.718/0.626	1.082/0.688–1.702/0.733	0.755/0.302–1.888/0.546	1.476/0.720–3.024/0.286	1.981/0.772–5.079/0.149	1.981/0.323–12.142/0.452
MD, early-onset	1.286/0.762–2.169/0.346	1.890/0.674–5.303/0.221	1.670/0.999–2.791/0.050	1.504/0.882–2.566/0.133	0.579/0.158–4.025/0.404	1.688/0.707–2.619/0.235	4.050/1.542–10.634/0.003*	2.700/0.368–19.816/0.311
MD, late-onset	0.636/0.334–1.212/0.167	1.188/0.349–4.043/0.783	0.966/0.537–1.737/0.908	0.825/0.438–1.554/0.551	0.848/0.261–2.759/0.785	0.747/0.728–4.191/0.208	0.742/0.150–3.673/0.714	1.485/0.130–16.911/0.749
MD, suicide	0.820/0.511–1.317/0.411	1.252/0.471–3.328/0.652	1.153/0.729–1.825/0.542	1.207/0.758–1.924/0.428	0.783/0.301–2.036/0.615	1.272/0.576–2.807/0.551	3.130/1.272–7.706/0.010*	3.913/0.739–20.733/0.086
MD, no suicide	1.285/0.661–2.495/0.459	1.471/0.373–5.807/0.579	1.182/0.600–2.326/0.628	1.567/0.807–3.043/0.183	1.051/0.277–3.983/0.942	2.020/0.745–5.476/0.161	1.839/0.450–7.514/0.390	2.452/0.212–28.303/0.438

MD = major depression; FH = family history; OR = odds ratio; CI = confidence interval.
* $P < 0.05$ when reference group is −182T/T−1287G/G.

this study, significant differences in genotypic and allelic frequencies of T-182C polymorphism were discovered in MD with FH group, early-onset MD group, MD with suicide concept group compared with the control group, indicating that the C allele possibly increased the risk of MD morbidity in these 3 groups.

In addition, the genotype–genotype interactions between the 2 loci of T-182C and G1287A in the *NET* gene for risk of MD and its sub-clinical phenotypes were evaluated. The results showed that, when referenced by the -182T/T-1287G/G combination, the OR value of -182C/C-1287G/A combination increased significantly in MD, MD early-onset, and MD suicide group, yet that of -182C/C-1287A/A combination increased significantly in MD positive FH group. These results revealed combined effects of T-182C and the G1287A polymorphism of *NET* gene on MD morbidity, that -182T/T-1287G/G combination might be a risk factor of MD morbidity and suicide concept and -182C/C-1287A/A combination might be a risk factor of MD FH.

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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