Association between *AKT1* gene polymorphisms and depressive symptoms in the Chinese Han population with major depressive disorder★

Chunxia Yang[1], Ning Sun[1], Yan Ren[1], Yan Sun[1], Yong Xu[1], Aiping Li[2], Kewen Wu[3], Kerang Zhang[1]

1 First Hospital of Shanxi Medical University, Taiyuan 030001, Shanxi Province, China
2 Department of Psychiatry, Three Hospital of Shanxi Medical University, Taiyuan 030053, Shanxi Province, China
3 Rongjun Kangning Psychiatric Hospital of Shanxi Province, Taigu 030800 , Shanxi Province, China

Abstract

For this study, 461 Chinese Han patients with depressive disorder were recruited. The *AKT1* genotype and allele distribution were determined by PCR amplification and direct sequencing. UNPHASED software was used to analyze associations between the 17-item Hamilton Depression Rating Scale, total score, four factors and the *AKT1* rs2494746 and rs3001371 polymorphisms. The results indicate that there is a significant association between suicidal ideation and anxiety symptoms in depressed patients and the rs2494746 polymorphism. The other *AKT1* polymorphism, rs3001371, was significantly associated with work and activities. Patients with the rs3001371-A allele had a significantly more severe illness compared to patients with the rs3001371-G allele. Thus, *AKT1* polymorphisms appear to be associated with depression severity, anxiety symptoms, work and activities, and suicide attempts in patients with depressive disorder.

Key Words: *AKT1*; major depression; symptoms; polymorphism; neural regeneration

INTRODUCTION

Major depressive disorder (MDD) is clinically characterized by symptoms such as anxiety, insomnia, loss of weight, cognitive dysfunction and psychomotor retardation[1-5]. Most studies seeking to elucidate the biological basis of the syndrome have primarily focused on neurochemical alterations. However, an increasing number of studies have begun to examine the molecular genetics of the disease. Overexpression of activated *AKT1*/Akt protects against apoptosis, whereas dominant negative AKT1/Akt does not[6-7], implying that AKT1/Akt is important for neuronal survival. Moreover, *in vivo* studies have revealed that activated *AKT1*/Akt in the hippocampus protects neurons against hypoxic stress and nitric oxide toxicity[8-9]. These AKT1/Akt targets likely function in a concerted manner to promote cell survival[10-11].

Human *AKT1* (v-akt murine thymoma viral oncogene homolog 1), the gene which maps to chromosome 14q32.3, is an isoform of the serine/threonine protein kinase AKT (also known as protein kinase B)[12], which was first reported as a susceptibility gene in schizophrenia by Emamian *et al* [13]. They found a significantly lower level of AKT1

protein in the peripheral lymphocytes of schizophrenics, and they also demonstrated lower levels of the protein in post-mortem samples of the frontal cortex and hippocampus. Weak evidence for an association between *AKT1* and bipolar disorder was reported by Toyota by screening all exons and flanking introns in 22 unrelated patients with bipolar disease[14]. Numerous studies have suggested that in patients with MDD, neuroprotective defense mechanisms against various stressors are impaired, and that antidepressants can enhance the neuroprotective responses[15]. Postmortem studies of cortical regions obtained from depressed subjects have also shown reductions in AKT function[16]. Attenuation of the kinase activity of AKT1, combined with the dysregulation of PTEN and PI3K in depressed suicide victims, may result in insufficient phosphorylation of lipid second messengers[17]. A recent review examined the role of AKT and glycogen synthase kinase-3 in various psychiatric illnesses, focusing on interactions with dopaminergic and serotoninergic pathways, as well as the effects of antipsychotics, antidepressants and lithium[18]. Although it is estimated that 40−70% of all cases of major depression can be attributed to genetic factors[19-20], little attention has been paid to the genetic contributions to the

illness. In this study, we investigate the potential contribution of *AKT1* gene polymorphisms to the severity of depressive symptoms and symptom clusters in a population of patients with MDD.

RESULTS

Quantitative analysis of subjects

For this study, 461 patients were recruited. Only subjects with a minimum baseline score of 7 on the Hamilton Rating Scale for Depression (HAMD, 17 items) were included in this study. Patients with prior (> 2 weeks) antidepressant treatment were excluded. Sequencing of PCR products from the genomic DNA of patients was performed. Due to difficulties in obtaining sequences from some samples, rs2494746 sequences were obtained for 447 patients, and rs3001371 sequences were obtained for 449 patients.

Analysis of *AKT1* genotypes and alleles

The genotypic distributions of the rs2494746 and rs3001371 polymorphisms were in Hardy-Weinberg equilibrium in the case group, while the distribution of the rs2494738 polymorphism deviated significantly from the Hardy-Weinberg equilibrium ($P < 0.01$). To test whether the violation of the Hardy-Weinberg equilibrium at rs2494738 was caused by true association with MDD or other reasons, such as genotyping errors, we performed a test developed by Li *et al* [21]. The result showed that the observed Hardy-Weinberg equilibrium deviation at rs2494738 was probably caused by an unknown reason instead of a true association with MDD. Therefore, it was omitted from further statistical analysis.

As shown in Tables 1–3, an association between the HAMD total score and rs2494746 was observed in both genotypic and allelic tests ($P < 0.05$).

Table 1   Hamilton Rating Scale for Depression (HAMD) total and clusters scores and the *AKT1* gene alleles and genotypes of patients with major depression

Marker	rs2494746		rs3001371	
	Allele	Genotype	Allele	Genotype
HAMD total score	0.005[a]	0.013[a]	0.122	0.248
Anxiety/physical symptoms	0.013[a]	0.051	0.710	0.923
Insomnia symptoms	0.413	0.557	0.300	0.555
Loss of weight symptoms	0.597	0.874	0.938	0.705
Retardation symptoms	0.032[a]	0.039[a]	0.100	0.183
1 Depressed mood	0.982	0.675	0.536	0.526
2 Feelings of guilt	0.034[a]	0.074	0.867	0.591
3 Suicide	0.014[a]	0.056	0.145	0.324
4 Insomnia early	0.702	0.849	0.815	0.456
5 Insomnia middle	0.396	0.646	0.725	0.933
6 Insomnia late	0.477	0.551	0.070	0.133
7 Work and activities	0.115	0.292	0.001[a]	0.002[a]
8 Retardation: psychomotor	0.118	0.028[a]	0.344	0.599
9 Agitation	0.242	0.503	0.477	0.204
10 Anxiety (psychological)	0.082	0.226	0.544	0.777
11 Anxiety somatic	0.067	0.202	0.654	0.509
12 Somatic symptoms (gastrointestinal)	0.734	0.321	0.814	0.458
13 Somatic symptoms general	0.773	0.293	0.034[a]	0.086
14 Genital symptom	0.066	0.198	0.414	0.650
15 Hypochondriasis	0.299	0.338	0.373	0.553
16 Loss of weight	0.597	0.874	0.938	0.705
17 Insight	0.116	0.244	0.654	0.866

[a]$P < 0.05$. 1–17 represents HAMD-17.

Table 2   Hamilton Rating Scale for Depression (HAMD) total and clusters scores and the *AKT1* genotype in patients with major depression

SNP	HAMD scores	Genotype			χ^2	*P*-value	Adjusted *P*-value[a]
		Mean score (scores)					
rs2494746	HAMD total score	CC ($n = 62$)	CG ($n = 196$)	GG ($n = 189$)	8.59	0.014	0.026
		17.97 (4.88)	18.22 (5.31)	19.58 (4.85)			
rs3001371	Work and activities	AA ($n = 148$)	AG ($n = 228$)	GG ($n = 73$)	12.46	0.002	0.003
		2.26 (0.70)	2.06 (0.79)	1.89 (0.84)			

a: The adjusted *P*-value after 10 000 permutation tests. UNPHASED software was utilized. *n* represents the number of patients carrying CC, CG or GG genotypes. The value in brackets represents the theoretical frequency using chi-square test. SNP: Single-nucleotide polymorphism.

Table 3   Hamilton Rating Scale for Depression (HAMD) total and clusters scores and the *AKT1* gene alleles in patients with major depression

SNP	HAMD scores	Allele		χ^2	*P*-value	Adjusted *P*-value[a]
		Mean score (scores)				
rs2494746		C ($n = 320$)	G ($n = 574$)			
	HAMD total	18.13 (5.13)	19.12 (5.05)	7.827	0.005	0.010
	Anxiety symptoms	4.51 (2.03)	4.86 (2.01)	6.133	0.013	0.025
	Suicide	0.92 (0.80)	1.06 (0.86)	5.977	0.014	0.030
rs3001371		A ($n = 524$)	G ($n = 374$)			
	Work and activities	2.19 (0.74)	2.00 (0.81)	11.680	0.001	0.001

a: The adjusted *P*-value after 10 000 permutation tests. UNPHASED software was utilized. *n* represents the number of patients carrying C and G alleles. The value in brackets represents the theoretical frequency using chi-square test. SNP: Single-nucleotide polymorphism.

Among the HAMD clusters, anxiety symptoms were associated with rs2494746 ($\chi^2 = 6.133$, corrected $P = 0.025$), and suicide attempt was associated with rs2494746 ($\chi^2 = 5.977$, corrected $P = 0.030$). Patients with the rs2494746-G/G genotype had significantly higher HAMD total scores relative to patients with the rs2494746-C/G or rs2494746-C/C genotypes ($\chi^2 = 8.59$, corrected $P = 0.026$). Patients with the rs2494746-G allele also had significantly higher HAMD total scores compared to patients with the rs2494746-C allele ($\chi^2 = 7.827$, corrected $P = 0.010$).

As shown in Tables 1–3, among the HAMD clusters, work and activities were significantly associated with rs3001371 genotypes ($\chi^2 = 12.46$, corrected $P = 0.003$) and alleles ($\chi^2 = 11.68$, corrected $P = 0.000\,9$). Patients with the rs3001371-A/A genotype had significantly higher HAMD total scores compared to patients with the rs2494746-A/G or rs3001371-G/G genotypes. Patients with the rs3001371-A allele also had significantly higher HAMD total scores compared to patients with the rs3001371-G allele.

As displayed in Tables 1–3, there were no significant differences in other HAMD cluster scores with respect to genotypes or alleles for either rs3001371 or rs2494746 after permutation testing.

DISCUSSION

One of the two *AKT1* polymorphisms in this study, rs2494746, was found to be associated with the severity of major depression, as measured by the HAMD total score. There was also a significant association between suicidal ideation and anxiety symptoms and this polymorphism in patients with major depressive disorder. Because the two *AKT1* polymorphisms were not in one block, based on Gabriel's criteria, they should make different genetic contributions to the disease. The other *AKT1* polymorphism, rs3001371, was significantly associated with work and activities, but not with the total score. This is the reason why we did not perform a haplotype analysis for these two SNPs in our study.

In the present study, HAMD-17 was chosen for patient assessment because of its widespread use in psychiatry[22]. In our sample, the higher the copy number of the *AKT1* rs2494746-G allele, the higher the HAMD total score and cluster score for suicide. Sarchiapone reported that the severity of depressive symptoms made significant and significantly independent contributions to the risk of a suicide attempt[23]. In depressed suicide victims, an alteration in 5HT1A-coupled G-protein function has been demonstrated, as well as in the downstream cascade involving AKT[7]. Our results are consistent with a postmortem study which found a reduction in AKT function in cortical regions in subjects with MDD[16].

Another important finding of this study was the association between the rs2494746 polymorphism in the *AKT1* gene and the anxiety symptom cluster in patients with MDD. Individuals with the rs2494746-G allele or the rs2494746-G/G genotype had significantly higher anxiety scores. An international epidemiological study of mood disorders (38 000 community subjects from ten countries) conducted by the Cross-National Collaborative Group reported that patients with major depression were at increased risk for comorbidity with anxiety disorders at all sites[24]. Furthermore, anxiety and depression were considered to be influenced by the same genetic factors[25-26]. Our observations strongly support this result.

Work and activities is one of the most frequently observed depressive symptom in patients with MDD[27]. We found a significant association between rs3001371, not only in the genotypic test, but also in the allelic test, and the work and activities symptom in patients with MDD. MDD is more heterogeneous than dimensional phenotypes as defined by clusters of symptoms. For example, it has been reported that serotonin transporter gene promoter polymorphism (5-HTTLPR) variants are associated with anxiety symptoms among mood disorder subjects, but not with mood disorders themselves[28].

In conclusion, a significant association was found between the rs2494746 polymorphism in the *AKT1* gene and the severity of major depression in a large sample of patients with unipolar depression. The positive findings in this study suggest that *AKT1* polymorphisms contribute to the observed clinical manifestations (anxiety symptoms, work and activities, and suicide attempts) in MDD. This report is, to our knowledge, the first to associate the *AKT1* gene with dimensional behavioral phenotypes in patients with major depression. Confirmation of the present findings in different populations should provide additional support for an important role of the AKT pathway in mood and behavior.

SUBJECTS AND METHODS

Design
Genetic association study.

Time and setting
Experiments were performed at the Laboratory of Molecular Biology, Chinese Academy of Medical Sciences, from March to September 2008.

Subjects
In this study, 461 patients were recruited, 29.14 ± 8.82 years of age, including 224 males and 237 females. Most patients (417, or 90.5%) were undergoing their first depressive episode, and the rest (44, or 9.5%) were undergoing a recurrent depressive episode. They were Chinese Han depressive disorder patients from the Outpatient Clinic of the Department of Mental Health, First Hospital, Shanxi Medical University. All patients were interviewed in the presence of family members and at least two consulting psychiatrists, and received the Chinese Version of the Modified Structured Clinical Interview for DSM-IV-TR Axis I Disorders-Patient Edition (SCIDI/P, 11/2002 revision). Diagnosis was performed

according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for MDD (American Psychiatric Association, 2000). The inter-rater reliability kappa value for the SCID was 0.81. Only subjects with a minimum baseline score of 7 on the HAMD (17 items) were included in this study. Patients with prior (> 2 weeks) antidepressant treatment were excluded. Potential participants that were pregnant, or had significant medical conditions, abnormal laboratory baseline values, unstable psychiatric features (*e.g.* suicidal), a history of alcoholism or drug abuse, epilepsy, head trauma with loss of consciousness, or neurological illness were also excluded. All patients were free of any other axis I diagnosis, including schizophrenia.

Depression severity was rated by the total of the 17-item Hamilton Depression Rating Scale. The following clusters of HAMD items were computed for each patient and analyzed: anxiety/physical (items 10, 11, 12, 15 and 17), insomnia (items 4, 5 and 6), loss of weight (item 16), and psychomotor retardation (items 1, 7, 8 and 14).

Methods

Genotyping and PCR

Based on the HapMap data generated for the Chinese Han population from Beijing, 18 tag SNPs were classified into two haplotype blocks based on Gabriel's criteria using the Haploview program[29]. Three tag SNPs were genotyped in this study; rs2494746 present in block 1, rs3001371 in block 2, and rs2494738 between these 2 blocks (Figure 1).

Genomic DNA was extracted from peripheral blood leukocytes using the standard phenol/chloroform procedure. All three SNPs were detected by PCR-based genotyping. The primers used for PCR amplification were designed by Primer 5.0 software and the specificity of each potential primer was validated using NCBI

BLASTN (http://www.Ncbi.nlm.nih.gov/BLAST/). PCR amplification was performed in a 25-µL reaction volume containing 2.5 µL 10 × GC buffer (Tiangen, Beijing, China), 200 µmol/L dNTP, 0.4 µmol/L of each primer, 1.0 unit of Tag DNA polymerase (Tiangen) and 60 ng of genomic DNA. The conditions used for PCR amplification included denaturation at 95°C for 10 minutes, followed by 35 cycles of 95°C for 30 seconds, 54–63°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. PCR products were purified using a MultiScreen-PCR plate. The purified PCR products were bidirectionally sequenced using the ABI 3700 DNA sequencer (Pekin-Elmer, Applied Biosystems, Foster City, CA, USA).
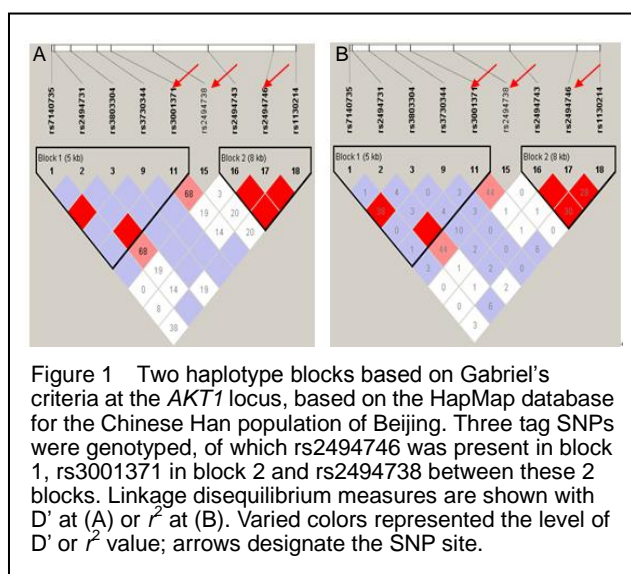

Figure 1　Two haplotype blocks based on Gabriel's criteria at the *AKT1* locus, based on the HapMap database for the Chinese Han population of Beijing. Three tag SNPs were genotyped, of which rs2494746 was present in block 1, rs3001371 in block 2 and rs2494738 between these 2 blocks. Linkage disequilibrium measures are shown with D' at (A) or r^2 at (B). Varied colors represented the level of D' or r^2 value; arrows designate the SNP site.

Genotypes of the SNPs were identified using the Chromas program (version 2.31). The primer sequences and the length of the PCR products are as follows:

AKT1 SNP ID	Location	Polymorphisms	Primer sequence (5'→3')	Product size (bp)	Annealing temperature (°C)
rs2494746	intron_2	C/G	F: TAG CAG ATG GGT TTC ACA R: CAG GCA GCA GAC TAT GTG	516	54
rs2494738	intron_2	A/G	F: CAT TCT TGA GGA GGA AGT AGC G R: GGC CGA CCC TGG TTG ATT	494	63
rs3001371	intron_4	A/G	F: GGA CCA CTG TCA TCG AAC G R: TCA CGA AGC CCT CTT GGA C	740	58

F: Forward; R: reverse.

Statistical analysis

Hardy-Weinberg equilibrium was tested with the chi-square (χ^2) goodness-of-fit test for all SNPs. The UNPHASED program (Frank Dudbridge, MRC Biostatistics Unit, Cambridge, UK)[30] was used for allelic and genotypic association tests of the quantitative trait. Linkage disequilibrium between each pair of SNPs was estimated with the UNPHASED program, and two linkage disequilibrium measures, D' and r^2, were used to express the strength of linkage disequilibrium between SNPs. The significance level was set at 0.05 and a permutation test was performed to correct for multiple testing.

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