Associations between Brain-derived Neurotrophic Factor G196A Gene Polymorphism and Clinical Phenotypes in Schizophrenia Patients

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Background: Brain-derived neurotrophic factor (BDNF) had been chosen as a candidate gene for schizophrenia. This study investigated the relationships between BDNF G196A gene polymorphism and clinical phenotypes in schizophrenia patients in the Taiwanese population.

Methods: During a one year period, 132 schizophrenic patients and 103 healthy controls were recruited. Psychiatric diagnoses were made according to DSM-IV criteria. Genotyping of the G196A polymorphism of BDNF was performed by polymerase chain reaction amplification and restriction fragment length polymorphism.

Results: The data showed that the BDNF G196A genotypes and their allele distributions did not differ between patients with schizophrenia and healthy controls. No significant differences were noted in the BDNF G196A genotypes and allele distribution between schizophrenia patients with and without a family tendency for schizophrenia or between those with an age of onset before or after 25 years old. However, there was a significant difference in BDNF G196A genotype distribution between schizophrenia patients with and without a suicide history.

Conclusions: These analytical results suggest that BDNF G196A gene polymorphism is associated with a susceptibility to a suicide history in schizophrenia patients in the Taiwanese population. Further study with a larger number of samples is needed to prove these findings.

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Key words: brain-derived neurotrophic factor, schizophrenia, suicide

Functions related to brain-derived neurotrophic factor (BDNF) include influencing axonal growth,[1] mediating nerve cell survival,[2] and participating in the local response to various neuronal stressors.[3] BDNF also coordinates cortical neuronal migration and connectivity. Thus, BDNF is active during a critical developmental period and can plausibly influence a predisposition to develop schizophrenia. Currently, BDNF is regarded as a candidate gene for schizophrenia.

BDNF dinucleotide (GT) repeat polymorphism has been applied in studies of schizophrenia in many different ethnic populations.[4-9] Five of these studies showed no association of this dinucleotide polymor-

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phism with schizophrenia.[4-8] However, Muglia et al.[9] identified a positive correlation for an Italian population.

Another BDNF gene single-nucleotide polymorphism, 196G/A polymorphism, has also been applied in associated studies of schizophrenia.[10-14] Egan et al.[11] domonstrated a role for BDNF V66M (val66-to-met, 196G/A) polymorphism in human memory and hippocampal function. Recently, Neves-Pereira et al.[15] pointed out that the BDNF gene is a risk factor for schizophrenia in a Scottish population by using a haplotype analysis of BDNF dinucleotide GT repeat polymorphism and 196G/A polymorphism simultaneously and explained the important relationships between other candidate genes in schizophrenia (e.g., involving glutamate and N-methyl-D-asparate receptors) and BDNF.

In the past decades, there have been many studies which discussed the associations of subtypes,[16,17] symptoms[18-20] and demographic variables in schizophrenia, such as age of onset.[18,20-22] Recent reports suggested that more attention should be focused on the impact of BDNF alleles and genotypes on clinical features.[23] Ethnic differences in the frequency of BDNF polymorphism could explain the different risks for disease among different populations.[24]

In Taiwan, Hong et al.[10] suggested that BDNF Val66Met polymorphism might be related to Taiwanese schizophrenia pathogenesis in clozapine responders. Liou et al.[13] found that schizophrenia patients with tardive dykinesia who were heterozygous for the BDNF genotypes had significantly higher orofacial scores on an abnormal involuntary movement scale. Anttila et al.[14] also showed that BDNF G196A polymorphism was not associated with treatment response to typical neuroleptics in Finland.

Therefore, in this study, we investigated the relationships between BDNF G196A gene polymorphisms and other clinical phenotypes (including suicide history, family tendency and age of onset) in Taiwanese patients with schizophrenia.

METHODS

Subjects and design

This one year study, from December 2003 to November 2004, was conducted at Chang Gung Memorial Hospital (CGMH) in Kaohsiung, Taiwan.

Subjects with schizophrenia were psychiatric inpatients or outpatients at CGMH. Schizophrenia was diagnosed by one psychiatrist using a semi-structured clinical interview with DSM-IV criteria.[25] Subjects did not have any systemic diseases, including heart, liver, or thyroid disease. The investigated clinical phenotypes in this study included suicide history, family tendency and age of onset. Age at disease onset was set before or after 25 years old (patients with late age of onset respond better to neuroleptic treatment than patients with early onset).[6] The suicide history signified that patients had a history of attempting suicide before entering this study. Family tendency was defined as a patient having more than one first-degree relative with a history of schizophrenia.

The healthy control group, which consisting of staff members of the psychiatric ward, were screened by the same psychiatrist to rule out any psychiatric disease. All participants gave written informed consent after receiving a full explanation of the study.

Genotyping for BDNF polymorphisms

Venous blood, 10 ml, was obtained from each sample in EDTA-containing tubes. Genomic DNA was extracted from peripheral blood leukocytes.[26] Genotyping of the G196A polymorphism of BDNF was performed by restriction fragment length polymorphism (RFLP), modified from Ventriglia et al.[27] Briefly, the 196 G/A (= V66M) polymorphism was achieved using the following pair of primers: Forward primer: 5'-CTG GAG AGC GTG AAT GGG CC-3'. Reverse primer: 5'-TCC AGC AGA AAG AGA AGA GGA GGC-3'. Polymerase chain reaction (PCR) amplifications were done. The PCR conditions included denaturing at 94°C 10 min, followed by 35 cycles with profiling at 94°C for 60 sec, 58°C for 60 sec, and 72°C for 60 sec, with a final extension at 72°C for 10 min. Enzymatic digestion was done by restriction enzyme *PmaCI* 5U (Roche).

Statistical analysis

The genotype frequencies were investigated with a chi-square (X^2) test for goodness- of-fit to test for the Hardy-Weinberg equilibrium. Pearson chi-square (X^2) tests or Fisher's exact test was used to evaluate the differences in genotype and allele frequencies in the schizophrenia patients and healthy controls and the differences in genotype frequencies

of clinical phenotypes (e.g., age of onset, family tendency or suicide history) in patients with schizophrenia. An alpha value of $p < 0.05$ was used for statistical significance.

RESULTS

A total of 132 patients with schizophrenia (mean age = 33.6 ± 10.2 years old; male/female = 76/56; age of illness onset = 27.7 ± 10.0 years old; duration of illness = 5.9 ± 5.0 years) and 103 healthy control subjects (mean age = 29.1 ± 5.2 years old; male/female = 43/60) were recruited. Table 1 shows the genotypic and allelic distributions of BDNF G196A polymorphism in all participants.

There were no significant deviations from the Hardy-Weinberg equilibrium in either schizophrenia patients or healthy controls for the investigated BDNF G196A polymorphism. The G/A genotype had higher frequencies than the GG genotype or AA genotype in both patients and controls (Table 1). However, no significant difference was noted between patients and controls in genotype frequency ($X^2 = 0.039$, df = 2, $p = 0.981$). Similarly, the G allele had higher frequencies than the A allele in both patients and controls (Table 1). However, there was no significant difference between patients and controls in allele frequency ($X^2 = 0.032$, df = 1, $p = 0.858$).

Further investigations were performed for associations between clinical phenotypes and BDNF G196A genotype in patients with schizophrenia. We found that there were no significant differences in patients with/without a family tendency ($X^2 = 1.200$, df = 2, $p = 0.549$) and disease onset before and after 25 years old ($X^2 = 4.883$, df = 2, $p = 0.087$). However, there was a significant difference for BDNF G196A genotype distribution between schizophrenia patients with a suicide history and without a suicide history ($X^2 = 6.565$, df = 2, $p = 0.038$). We found that patients with a suicide history had lower frequencies of the GG genotype (25%) and GA genotype (37.5%) and a higher frequency of the AA genotype (37.5%) than patients without a suicide history (Table 1). In addition, no significant differences were observed for allele frequency in patients with/without a suicide history ($X^2 = 2.101$, df = 1, $p = 0.147$), family tendency ($X^2 = 0.256$, df = 1, $p = 0.613$) or age of onset ($X^2 = 2.967$, df = 1, $p = 0.085$).

DISCUSSION

Our results showed that there were no significant differences between patients and controls in BDNF G196A genotype and allele frequencies. This means that BDNF G196A polymorphism is unlikely to cause more genetic susceptibility to schizophrenia in Taiwanese. In addition, we found that there was a significant difference in BDNF G196A genotype distribution between schizophrenia patients with and without a suicide history. Taiwanese schizophrenia patients with a Chinese ethnic background with a

Table 1. Genotype and Allele Distributions of BDNF G196A Polymorphism in Schizophrenia Patients and Healthy Controls

BDNF G196A	Genotypes				Alleles		
	GG (%)	GA (%)	AA (%)	*p* value	G (%)	A (%)	*p* value
Controls (n = 103)	27 (26.2%)	59 (57.3%)	17 (16.5%)	0.981	113 (54.9%)	93 (45.1%)	0.858
Patients (n = 132)	36 (27.3%)	75 (56.8%)	21 (15.9%)		147 (55.7%)	117 (44.3%)	
Age of onset							
≦25 (n = 61)	11 (18.0%)	39 (63.9%)	11 (18.0%)	0.087	61 (50.0%)	61 (50.0%)	0.085
＞25 (n = 71)	25 (35.2%)	36 (50.7%)	10 (14.1%)		86 (60.6%)	56 (39.4%)	
Family tendency							
Positive (n = 15)	4 (26.7%)	10 (66.7%)	1 (6.7%)	0.549	18 (60.0%)	12 (40.0%)	0.613
Negative (n = 117)	32 (27.4%)	65 (55.6%)	20 (17.1%)		129 (55.1%)	105 (44.9%)	
Suicide History							
Positive (n = 16)	4 (25.0%)	6 (37.5%)	6 (37.5%)	0.038*	14 (43.8%)	18 (56.3%)	0.147
Negative (n = 116)	32 (27.6%)	69 (59.5%)	15 (12.9%)		133 (57.3%)	99 (42.7%)	

* : $p < 0.05$

suicide history had a lower frequency of the GA genotype and a higher frequency of the AA genotype. This is the first study showing a positive association between BDNF G196A gene polymorphism and schizophrenia patients with a suicide history. However, research with a larger number of samples is needed to prove this finding.

In conclusion, these results show that BDNF G196A gene polymorphism is not associated with susceptibility to schizophrenia in the Taiwanese population, but is associated with a suicide history in this disorder.

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腦源神經滋養因子 G196A 基因多型性與精神分裂症臨床表徵之相關性研究

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背 景： 腦源神經滋養因子基因是精神分裂症的候選基因。此研究將調查腦源神經滋養因子 G196A 基因多型性與台灣精神分裂症患者臨床表徵之相關性。

方 法： 在一年中共有 132 位精神分裂症患者與 103 位健康的對照組加入。精神科的診斷依據 DSM-IV 標準。而腦源神經滋養因子 G196A 基因多型性的確立則由分子實驗室的方法得到。

結 果： 精神分裂症患者的腦源神經滋養因子 G196A 基因多型性與正常對照組的基因型分佈並無差異。此外，腦源神經滋養因子 G196A 基因多型性與精神分裂症患者的家族史及發病年齡早晚亦無相關，然而卻與自殺史有相關。

結 論： 此結果顯示精神分裂症患者是否較易自殺，可能與腦源神經滋養因子 G196A 基因多型性有關，但未來仍需更多樣本加以證實。

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關鍵詞： 腦源神經滋養因子，精神分裂症，自殺

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