

A Negative Study of the Association between Tumor Necrosis Factor Alpha-308G/A Polymorphism and Schizophrenia among Taiwanese Patients

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Objectives: Tumor necrosis factor alpha (TNF- α) has been related to the pathogenesis of schizophrenia through its involvement in the processes of neurodevelopment and neurodegeneration. We examined the association between the TNF- α -308G/A polymorphism and schizophrenia in Taiwanese samples. **Methods:** The association analysis was performed using both a case-control and a family-based association design. The case-control sample comprised 124 patients and 119 controls; the family-based sample included 80 parent-offspring trio families. **Results:** No significant difference was found in genotype or allele frequencies between patients with schizophrenia and controls. No significant transmission distortion of the polymorphism was found in the family sample. **Conclusion:** Our results suggest that -308 G/A polymorphism in the TNF- α gene does not confer increased susceptibility to schizophrenia in this population of Han Chinese ethnicity from Taiwan.

Key words: association, polymorphism, schizophrenia, tumor necrosis factor alpha (Taiwanese J Psychiatry 2008;22:141-7)

Introduction

Schizophrenia is a common severe neuropsychiatric disorder that affects 1% of the general population. Although the pathogenesis of schizophrenia remains unclear, immune alterations associated with this disease have been studied for decades [1]. Tumor necrosis factor alpha (TNF- α) is

one of the major cytokines that mediates primary host response during inflammation. It has been proposed that TNF- α is related to the pathogenesis of schizophrenia through the processes of neurodevelopment and neurodegeneration [2]. Significant increases in the plasma concentration of TNF- α have been reported in patients with schizophrenia [3].

The TNF- α gene is located in the region

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6p21.1-21.3, near the HLA region, a locus associated with genetic susceptibility to schizophrenia [4]. For the TNF- α gene promoter region, there is a functional single-nucleotide polymorphism (SNP) located at nucleotide position -308, which involves a common variant with guanine (G) (TNF1) and a less common one with adenine (A) (TNF2). Higher transcriptional activity has been revealed for the -308A allele in comparison to the -308G variant [5]. Recent studies found an association between this SNP and schizophrenia, with a significantly increased -308A allele frequency in patients with schizophrenia compared to controls [6,7]. In contrast, other studies have demonstrated that the frequency of the -308A allele was significantly lower in schizophrenia [8-10], while no positive results were observed in other samples [11-15]. Given these inconsistent findings, we sought to clarify the possible role of TNF- α in the etiopathogenesis of schizophrenia by examining the association between the TNF- α -308G/A polymorphism and schizophrenia in Taiwanese samples using both a case-control and a family-based association design.

Materials and Methods

Approval was obtained from the Ethics Committee for Human Research of National Taiwan University Medical Center. After listening to a complete description of the project, patients, their relatives, and controls provided informed consent prior to their inclusion in the study. A sample of 10 ml whole blood was collected for DNA extraction from all recruited subjects.

The Case-control sample

A total of 124 patients with schizophrenia (68 males, mean age \pm SD: 30.0 ± 6.8 years, mean age of onset \pm SD: 21.9 ± 5.5 years) and 119 nor-

mal controls (50 males, mean age \pm SD: 32.8 ± 7.2 years) participated in this study. All of the subjects were genetically unrelated Han Chinese. The patients with schizophrenia were all participants in a prospective study of schizophrenia in Northern Taiwan called the multidimensional psychopathological group research projects (MPGRP). Briefly, from August 1, 1993 to June 30, 1997, all patients consecutively admitted to the acute wards of three hospitals, National Taiwan University Hospital, Taipei City Psychiatric Center, and Taoyuan Psychiatric Center were recruited if they met the DSM-IV criteria for schizophrenia. Patients with other axis I diagnoses (substance abuse, organic mental disorders, affective disorders), neurological illness (epilepsy), or systemic illness were excluded. A clinical interview using the Positive and Negative Syndrome Scale (PANSS) [16] was conducted within one week after patients were admitted due to acute exacerbation. Among the 234 patients recruited at the index admission, only 124 signed informed consent for blood collection and were entered into this study. No significant differences were found between patients entering this study and those who were excluded in terms of gender distribution, age, age at onset and the scores of total symptoms, positive subscale, negative subscale and general psychopathology subscale of the PANSS (data not shown).

The normal control subjects were mostly recruited from hospital staff ($n=104$) and patients visiting the physical examination ward ($n=15$) of National Taiwan University Hospital without matching for cases. After informed consent was obtained, control subjects underwent a screening interview schedule to rule out major psychiatric illness and substance abuse. Because most of the controls were within the characteristic age range of schizophrenia onset, we could not exclude potential cases completely. However, considering

the disease prevalence of schizophrenia was 0.3% in our population [17], the number of potential cases was likely too small to influence the association results.

The family sample

Schizophrenic probands were recruited from the same hospitals using the same inclusion and exclusion criteria as the case-control sample. The parents and siblings of each proband were also recruited. All of the patients and their first-degree relatives were interviewed with the Chinese version of the Diagnostic Interview for Genetic Studies (DIGS-C) [18]. This family sample comprised 80 parent-offspring trio families, including 80 schizophrenic probands (41 males) with a mean age of 30 years old, 149 of their parents (72 males) with a mean age of 58 years old, and 42 of their healthy siblings (21 males) with a mean age of 31 years old. Most of the families ($n=69$) were complete trios while 11 families had only one parent.

Genotyping

Genomic DNA was extracted from whole blood using standard procedures. The SNP at position -308 in the TNF- α promoter region was screened by polymerase chain reaction (PCR) using the methods described by Wilson et al [19]. Oligonucleotides primers were (forward) 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and (reverse) 5'-TCCTCCCTGCTCCGATTCCG-3'. Conditions for PCR were as previously described with minor modifications [6]. The digested samples of amplified DNA with *NcoI* restriction enzyme (New England Biolabs, MA, USA) were electrophoresed on 4% MetaPhor agarose gel and stained with ethidium bromide. The product size after digestion was: TNF1 allele (-308G) = 87bp/20bp, TNF2 allele (-308A) = 107 bp.

The statistical analysis

The presence of Hardy-Weinberg equilibrium was examined by the chi-square (χ^2) test for goodness of fit. Allele and genotype frequencies in each group were compared using the χ^2 test and Fisher's exact test. Between-genotype differences in continuous variables were evaluated using the Student's *t*-test or analysis of variance (ANOVA). The critical *p*-value was set at 0.05 (two-tailed). All data for the case-control sample were analyzed using SPSS version 10.0 (SPSS, IL, USA). Family-based association analysis was performed using the TDT/S-TDT program [20].

Results

Case-control sample

The genotype distributions and allele frequencies of the SNP for the patients and controls are compared in Table 1. The genotype distribution of the SNP for patients and controls was in Hardy-Weinberg equilibrium. Although the frequency of the minor allele (-308A) was somewhat higher in the schizophrenics compared to the controls, the genotype and allele distributions in these patients were not significantly different from those in the controls. However, under the dominant hereditary model, there were significantly more A/A or G/A genotypes in the patients than in the controls ($p = 0.045$).

Among the genotypes, there were no differences in clinical variables, such as onset age ($F = 0.50$, $p = 0.611$; data not shown), PANSS-positive subscale, negative subscale, general psychopathology subscale, and total scores ($F = 0.21$, $p = 0.809$; $F = 0.84$, $p = 0.433$; $F = 0.40$, $p = 0.673$; $F = 0.68$, $p = 0.508$, respectively; data not shown). No evidence of association was found between this SNP and the severity of clinical symptoms in the acute exacerbation stage.

Table 1. Genotype distribution and allele frequencies of the TNF- α gene polymorphism at position -308 among patients with schizophrenia and controls

	Patients with Schizophrenia (n = 124)	Controls (n = 119)
Genotype distribution		
GG	70 (56.5)	82 (68.9)
GA	50 (40.3)	34 (28.6)
AA	4 (3.2)	3 (2.5)
	$\chi^2 = 4.04, p = 0.133$	
GG	70 (56.5)	82 (68.9)
AA or GA	54 (43.5)	37 (31.1)
	$\chi^2 = 4.02, p = 0.045$	
Allele frequency		
G	190 (76.6)	198 (83.2)
A	58 (23.4)	40 (16.8)
	$\chi^2 = 3.27, p = 0.071$	

Odds ratio (-308A vs. -308G) = 1.511 (95%CI = 0.964-2.368)

Table 2. Family-based association analysis using TDT/S-TDT program 1.1

Allele	N	Z	P-value
A	38	1.161	0.2456-
G		1.161	0.2456+

-: under-transmitted to affected individuals

+: over-transmitted to affected individuals

N: Number of informative families

The family sample

The genotype distribution of the SNP in the family sample was in Hardy-Weinberg equilibrium. Table 2 shows the results of the family-based association analysis using TDT/S-TDT. No significant transmission distortion of the SNP was found in the family sample.

Discussion

This study failed to detect a positive association between -308 G/A polymorphism in the promoter region of the TNF- α gene and schizophrenia in a Taiwanese sample. Only a borderline significant difference in genotype distribution was found when the dominant model was applied. We could not replicate the previous findings of association between -308 G/A and patients with schizophrenia in samples of Italians [6], Brazilians [7], Polish [8], Germans [10], and Chinese Singaporeans [9]. However, this result is similar to findings from studies of other Asian samples [11-15]. In addition, the odds ratio of -308A versus -308G obtained in our study is close to that reported by Tsai et al. [13] in a previous study of another Taiwanese sample (1.51; 1.53, respectively). These findings suggest that an ethnic difference may account for the variation in etiological significance of this SNP in patients with schizophrenia from different populations.

It is possible that this study generated a false negative result due to the small genetic effect of the -308G/A polymorphism of the TNF- α gene in the Taiwanese population, or because of the relatively small sample size and inadequate statistical power. The statistical power of this study was 0.20 with an alpha of 0.05 and the minor allele frequency difference between cases and controls was 0.066. With the assumption of adequate power of 0.8, the estimated required sample size for cases and controls would both be 606. For our family sample, with the assumptions of disease frequency of 0.003 [17], disease allele frequency of 0.234, alpha of 0.05, the power would reach 0.8 when the allelic odds ratio reaches 2.15. This implies that the study had inadequate power for detecting of gene effect below 2.15.

Clinical implication

1. Our study does not support the notion that -308 G/A polymorphism in the TNF- α gene plays a major role in the susceptibility to schizophrenia among the Han Chinese population of Taiwan.
2. Further study with a larger sample size is needed in order to confirm these findings.

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精神分裂症與腫瘤壞死因子之基因多型性分析

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目的：研究腫瘤壞死因子 (TNF-*a*) 基因之 -308G/A 單核苷酸多型性與精神分裂症之相關性。**方法：**本研究採用病例－對照相關研究法，及家系關聯性分析研究法來分析 TNF-*a* 基因 -308G/A 單核苷酸多型性與精神分裂症之相關性。病例－對照相關研究組包括 124 名病人及 119 名對照組；家系關聯性分析研究組包括 80 名病人，149 名父母，及 42 名健康手足。**結果：**病例－對照相關研究的結果

顯示，在 TNF-*a* 基因之 -308G/A 單核苷酸多型性上，精神分裂症與對照組之間其對偶基因頻率或基因型頻率並沒有任何差異；在家系關聯性分析研究中，亦未發現此一單核苷酸多型性有傳遞不平衡之現象。**結論：**本研究顯示 TNF-*a* 基因之 -308G/A 單核苷酸多型性在台灣精神分裂症的病源學上並不扮演決定性角色。

關鍵詞：關聯研究，單核苷酸多型性，精神分裂症，腫瘤壞死因子
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