

Absence of significant associations between four AKT1 SNP markers and schizophrenia in the Taiwanese population

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AKT1 (V-akt murine thymoma viral oncogene homolog 1) is a protein kinase isoform of AKT. Five single-nucleotide polymorphisms, rs3803300, rs1130214, rs3730358, rs2498799 and rs2494732, at the genomic region of AKT1 have been reported to be significantly associated with schizophrenia. We tested for the presence of these five single-nucleotide polymorphisms in a Taiwanese population by genotyping 218 co-affected schizophrenia families. Both single locus and haplotypes analyses showed no association of these single-nucleotide polymorphisms with schizophrenia. These findings fail to support AKT1 as a susceptibility gene for schizophrenia in the Taiwanese population. *Psychiatr Genet* 16:39–41 © 2006 Lippincott Williams & Wilkins.

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Introduction

AKT1 (V-akt murine thymoma viral oncogene homolog 1) is an isoform of serine/threonine protein kinase AKT (also known as protein kinase B), which was isolated from an AKR mouse thymoma cell line transforming with murine retrovirus AKT8 (Staal, 1987).

The AKT1 gene was first reported as a susceptibility gene in schizophrenia by Emamian *et al.* (2004), who found a significant reduction of AKT1 in the viral-transformed peripheral lymphocytes of schizophrenic patients, and also confirmed this result in post-mortem studies of the human brain frontal cortex and hippocampus. Besides the proteomic findings, five single-nucleotide polymorphisms (SNPs) of AKT1 markers located at the chromosome 14q32.32 spanning from the promoter to the intron 11 were genotyped in 265 schizophrenia proband families. Their study revealed significant haplotype associations close to the promoter region of the AKT1 gene. The

substrate of AKT1, glycogen synthase kinase-3 β , decreased its phosphorylation of the serine 9 residue. The mRNA levels of AKT1 at the dorsolateral prefrontal cortex were also decreased in the schizophrenia patients (Kozlovsky *et al.*, 2004). Two separate genotyping studies of these five SNPs of AKT1 in the Japanese population, however, revealed contradictory results (Ikeda *et al.*, 2004; Ohtsuki *et al.*, 2004). In order to determine whether the AKT1 gene is a schizophrenia-susceptibility gene in the Taiwanese population, we genotyped these five SNPs in 218 affected sib-pair schizophrenia families.

Study participants and methods

This research project was approved by the Institutional Review Board of the National Taiwan University Hospital. All genomic DNA samples were collected from families with at least two affected siblings after obtaining written, informed consent. The participants were recruited from two research programs: the Multidimensional

Psychopathology Study of Schizophrenia (MPSS) (Hwu *et al.*, 2002) from 1993 to 2001 and the Taiwan Schizophrenia Linkage Study (TSLs) (Hwu *et al.*, 2005) from 1998 to 2002. The 86 families of MPSS participants were interviewed by the research psychiatrists using the Psychiatrist Diagnostic Assessment (PDA) (Hwu, 1999). The 132 TSLs families were interviewed by well trained assistants using the Mandarin Chinese version of the Diagnostic Interview for Genetic Studies (DIGS) (Chen, 1999). For both studies, the final diagnostic assessment was formulated by integrating either the PDA or the DIGS data with clinical information from medical records using the Specialist Diagnostic Assessment Sheet, based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition.

A total of 218 families participated in this study. Among these families, there were 199 complete families (at least one parent and one affected sibling), and 19 families without parents participated. A total of 1006 individuals were investigated, but only 854 participants had a DNA sample and were genotyped. All affected individuals were diagnosed as having schizophrenia only. No schizoaffective or non-affective psychotic disordered patients were included.

All SNP markers were genotyped by the method of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). A DNA fragment (100–300 bp) encompassing the SNP site was amplified using the polymerase chain reaction GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instructions. After polymerase chain reaction amplification and neutralization of the deoxynucleotide triphosphate (dNTP), the primer extension was performed by adding the probe, Thermo Sequenase (Amersham Pharmacia, Piscataway, New Jersey, USA) and appropriate dideoxynucleotide triphosphate (ddNTP)/dNTP mixture. Different extension products were differentiated by mass through MALDI-TOF.

We used the procedure ALLELE in SAS/GENETICS release 8.2 (SAS Institute, 2002) to assess Hardy–Weinberg equilibrium. Family relationships were verified by PED-CHECK version 1.1 (O'Connell and Weeks, 1998) and UNKNOWN version 5.23 (Terwilliger and Ott, 1994) to detect deviations from Mendelian inheritance. Both single-point and haplotype association analyses were carried out using TRANSMIT version 2.5.4 (Clayton, 1999).

Results

All of the family relationships and genotypes were verified by checking Mendelian inheritance and using the procedure ALLELE in SAS/GENETICS release 8.2 for Hardy–Weinberg equilibrium. The SNP markers were all compatible with the Hardy–Weinberg equilibrium distribution, except for SNP1 (rs3803300). The other four SNPs (SNP 2–SNP 5) were validated and showed a minor allele frequency greater than 10% and a missing rate of less than 2% in this Taiwanese cohort (Table 1).

We used haploview software to construct haplotype blocks constituted by 'strong LD' markers according to the criteria proposed by Gabriel *et al.* (2002). The results show that only one haplotype block formed by SNP4 and SNP5 ($D' = 0.96$) was found among the four markers. The TRANSMIT program version 2.5.4 (Clayton, 1999), which can utilize data from all families even when parental genotypes are unknown, was used to analyze the associations between single SNPs, the haplotypes and schizophrenia. No significant association was found between a single locus or haplotype and schizophrenia (Table 1). Similar results were also obtained from single locus: SNP 2 ($n = 35$, $Z = -0.277$, $P = 0.782$), SNP 3 ($n = 21$, $Z = -0.14$, $P = 0.889$), SNP 4 ($n = 81$, $Z = 0.32$, $P = 0.748$), and SNP 5 ($n = 74$, $Z = 0.37$, $P = 0.714$), and from haplotypes: C–T ($n = 85$, $Z = 0.8$, $P = 0.422$), T–C ($n = 79$, $Z = -0.45$, $P = 0.65$), C–C ($n = 50$, $Z = -0.449$, $P = 0.65$), and T–T ($n = 1$) association analyses performed by family-based tests, using the FBAT program (Horvath *et al.*, 2004).

Table 1 Frequencies of the single nucleotide polymorphisms and haplotypes of the AKT1 gene and associations (analyzed by Transmit program 2.5.4.) with schizophrenia

Report SNP (primer ID)	SNP_ID	Position	Genetic region	Allele type	MF	HW test		Haplotypes		Schizophrenia ($n = 214$)		
						P	χ^2	P	Haplotype (rs2498799– rs2494732)	Haplotype frequency	χ^2	P
SNP2	rs1130214 (9128)	10323141	AKT1 (promoter)	C/A	0.1098	0.6157	0.177	0.674	C–T	0.5594	1.635	0.201
SNP3	rs3730358 (9127)	10321808	AKT1 (intron 3)	G/A	0.0538	0.2056	0.267	0.605	T–C	0.2755	0.124	0.724
SNP4	rs2498799 (9132)	10321157	AKT1 (exon 9)	T/C	0.4414	0.6591	0.829	0.363	C–C	0.1573	1.283	0.257
SNP5	rs2494732 (9131)	10321086	AKT1 (intron 11)	C/T	0.2966	0.9365	0.464	0.496	T–T	0.0077	2.834	0.092

MF, minor allele frequency; HW, Hardy–Weinberg; n , number of families.

Discussion

Although chromosome 14q has been reported in a genome-wide scan as a susceptible locus for schizophrenia (Bailer *et al.*, 2000), AKT1 was the first candidate gene for schizophrenia in the chromosome 14q32.32 region reported from SNP2–SNP3–SNP4 (T–C–G) haplotype association analyses (Emamian *et al.*, 2004). The single locus result was similar to the result obtained from this Taiwanese sample. The minor allele frequencies in the study by Emamian *et al.* and us are the following: SNP2 0.29 and 0.11, SNP3 0.17 and 0.054, SNP4 0.25 and 0.44, and SNP5 0.44 and 0.3, respectively. While Emamian *et al.* (2004) reported that the risk haplotypes in the SNP2–SNP3 had a linkage disequilibrium of around 0.67, this study found an SNP4–SNP5 block with *D'* of 0.98. This result suggests inheritance differences between different ethnic groups.

If the AKT1 gene has a major role in schizophrenia susceptibility, we would expect studies to consistently demonstrate its association with the disease development among members of an ethnic group. In the Japanese population, however, inconsistent results for this association have been reported (Ikeda *et al.*, 2004; Ohtsuki *et al.*, 2004). The present study also could not confirm the significance of Emamian *et al.*'s (2004) finding of an association between AKT1 haplotypes and schizophrenia. This suggests that AKT1 does not have an important role in the predisposition to schizophrenia in a Taiwanese population.

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