Lack of association between the norepinephrine transporter gene and major depression in a Han Chinese population

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Objective: Although the physiological mechanisms contributing to the development of major depression remain unclear, several lines of evidence suggest that the catecholaminergic system involving the norepinephrine transporter (NET) is implicated in the etiology of major depression. This study aims to determine whether major depression is associated with the NET gene in a Han Chinese population. **Methods:** We analyzed the NET promoter T-182C polymorphism and another silent polymorphism G1287A in exon 9 of the NET gene with a polymerase chain reaction (PCR)-based method in 216 patients with major depression and 210 unrelated, age- and sex-matched healthy control subjects. We interviewed all subjects with the Chinese Version of the Modified Schedule of Affective Disorders and Schizophrenia-Lifetime; major depressive disorder was diagnosed according to DSM-IV criteria. In addition, to reduce the clinical heterogeneity, we performed a subtype analysis with clinically important variables, such as family history of major affective disorder and age at onset of major depression. **Results:** No significant difference was observed between the patients and healthy control subjects in the genotype distributions and allele frequencies for the investigated NET polymorphisms. Similarly, no significant differences were found between more homogeneous subgroups of patients and normal control subjects. **Conclusions:** This study suggests that the investigated polymorphisms in the NET gene are not major risk factors in increasing susceptibility to either major depression or its clinical subtypes in a Han Chinese population. However, larger replication studies with different ethnic samples are needed.

Objectif : Même si l'on ne connaît pas clairement les mécanismes physiologiques contribuant à l'apparition de dépressions sévères, plusieurs sources de données probantes laissent croire que le système catécholaminergique mettant en cause le transporteur de norépinéphrine (TNE) joue un rôle dans l'étiologie des dépressions sévères. Cette étude vise à déterminer si les dépressions sévères sont associées avec le gène TNE dans une population chinoise Han. **Méthodes :** Nous avons analysé l'agent promoteur T-182C du polymorphisme de TNE, et un autre polymorphisme silencieux G1287A dans l'exon 9 du gène TNE au moyen de la méthode fondée sur la réaction en chaîne de la polymérase chez 216 patients souffrant de dépression sévère et 210 sujets témoins en santé, non apparentés, de même âge et de même sexe. Nous avons interviewé tous les sujets avec la version chinoise du Guide modifié pour le diagnostic des troubles affectifs et de la schizophrénie; les troubles dépressifs majeurs ont été diagnostiqués selon les critères du DSM-IV. De plus, pour réduire l'hétérogénéité clinique, nous avons effectué une analyse de sous-type avec des variables cliniques importantes telles que les antécédents familiaux de troubles affectifs majeurs et l'âge de l'apparition d'une dépression majeure. **Résultats :** Aucune différence significative n'a été observée entre les patients et les sujets témoins en santé dans les distributions des génotypes et les fréquences des allèles pour les polymorphismes de TNE étudiés. De même, aucune différence significative n'a été trouvée entre les sous-groupes plus homogènes de patients et les sujets témoins normaux. **Conclusions :** Cette étude indique que les polymorphismes étudiés dans le gène TNE ne sont pas des facteurs de risque importants d'augmentation de la sensibilité à une dépression sévère ou à ses sous-types cliniques dans une population chinoise Han. Toutefois, des études de réplication plus vastes avec des échantillonnages ethniques différents devront être réalisées.

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Introduction

Major depressive disorder is a common and serious clinical problem that reduces patients' productivity and quality of life and increases their mortality. The original hypothesis for the biological cause of depression is associated with functional deficits in the norepinephrine (NE) and/or serotonin neurotransmitter systems.[1–3] Comparison of NE metabolites in the cerebrospinal fluid (CSF) of patients with depression and healthy control subjects has yielded heterogeneous results. The most consistent set of findings in the metabolite literature is that treatment with antidepressant drugs causes a decrease in CSF levels of the NE metabolite 3-methoxy-4-hydroxyphenyglycol (MHPG) in patients with major depression.[4–7] In addition, catecholamine depletion studies have demonstrated that patients with depression who respond to desipramine or mazindol (predominantly NE action) are at greater risk of relapse when NE synthesis is inhibited.[8,9] Taken together, these results suggest a decrease in the level of NE in people with depression, which is consistent with the hypothesis that central nervous system (CNS) noradrenergic dysfunction plays an important role in the pathophysiology of major depressive disorder.

The norepinephrine transporter (NET), a Na^+/Cl-dependent substrate specific transporter,[10] terminates NE transmission by recycling NE from the synaptic cleft. Thus, blockade of the NET enhances the action of NE at the synapse. Participation of the NET in modulating depressive symptomatology is demonstrated by the therapeutic benefit derived from the administration of NET inhibitors, such as tricyclic antidepressants and serotonin noradrenalin reuptake inhibitors (SNRIs).[11,12] Reboxetine, a unique selective NE reuptake inhibitor with little or no effect on serotonin or dopamine neurotransmission has been observed to be at least as effective as specific serotonergic reuptake inhibitors in the treatment of major depression.[13–15] Further, reduced levels of the norepinephrine transporter have been detected in the locus coeruleus of postmortem patients with major depression.[16] Since noradrenergic neurotransmission can be regulated by changes in NET expression, it seems reasonable that NET genetic variants may be responsible for central NE system dysregulation, putatively contributing to major depression and/or treatment response.

The NET gene has been mapped to the chromosome 16q12.2, which spans 45 kb and comprises 14 exons.[17,18] Stöber and colleagues[19] have systematically screened the whole coding region of the NET gene and identified 13 DNA sequence variants (i.e., 5 infrequent missense, 3 silent and 5 intronic mutations). Among them, a silent G1287A polymorphism, located at exon 9 of the NET gene, was a particularly interesting candidate because it has higher heterozygosity than the other markers.[19,20] Zill and colleagues[21] have identified the T-182C polymorphism located in the 5′ flanking promoter region of the NET gene. Because the 5′ flanking promoter region of the NET gene contains several cis-elements that play a critical role in transcription regulation,[22,23] changes in this promoter DNA structure may lead to an altered transcriptional activity responsible for predisposition to major depression. Thus, this NET T-182C polymorphism is also an important candidate for genetic studies.

In the present study, we investigated the association between either T-182C or G1287A polymorphism of the NET gene and depression by comparing the frequency of these polymorphisms in Han Chinese patients with major depression with healthy control Han Chinese subjects. In addition, it has been postulated that focusing the investigation on specific subclinical phenotypes may increase the strength to detect genes involved in complex disorders, for example, affective disorders.[24] Thus, to reduce the clinical heterogeneity, this study further examined the patient subgroups according to clinical variables, including family history of major affective disorder, age at onset of major depression, first/recurrent major depression and severity of major depressive episode.

Methods

Subjects

This study was approved by the Institutional Review Board for the Protection of Human Subjects at the Tri-Service General Hospital, a medical teaching hospital of the National Defence Medical Center in Taipei, Taiwan. We obtained written informed consent from all participants and fully explained the procedures of the study. To minimize the effect of ethnic differences in gene frequencies, the study participants were from the Han Chinese population in northern Taiwan. All participants were unrelated and were born and living in Taiwan; all of their biological grandparents were of Han Chinese ancestry. In total, we recruited 426 subjects.

The patient group comprised 216 patients with major depression (90 men and 126 women; mean age 39.63, standard deviation (SD) 14.79, range 16–65 years), who were recruited from clinical settings (172 inpatients and 44 outpatients). Each patient was initially evaluated by an attending psychiatrist and then interviewed by a well-trained psychologist, using the Chinese Version of the Modified Schedule of Affective Disorder and Schizophrenia-Lifetime[25,26] to reach the *Diagnostic and statistical manual of mental disorders*, fourth edition (DSM-IV)[27] diagnosis. The interrater reliability kappa values of the Chinese Version of the Modified Schedule of Affective Disorders and Schizophrenia-Lifetime were as follows: major depression, 0.79; bipolar disorder, 0.71; anxiety disorder, 0.86; schizophrenia, 0.95; and substance abuse and dependence, 0.82.[28] All study patients met the DSM-IV criteria for major depressive disorder on the basis of interviews and a best-estimate procedure that used all available information, including clinical observations, medical records and family information. They were recruited either in their first or recurrent major depressive episode. The severity of major depressive episode was assessed with the 17-item version of the Hamilton Depression Rating Scale (HAM-D). Only subjects with a minimum score of 18 on the HAM-D entered the study. Seventy-one patients had moderate major depressive episodes (HAM-D scores 18–24); the remaining 145 patients met the criteria for severe major depression (HAM-D scores > 24).

First-episode major depression was diagnosed in 29.6% of patients (all were enrolled from our psychiatric ward), and the other 70.4% had recurrent episodes (range 2–10, mean 2.57 [SD 1.03]). We excluded individuals with a history of substance dependence, severe medical illness, organic brain disease or any concomitant major psychiatric disorders. Further, patients were classified into 4 homogeneous clinical subgroups: major depression with family history, major depression without family history, early-onset major depression and late-onset major depression (Table 1). Family history here indicates one or more first-degree relatives affected with bipolar disorder or major depression. We defined early-onset as 18 years or under at the onset of initial depressive episode; we defined late onset as age 18 or older.

The normal control group included 210 healthy volunteers (104 men and 106 women; mean age 41.08 [SD 13.69], range 18–65 yr), recruited from the community. We used the Chinese Version of the Modified Schedule of Affective Disorder and Schizophrenia-Lifetime[25,26] to screen for psychiatric conditions in the control group. Subjects were free of past or present major or minor mental illness (affective disorder, schizophrenia, anxiety disorder, personality disorder, substance use disorders), and there was no family history of psychiatric disorder in the control subjects' first-degree relatives.

Genotyping

Polymerase chain reaction (PCR) was used to amplify 2 polymorphisms of NET gene for T-182C[21] and G1287A.[19] The primers' design and cycling protocol were modified from those described in the above-cited studies, and the PCR protocols were performed in a PerkinElmer 9700 thermal cycler (Boston, MA, USA). The forward primer 5′AGTGTCCGAGAAGGCTCCTGTG and reverse primer 5′-GCGCCAGAAGCATGGATG-3′ were used for genotyping the NET promoter T-182C polymorphism. Additionally, another forward primer 5′CTGCAGGAGGCTCTAGGAACC-3′ and reverse primer 5′GGAGGACTGGGAGCTGAGG-3′ were used for genotyping the G1287A polymorphism in exon 9 of the NET gene. All PCR products for direct sequencing were bidirectionally sequenced with a BigDye terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, Calif, USA), according to the manufacturer's instructions. We used bidirectional direct sequencing with a model 3730 DNA analyzer (Applied Biosystems) to identify the 2 NET gene mutation sites.

Statistical analysis

We used the independent samples *t* test to determine the difference in mean age between patients with major depression and normal control subjects, and we used Pearson chi-square analysis to compare sex difference between the patient group and the control group. We assessed Hardy–Weinberg equilibrium for each group, and the frequencies of genotype and allele were also compared between patients and control subjects, using the Pearson chi-square analysis. Fisher's exact test was substituted for the Pearson's chi-square when sample sizes were smaller than expected (fewer than 5 subjects). We applied multiple logistic regression analysis to correct the effects of possible covariates, age, sex and the other NET polymorphism, on the risk of major depression. Power analysis was performed with the G-power.[29] All tests were 2-tailed and alpha level was set at 0.05. We analyzed the level of linkage disequilibrium between the 2 polymorphism sites, T-182C and G1287A, using the FastEH program.[30]

Results

There was no significant difference in mean age and sex between the patients with major depression and the healthy control subjects (Table 1). With regard to patient subgroups, the mean age of early-onset major depression was significantly different from that of the control subjects ($p < 0.001$). In addition, significant differences in sex were found between control subjects versus subjects with major depression with a family history of major affective disorders ($p = 0.047$) and control subjects versus subjects with late-onset major depression ($p = 0.025$). Genotype distributions of T-182C and G1287A polymorphisms of the NET gene were in

Table 1: Comparison of the mean age and sex between patients and control subjects and between control subjects and patient subgroups

Group	*n*	Age, yr; mean (and SD)	*p*†	Sex; no. (%)		*p*†
				Men	Women	
Major depression	216	39.63 (14.79)	0.300	90 (41.7)	126 (58.3)	0.104
MD, positive FH*	55	38.89 (14.17)	0.297	19 (34.5)	36 (65.5)	0.047
MD, negative FH	161	39.89 (15.04)	0.430	71 (44.1)	90 (55.9)	0.300
MD, early-onset	38	24.32 (6.33)	< 0.001	22 (57.9)	16 (42.1)	0.342
MD, late-onset	178	42.92 (14.01)	0.198	68 (38.2)	110 (61.8)	0.025
MD, first episode	64	38.33 (14.30)	0.166	27 (42.2)	37 (57.8)	0.304
MD, recurrent episode	152	40.19 (15.01)	0.559	63 (41.4)	89 (58.6)	0.128
MD, moderate (HAM-D score 18–24)	71	37.81 (14.12)	0.089	31 (43.7)	40 (56.3)	0.393
MD, severe (HAM-D score > 24)	145	40.51 (15.08)	0.712	59 (40.7)	86 (59.3)	0.101
Healthy control subjects	210	41.08 (13.69)		104 (49.5)	106 (50.5)	

FH = family history; HAM-D = Hamilton Depression Rating Scale; MD = major depression; SD = standard deviation.
*Family history indicates bipolar disorder or major depression in a first-degree relative. First depressive episode developed before age 18 defined as early-onset, and the onset age 18 or later defined as late-onset.
†Compared with the control group.

the Hardy–Weinberg equilibrium, both in the patients and in the control subjects ($p > 0.1$; data not shown). The results of the genotype distributions and allele frequencies for these 2 polymorphisms in patients and control subjects are summarized in Table 2 and Table 3. No statistically significant differences were evident for the allele or the genotype frequencies between patients and control subjects. Moreover, no significant difference in the genotype distribution or in the allele frequency was found between the clinical subgroups and the control subjects (Table 2, Table 3). Using multiple logistic regression analyses, we confirmed that the association between either major depression or its clinical subtypes and the investigated NET polymorphisms persisted in its negative association after we corrected for age, sex and genotype (Table 4). We calculated pairwise linkage disequilibrium between the 2 investigated polymorphisms, using the FastEH program. We found that the 2 polymorphism sites were not in linkage disequilibrium with each other (healthy control: $\chi^2 = 0.45$, $df = 1$, $p = 0.502$, $D' = 0.999$; major depression: $\chi^2 = 0.02$, $df = 1$, $p = 0.888$, $D' = 0.999$). Accordingly, haplotype 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 major depression and its subclinical phenotypes, we analyzed the 8 combinatorial genotypes of the 2 loci by logistic regression (Table 5). One combinatorial genotype, 182 C/C – 1287 A/A was excluded, because of the absence of individuals with that genotype in the major depression group. Compared with those having both -182 T/T and 1287 G/G genotypes, subjects carrying another combinatorial genotype have no significant risk for major depression and its subclinical phenotypes ($p > 0.05$).

Power

In our total sample size ($n = 426$), we had a power of 0.44 to detect a small effect, 0.99 to detect a medium effect and 0.99

Table 2: Genotype distributions and allele frequencies of the promoter T-182C polymorphism in the NET gene between patients with major depression or its clinical subtypes and control subjects

		Genotype (%)						Allele (%)				
Group	*n*	T/T	T/C	C/C	χ^2	df	*p**	T	C	χ^2	df	*p**
Major depression	216	109 (50.5)	94 (43.5)	13 (6.0)	1.165	2	0.559	312 (72.2)	120 (27.8)	0.888	1	0.346
MD, positive FH	55	27 (49.1)	26 (47.3)	2 (3.6)	0.614†	2	0.758†	80 (72.7)	30 (27.3)	0.492	1	0.483
MD, negative FH	161	82 (50.9)	68 (42.2)	11 (6.8)	1.310	2	0.520	232 (72.0)	90 (28.0)	0.669	1	0.413
MD, early-onset	38	23 (60.5)	13 (34.2)	2 (5.3)	2.909†	2	0.252†	59 (77.6)	17 (22.4)	2.158	1	0.142
MD, late-onset	178	86 (48.3)	81 (45.5)	11 (6.2)	0.369	2	0.832	253 (71.1)	103 (28.9)	0.292	1	0.589
MD, first episode	64	31 (48.4)	30 (46.9)	3 (4.7)	0.333†	2	0.849†	92 (71.9)	36 (28.1)	0.313	1	0.576
MD, recurrent episode	152	78 (51.3)	64 (42.1)	10 (6.6)	1.377	2	0.502	220 (72.4)	84 (27.6)	0.807	1	0.369
MD, moderate	71	32 (45.1)	33 (46.5)	6 (8.5)	0.267	2	0.875	97 (68.3)	45 (31.7)	0.047	1	0.828
MD, severe	145	77 (53.1)	61 (42.1)	7 (4.8)	2.268	2	0.322	215 (74.1)	75 (25.9)	1.972	1	0.160
Healthy control subjects	210	95 (45.2)	101 (48.1)	14 (6.7)				291 (69.3)	129 (30.7)			

FH = family history; MD = major depression.
*Compared with the control group.
†Statistical analysis was performed with Fisher s exact test.

Table 3: Genotype distributions and allele frequencies of the G1287A polymorphism in the exon 9 of NET gene between patients with major depression or its clinical subtypes and normal control subjects

		Genotype (%)						Allele (%)				
Group	*n*	G/G	G/A	A/A	χ^2	df	*p**	G	A	χ^2	df	*p**
MD	216	100 (46.3)	95 (44.0)	21 (9.7)	1.993	2	0.369	295 (68.3)	137 (31.7)	1.526	1	0.217
MD, positive FH	55	24 (43.6)	22 (40.0)	9 (16.4)	2.071	2	0.355	70 (63.6)	40 (36.4)	0.016	1	0.899
MD, negative FH	161	76 (47.2)	73 (45.3)	12 (7.5)	2.771	2	0.250	225 (69.9)	97 (30.1)	2.565	1	0.109
MD, early-onset	38	16 (42.1)	19 (50.0)	3 (7.9)	0.245†	2	0.905†	51 (67.1)	25 (32.9)	0.224	1	0.636
MD, late-onset	178	84 (47.2)	76 (42.7)	18 (10.1)	2.348	2	0.309	244 (68.5)	112 (31.5)	1.559	1	0.212
MD, first episode	64	32 (50.0)	29 (45.3)	3 (4.7)	3.349†	2	0.182†	93 (72.7)	35 (27.3)	3.074	1	0.080
MD, recurrent episode	152	68 (44.7)	66 (43.4)	18 (11.8)	1.335	2	0.513	202 (66.4)	102 (33.6)	0.363	1	0.547
MD, moderate	71	30 (42.3)	31 (43.7)	10 (14.1)	0.921	2	0.631	91 (64.1)	51 (35.9)	0.002	1	0.966
MD, severe	145	70 (48.3)	64 (44.1)	11 (7.6)	3.065	2	0.216	204 (70.3)	86 (29.7)	2.838	1	0.092
Healthy control subjects	210	83 (39.5)	104 (49.5)	23 (11.0)				270 (64.3)	150 (35.7)			

FH = family history; MD = major depression.
*Compared with the control group.
†Statistical analysis was performed with Fisher's exact test.

to detect a large effect in the T-182C or G1287A genotype distributions. When given a power of 0.80, we were able to detect an effect size of 0.15 for detecting a significance difference in genotype distributions. In the allele frequencies of these 2 polymorphisms (n = 852), this study had a power of 0.83 to detect a small effect, 0.99 to detect a medium effect and 0.99 to detect a large effect. With regard to the patient subgroups, the statistical power was considerably lower because of the limited sample size. In this power analysis, effect size conventions were determined according to the method by Buchner and others[29]: small effect size = 0.10, medium effect size = 0.30 and large effect size = 0.50 (α = 0.05).

Discussion

The norepinephrine transporter gene is a plausible candidate gene for major depression, and it provides an avenue for investigating the susceptibility to major depression and/or response to antidepressant therapy.[31] In spite of the strong rationale for this study, the results showed no association between either major depression or its clinical subtypes and the promoter T-182C or the exonic G1287A polymorphism of the NET gene in Han Chinese subjects.

Although T-182C polymorphism located in the NET 5′ flanking promoter region may lead to an altered transcrip-

Table 4: Multiple logistic regression analysis of the NET gene (T-182C and G1287A) for risk of major depression and its clinical subtypes

Group	-182 T/C; OR/95% CI /p value	-182 C/C; OR/95% CI /p value	1287 G/A; OR/95% CI /p value	1287 A/A; OR/95% CI /p value	Sex; OR/95% CI /p value	Age; OR/95% CI /p value
MD (n = 216)	0.835/0.560–1.245/0.376	0.807/0.359–1.811/0.603	0.764/0.510–1.145/0.192	0.778/0.399–1.516/0.460	0.743/0.506–1.092/0.130	0.991/0.977–1.005/0.190
MD, late-onset (n = 178)	0.913/0.599–1.392/0.673	0.846/0.361–1.980/0.699	0.732/0.477–1.122/0.152	0.787/0.391–1.585/0.503	0.640/0.426–0.962/0.032	1.007/0.992–1.023/0.340
MD, positive FH (n = 55)	0.901/0.484–1.680/0.744	0.498/0.106–2.350/0.379	0.714/0.371–1.371/0.311	1.321/0.529–3.295/0.551	0.538/0.289–1.003/0.051	0.988/0.965–1.010/0.279
MD, negative FH (n = 161)	0.810/0.526–1.248/0.340	0.901/0.386–2.103/0.809	0.775/0.502–1.195/0.249	0.599/0.277–1.296/0.193	0.820/0.541–1.242/0.348	0.993/0.978–1.008/0.341
MD, first episode (n = 64)	0.946/0.529–1.691/0.850	0.621/0.166–2.329/0.480	0.714/0.399–1.277/0.256	0.336/0.094–1.204/0.094	0.742/0.420–1.312/0.305	0.984/0.963–1.005/0.131
MD, recurrent episode (n = 152)	0.786/0.505–1.224/0.287	0.887/0.372–2.117/0.787	0.783/0.501–1.225/0.285	1.010/0.498–2.050/0.977	0.745/0.487–1.140/0.175	0.994/0.979–1.009/0.417
MD, moderate (n = 71)	0.964/0.545–1.705/0.900	1.290/0.456–3.654/0.631	0.831/0.465–1.486/0.533	1.236/0.521–2.936/0.630	0.789/0.458–1.360/0.394	0.983/0.963–1.003/0.101
MD, severe (n = 145)	0.771/0.495–1.202/0.251	0.602/0.230–1.578/0.302	0.727/0.464–1.139/0.164	0.582/0.263–1.290/0.183	0.710/0.461–1.092/0.119	0.994/0.979–1.010/0.464

CI = confidence interval; FH = family history; HAM-D = Hamilton depression rating scale; MD = major depression; OR = odds ratio.
Reference group is -182 T/T, 1287 G/G and female, respectively.
The p values and ORs of early-onset MD (n = 38) are not shown in this table, but the risk of the NET gene for this group is not significant (p > 0.1).

Table 5: 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	-182 T/T – 1287 G/A OR/95% CI/ p value	-182 T/T – 1287 A/A OR/95% CI/ p value	-182 T/C– 1287 G/G OR/95% CI/ p value	-182 T/C – 1287 G/A OR/95% CI/ p value	-182 T/C– 1287 A/A OR/95% CI/ p value	-182 C/C– 1287 G/G OR/95% CI/ p value	-182 C/C– 1287 G/A OR/95% CI/ p value
Major depression (n = 216)	0.867/0.489–1.535/0.624	0.800/0.259–2.469/0.698	0.956/0.521–1.754/0.883	0.592/0.327–1.073/0.084	0.800/0.350–1.830/0.597	0.800/0.259–2.469/0.698	0.667/0.190–2.345/0.527
MD, late-onset (n = 178)	0.8 63/0.470–1.586/0.635	0.774/0.225–2.668/0.685	1.057/0.558–2.002/0.865	0.656/0.349–1.234/0.191	0.916/0.390–2.153/0.840	1.018/0.325–3.189/0.976	0.509/0.118–2.192/0.364
MD, positive FH (n = 55)	0.933/0.374–2.327/0.882	1.597/0.349–7.313/0.547	1.222/0.477–3.130/0.677	0.613/0.223–1.679/0.341	1.608/0.521–4.968/0.409	0.524/0.058–4.779/0.567	na/na/0.567
MD, negative FH (n = 161)	0.838/0.455–1.544/0.571	0.610/0.165–2.260/0.460	0.893/0.465–1.716/0.734	0.607/0.321–1.149/0.125	0.544/0.207–1.428/0.216	0.886/0.273–2.877/0.841	0.875/0.246–3.112/0.837
MD, first episode (n = 64)	0.877/0.378–2.038/0.761	0.860/0.158–4.665/0.861	1.256/0.537–2.937/0.599	0.744/0.314–1.764/0.502	0.182/0.022–1.516/0.115	0.802/0.148–4.340/0.798	0.465/0.051–4.220/0.496
MD, recurrent episode (n = 152)	0.857/0.460–1.598/0.628	0.815/0.237–2.806/0.746	0.854/0.435–1.677/0.647	0.559/0.286–1.090/0.088	1.033/0.438–2.436/0.941	0.815/0.237–2.806/0.746	0.761/0.198–2.923/0.690
MD, moderate (n = 71)	0.762/0.328–1.771/0.528	1.177/0.268–5.174/0.829	0.894/0.369–2.164/0.804	0.762/0.328–1.766/0.526	1.227/0.418–3.605/0.709	1.125/0.256–4.937/0.876	1.360/0.300–6.159/0.690
MD, severe (n = 145)	0.913/0.489–1.704/0.774	0.680/0.183–2.534/0.566	1.000/0.515–1.941/0.999	0.545/0.278–1.069/0.078	0.595/0.225–1.575/0.296	0.680/0.183–2.534/0.566	0.374/0.071–1.983/0.248

CI = confidence interval; FH = family history; HAM-D = Hamilton depression rating scale; MD = major depression; na = not applicable; OR = odds ratio.
The combinatorial genotype of -182 C/C – 1287 A/A was not found in the MD group.
Reference group is *-182 T/T – 1287 G/G*.
Control subjects = 210. 5.
The p values and odds ratios of early-onset MD (n = 38) are not shown in this table, but the combinatory genotypes of NET gene for risk of this group are not significant (p > 0.1).

tional activity responsible for increasing susceptibility to major depression, our study failed to detect an association between this polymorphism and major depression (Table 2). Consistent with this finding is the previous report of an absence of an association between this genetic variant and major depressive illness in Caucasians.[21] In contrast, studies conducted by Inoue and others[32] and Ryu and others,[33] using subjects from 2 populations from Japan and Korea, respectively, obtained contradictory positive results. In Inoue's samples, the C/C genotype was significantly less associated with major depression.[32] Conversely, the T/T genotype was associated with lesser susceptibility to major depressive disorder in Ryu's samples.[33] Regarding the T-182C polymorphism, the -182C allele frequency in our control samples (0.31) were similar to Korean[33] and Caucasian[21] samples (0.30 and 0.32, respectively), but the frequency of the -182C allele was higher in Japanese samples (0.41).[32] This allele frequency difference may be partly responsible for the divergent association results. However, because the functional consequences of the T-182C polymorphism are obscure, these conflicting results are difficult to interpret precisely. In any case, they suggest that the influence of T-182C polymorphism on major depression may be ethnically dependent. Larger replication studies with different ethnic samples are needed to investigate whether there are ethnic differences in the influence of NET T-182C polymorphism on major depression.

In addition, we could not detect an association between major depression and exonic G1287A polymorphism (Table 3). Our finding of no association between G1287A polymorphism and major depression is in-line with those of the previous studies conducted in 2 Caucasian populations.[21,34] Also, the -1287A allele frequencies in our Han Chinese control subjects (0.36) were similar to Caucasians (0.34–0.35).[21,34] Notably, Jönsson and colleagues[35] reported that the NET G1287A polymorphism was associated with the concentration of NE metabolite (MHPG) in the CSF of healthy volunteers in another Caucasian population. They reported that CSF MHPG concentrations were higher in the G/G genotype than in the G/A and A/A genotypes. It has also been reported that the A/A genotype of the G1287A polymorphism is associated with a slower onset of antidepressant response to SNRI milnacipran in Japanese patients with major depression.[31] Given that the G1287A allele has no functional consequences,[19] these 2 findings indicate that the G1287A polymorphism may be in linkage disequilibrium with a causal allele. Thus, work is needed to find the causal allele and to determine the effects that it has on the structure and function of the NET. It is, however, possible that this linkage disequilibrium may not occur in all populations; accordingly, we did not detect an association between this silent polymorphism and major depression in Han Chinese. This issue should be investigated with different ethnic samples in the future. Investigations concerning the impact of this silent polymorphism on NET mRNA stability are warranted.

Since ethnic stratification among study samples may lead to resetting population gene frequencies, it might produce a false–positive or false–negative result by chance rather than reveal a direct causal relation, when we interpret the association between an allele and a disease.[36] However, all our subjects were unrelated Han Chinese subjects, matched for age and sex, and drawn from a population pool in the northern part of Taiwan that is known to be genetically homogeneous.[37,38] All of the biological grandparents of our recruited subjects were of Han Chinese ancestry. Therefore, it is less likely that ethnic stratification bias produced a false–negative result in our study. Further, the healthy control subjects were interviewed with the Chinese Version of the Modified Schedule of Affective Disorder and Schizophrenia-Lifetime[25,26] to rule out psychiatric disorders. Thus a false–negative result due to inclusion of affective disorders in our control group is also presently unlikely.

In our sample, the statistical power to detect a small effect size (0.10) was more than 0.80 for detecting a significant difference in allelic distributions. As judged by the statistical power, the present total sample size was estimated to have been sufficient to reveal any statistically significant difference. With regard to the genotype analysis, when given a power of 0.80, our study could identify an effect size of 0.15 for detecting a significance difference in genotype distributions. However, we realize that our findings that the genotypes in any of the analyzed polymorphisms do not confer susceptibility to major depression should be interpreted cautiously, because the possibility of a small contribution to the genetic liability (e.g., effect size = 0.10) cannot be excluded. Thus our negative findings require confirmation with a much larger study group, preferably in family-based samples, to be more conclusive. Nevertheless, this study suggests that the investigated NET polymorphisms do not play a major role in predisposition to major depression.

Several studies have reported that family history of major affective disorder (bipolar disorder or major depression or both) in first-degree relatives is associated with poorer long-term outcome in depressive patients.[39,40] Early-onset (before age 18 years) depression has a more malignant course and is associated with greater comorbidity than is late-onset (age 18 or older) major depression.[41,42] According to these findings, it would be important to examine the role of polymorphisms in the NET gene in these homogenous subgroups of patients with major depression. Our results showed that T-182C and G1287A polymorphisms in the NET gene were not associated with these more homogeneous subtypes of major depression (Table 2, Table 3). Likewise, patients subgrouped according to first or recurrent major depressive episode and moderate (HAM-D score 18–24) or severe depression (HAM-D score > 24) were exploratorily analyzed, respectively, and we could not find a positive association between these patient subgroups and the studied NET polymorphisms (Table 2, Table 3). Although the age and/or sex distributions of control subjects and some patient subgroups were significantly different (Table 1), these differences should not affect our results, because the association between patient subgroups and the investigated NET polymorphisms persisted in its negative association after we corrected for age and sex, using multiple logistic regression

analyses (Table 4). In addition, genotype-genotype interaction between the 2 loci of the NET gene have been evaluated by logistic regression of 8 combinatorial genotypes between major depression group and control subjects (Table 5). We confirm that our negative findings were not biased due to the interaction between these 2 genetic variants. However, concerning the subtypes of major depression, especially those suffering from early-onset major depression (n = 38), the statistical power was dramatically reduced because of the limited sample size; thus our results should be considered preliminary and must be qualified with a larger number of subjects with subtypes of major depression. Moreover, since patients with major depression had a high risk for anxiety disorders,[43,44] future studies designed to clarify the relation between the NET gene and major depression comorbid with anxiety disorders may also provide a useful basis for approaching the genetic heterogeneity.

In this study, we investigated only 2 polymorphisms within the 5′ promoter and coding region of the NET gene. Therefore, it is possible that other sequence variations in, for instance, the yet undetected 3′UTR regions, may be important in determining susceptibility to major depression. In addition, we found that T-182C and G1287A variants were not in linkage disequilibrium with each other. We believe that this result is reliable because these polymorphisms were approximately 41 kb apart. Finally, elevated agonist binding (supersensitivity) to norepinephrine receptors has been reported in depression,[45,46] and antidepressant therapies appear to cause a downregulation of noradrenergic receptors.[47,48] Further research might also focus on the role of allelic polymorphisms of genes encoding for various norepinephrine receptors.

Conclusion

The results presented here suggest that the investigated genetic variants of the NET gene do not play a major role in increasing susceptibility to major depression or its clinical subtypes. Prospective studies with a much larger group, preferably in family-based samples, are necessary to confirm the results of our study. Extended studies need to be carried out in different ethnic samples to investigate whether there are ethnic differences in the influence of NET polymorphisms on major depression.

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