Family-based transmission analysis of -308A/G polymorphism in TNF-alpha with Tourette syndrome in Chinese Han population

QI Feng-guang, WANG Pei-lin△, WANG Xiu-hai, LIU Shi-guo

(Department of Biology, Medical College of Qingdao University, Qingdao, 266021, China)

ABSTRACT Objective: To investigate the relationship between -308A/G polymorphism in the promoter region of TNF-alpha and susceptibility to Tourette syndrome in Chinese Han population. Methods: Genetic distribution of -308A/G polymorphism in TNF-alpha of 91 trios were evaluated by using case-control study method based on nuclear family including transmission disequilibrium test (TDT), haplotype relative risk (HRR) and haplotype-based haplotype relative risk(HHRR). Results: -308A/G polymorphism in TNF-alpha of 91 trios was in accordance with Hardy-Weinberg equilibrium in genotype distribution. (x^2<3.84;P>0.05). There was not biased transmission of allele from parents to their affected offspring in our informative samples in -308A/G polymorphism. HRR and HHRR had not any associations with TS at -308A/G polymorphism in TNF-alpha. Conclusion: -308A/G polymorphism in the promoter region of TNF-alpha was not associated with susceptibility to Tourette syndrome in Chinese Han population.

Key words: Tourette syndrome; TNF-α ; TDT; HRR;HHRR

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Introduction

Tourette's syndrome (TS) is a chronic, neurobehavioral disorder characterized by waxing and waning motor and phonic tics that persist for at least 12 months [1]. This disease etiology remains poorly understood. It is believed that susceptibility to TS is determined by the interactions of multiple genetic loci with unknown environmental factors. Over the past decade, the role of the immune system in TS has been increasingly investigated and the results suggest immune abnormalities associated with the pathogenesis of TS[2]. Kawikova[3]found decrease T regulatory cells in a combined group of children with Tourette's disorder (TD) and/or obsessive-compulsive disorder (OCD) compared to controls. Another investigative approach [4] has focused on the possible role of cytokines, glycopeptide signaling molecules that mediate key steps in cellular and humoral immunity. Peripheral cytokines can cross the blood-brain barrier and influence complex brain functions. Leckman[5] found increased serum TNF-α of TD compared to controls during illness exacerbation. In addition, animal experiments have shown that TNF-α treatment can result in an increase or decrease of monoamine concentrations in specific brain regions, suggesting the involvement of distinct brain areas in TD and OCD[6,7].

TNF-a, produced by monocytes, macrophages, T and B lymphocytes[8], is a proinflammatory cytokine and produces pleiotropic effects such as apoptosis, cell proliferation and cytokine production [9-11]. It had been suggested that there were increased transcription in -308A/G polymorphism of TNF-α [12,13]. But, there were opposite results in some studies [14-16]. Previous studies suggested that-308A/G polymorphism at the promoter regions of TNF-α genes had been associated with systemic lupus erythematosus (SLE), hepatocellular carcinoma (HCC) [17-19].

This study investigated the association between TNF-α polymorphism and TS. Therefore, the transmission disequilibrium test (TDT) analysis was performed to assess whether the TNF-α gene promoter polymorphism could be implicated in susceptibility to TS in Chinese Han population.

1 Material and methods

1.1 Patient selection

TS subjects were from the Affiliated Hospital of Qingdao University Medical College and Weifang people's hospital and Linyi city people's hospital. There are 91 trios including patients and their parents. All participants were informed consen and take part in this research. All patients were assessed using an assessment process and diagnosed according to diagnostic and statistical manual of mental disorders, 4th edition text revision[1]. Exclusion criteria: included diagnosis is not clear, no informed consent, or incomplete medical record data.

1.2 Genotyping

DNA was extracted from 5ml of venous EDTA blood using standard phenol chloroform rassays. -308A/G of TNF-α genotypes were determined by restriction fragment length polymorphism (RFLP) analysis from the DNA by an investigator unaware of the phenotype. PCR amplification was performed using specific primers (Shanghai Sangon Biological Engineering Technology & Service Co., Ltd., China).A 140 basepair polymerase chain reaction (PCR) product was generated using the forward 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and reverse. 5'-CACAGCATCAAGGATACCC-3' primers. PCR amplification was performed at: initial denaturation at 94℃ for 5 min, 35 cycles of denaturation at 94℃ for 30 sec, annealing at 62℃ for 1 min and

Auther: QI Feng-guang,(1982-), master, Mainly engaged in genetics research tel:15266239064

△Corresponding auther: WANG Pei-lin

E-mail:wangpeilin@163169.net

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extension at 72℃ for 1 min. A final extension at 72℃ for 10 min was added. PCR products were electrophoresed in a 3.0% agarose gel. Agarose gel were stained by ethidium bromide, and were visualized by UV transillumination. Alleles were assigned by comparison with a 100-bp-ladder marker.-308A/G. PCR products were digested with NCOI generating fragments of 120 bp and 20 bp. The undigested part was A allele.

1.3 Statistical analysis

All data was analysed by the Statistical Package for Social Sciences (Version12.0 for Windows; SPSS, Inc., Chicago, IL, USA) [20]. The genotype frequencies were given byproduct of the allele frequencies and the population was said to be in Hardy-Weinberg equilibrium (HWE). A test was performed by the HWE program [21]. The association analysis of genetic polymorphism and TS was carried out by TDT [22]. In this study, the numbers of allele transmissions from heterozygous parents to their affected offspring were used to estimate degrees of association. Therefore, all 91 trios were used TDT to assess the significance level of TNF-α .

2 Results

2.1 Hardy−Weinberg equilibrium test

Genotype distributions of TS trios were in accordance with H-W equilibrium. -308A/G polymorphism of TNF-a showed no significant differences in genotype and allele distributions between patient groups and parent groups. (Table 1)

Table 1 The allele and genotype frequency of patient groups and parent groups and H-W equilibrium

	Genotype frequency				Alleles frequency			HWE	
	AA	AG	GG	df	A	G	df	x^2	P
Patients	1(0.01)	9(0.10)	81(0.89)	1	11(0.06)	171(0.94)	1	1.519	0.218
Parents	0 (0.00)	18(0.10)	164(0.90)	1	18(0.05)	346(0.95)	1	0.493	0.483
x^2		2.01			0.291				
P		0.366			0.589				

2.2 TDT and HHRR test

The analysis of the association between polymorphism in TNF-a and TS was carried out by TDT. Each parent can be summarized by the transmitted and the non-transmitted allele (Table 2). There was not biased transmission of alleles from parents to their affected offspring in this informative samples. In order to enhance the effectiveness, two times specimens were expand by haplotype-based haplotype relative risk(HHRR). (Table 2) However, there was not biased transmission of allele from parents to their affected offspring in our samples.

Table 2 TDT and HHRR analysis

Transmitted allele	Non-transmitted allele		Total		
	A	G		x^2	P
A	a= 0	b= 11	W= 11		
G	c= 6	d=165	X=171		
Total	Y=6	Z=176	2N=364	1.54	0.21
x^2	1.47				
P	P>0.25				

2.3 HRR test

The haplotype relative risk(HRR)test[23] were used the haplotype relative risk (HRR)test for the association analysis,. First, genotype relative risk we used, which compared the genotype in the affected offspring with the control genotype derived from non-transmitted parental chromosomes. (Table 3) There was not the biased transmission of genotype from parents to their affected offspring. Second, the allele relative risk was used. There was not the biased transmission of allele from parents to their affected offspring in specimens.

Table 3 genotype relative risk and allele relative risk analysis

	Classification		Allele	genotype
	A	G	A(+)*	A(-)**
Transmitted	a=11	b=171	A=10	B= 81
Non-transmitted	c=6	d=176	C= 6	D=85
95%CI	0.8~3.9		0.98~3.13	
df	1		1	
x^2	1.54		1.10	
P	0.21		0.30	
HRR	1.89		1.75	

A (+)*=AG; AA A (-)*=GG

3 Discussion

This study investigated the association using case-control study method based on nuclear family. Family-based linkage/association studies have gained popularity, because they control for spurious associations between disease and specific marker alleles due to population admixture. The TDT had thus become one of the most frequently used statistical methods in genetics. This method eliminates no consistency of genetic background and avoids false positive result and phenomenon of layered group structure. It was used next to evaluate the allelic transmission from heterozygous parents to TS patients. TDT analyses also allowed us to detect the cosegregations of some alleles on the same chromosome in linkage with TS. To test risk of transmissions in polymorphism of TNF-α gene, The study was evaluated possible discrepancies in the haplotypes tested and results indicated no significant excess transmissions presence of a preferential parent's transmission to TS patients. These alleles may not be considered directly responsible for susceptibility to TS.

TNF-a is located within a region of chromosome 6. It was a potent pro-inflammatory cytokine exerting pleiotropic effects on various cell types and plays a critical role in the pathogenesis of chronic inflammatory diseases. Several abnormalities of the immune system have been reported in TS patients, the number of T reg cells in TS patients with symptoms is approximately 40% lower than in healthy control groups. Regulatory T cell numbers are also decreased in five out of six patients during exacerbation of their TS symptoms as compared with their controls. The suppressive capacity of T reg cell population in TS patients may be insufficient to control the activity of auto-aggressive cells, which may predispose TS patients to development of autoimmunity [24]. Furthermore, A growing body of research data indicates the involvement of autoimmunity in the pathogenesis of patients with TS. The data included the work on regarding their potential in generating disease in an animal model [25], the association with B lymphocyte. The results suggest that the allele of the ?308A/G are not likely to be independent risk factors for TS. To date, this is the first study to report an association between -308A/G polymorphism in the promoter region of TNF-a and TS in Chinese Han population.

In addition, the number of specimens is lesser, Therefore, the study needed collect larger specimens in more area and adopt update method (combined neural physiological test and single-photon emission computed body into photography .etc). It will contribute to help us better research the pathogenesis, genetic pattern of TS.

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在中国汉族人群中,以家系传递分析肿瘤坏死因子 a-308A/G 单核苷酸多态性与抽动秽语综合征

綦丰光 王培林△ 王修海 刘世国

(青岛大学医学院生物教研室 山东 青岛 266021)

摘要 **目的:**探讨在中国汉族人群中,肿瘤坏死因子 a (TNF-alpha,TNF-a)基因启动子区 -308A/G 单核苷酸多态性与抽动秽语综合征(Tourette syndrome,TS)的遗传易感性。方法:91 例 TS 患者及其父母组成的核心家系成员经聚合酶链式反应 - 限制性片段长度多态性(polymerase chain reaction-restriction fragment length polymorphism,PCR-RFLP)方法进行基因分型,评估所有研究对象的 TNF-a -308A/G 位点的等位基因频率和基因型频率的分布,进行传递不平衡检验(transmission disequilibrium test,TDT),单体型相对风险(haplotype-based haplotype relative risk,HHRR),单体型风险(haplotype relative risk HRR)的研究。**结果:**TS 患者及其父母的等位基因分布经 Hardy-Weinberg (H-W)平衡检验显示符合遗传平衡法则。(x^2<3.84;P>0.05) TDT 、HHRR 和 HRR 研究结果显示该多态性位点的等位基因频率和基因型频率均不存在传递不平衡。**结论:**我们的数据表明肿瘤坏死因子 a 启动子区 -308A/G 单核苷酸多态性位点不是中国汉族人群 TS 的易感基因位点。

关键词:图雷特综合症;肿瘤坏死因子 α ;传递不平衡检验;单体型相对风险;单体型风险

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作者简介:綦丰光,(1982-),男,硕士,主要从医学遗传学的研究

△通讯作者:王培林,教授,E-mail:wangpeilin@163169.net

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