Original Article

Association between Tourette Syndrome and the Dopamine D3 Receptor Gene Rs6280

Fan He[1,2], Yi Zheng[1,2], Huan-Huan Huang[1], Yu-Hang Cheng[1], Chuan-Yue Wang[1,2]

[1]Department of Psychiatry, Beijing Anding Hospital, Capital Medical University, Beijing 100088, China
[2]Center of Schizophrenia, Beijing Institute for Brain Disorders, Beijing 100088, China

Abstract

Background: Tourette syndrome (TS) is a complex, heterozygous genetic disorder. The number of molecular genetic studies have investigated several candidate genes, particularly those implicated in the dopamine system. The dopamine D3 receptor (DRD3) gene has been considered as a candidate gene in TS. There was not any report about the association study of TS and DRD3 gene in Han Chinese population. We combined a case–control genetic association analysis and nuclear pedigrees transmission disequilibrium test (TDT) analysis to investigate the association between DRD3 gene rs6280 single nucleotide polymorphisms (SNPs) and TS in a Han Chinese population.
Methods: A total of 160 TS patients was diagnosed by the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. The DRD3 gene rs6280 SNPs were genotyped by TaqMan SNP genotyping assay technique in all subjects. We used a case–control genetic association analysis to compare the difference in genotype and allele frequencies between 160 TS patients and 90 healthy controls. At the same time, we used TDT analysis to identify the DRD3 gene rs6280 transmission disequilibrium among 101 nuclear pedigrees.
Results: The genotype and allele frequency of DRD3 gene rs6280 SNPs had no statistical difference between control group (90) and TS group (160) ($\chi^2 = 3.647$, $P = 0.161$; $\chi^2 = 0.643$, $P = 0.423$) using Chi-squared test. At the basis of the 101 nuclear pedigrees, TDT analysis showed no transmission disequilibrium of DRD3 gene rs6280 SNPs ($\chi^2 = 0$; $P = 1$).
Conclusions: Our findings provide no evidence for an association between DRD3 gene rs6280 and TS in the Han Chinese population.

Key words: Dopamine D3 Receptor Gene; Single Nucleotide Polymorphism; Tourette Syndrome; Transmission Disequilibrium Test

Introduction

Tourette syndrome (TS), also known as multiple transient tic disorder or combined vocal and multiple motor tics, is a type of chronic neuropsychiatric developmental disorder that occurs in children and adolescents.[1] It is characterized by involuntary vocal tics and multiple motor tics of the head and body, particularly the face and extremities. These symptoms may occur simultaneously or during different disease periods, showing a sudden and rapid onset, purposelessness, stereotypy, and arhythmicity. The prevalence of TS has increased within recent years and is estimated to be 0.05%–3%; a large-scale epidemiological survey of 420,312 children and adolescents reported it to be 1.0%.[2] The ratio of the TS prevalence rate between males and females is 3–5:1, and most patients develop the disease between the ages of 2 and 15 years, with a mean age of 7 years.[3] Tic severity is closely related to age, with the most severe symptoms usually occurring at 8–12 years of age, causing a great impact on the children's self-esteem, self-confidence, peer relationships, and family relationships. About half of TS patients suffer social dysfunctions such as learning difficulties and interpersonal difficulties.[4]

It is widely acknowledged that TS results from the interaction between genetic and environmental factors,[5] as shown by pedigree and twin studies.[6,7] However, the etiological factor of TS still remains unclear. Indeed, antipsychotic agents, both typical dopamine antagonists and atypical dopamine antagonists, have been proven to improve the symptoms of TS. In addition, neuroimaging studies have found abnormalities in brain structures that are functionally related to the dopamine neurotransmission system. Therapeutic response to dopamine antagonists, as well as neuroimaging studies, supports a dopaminergic system abnormality in TS. Currently, the polymorphism of many dopaminergic system-related genes has been identified, which may be associated with TS. The genes are the dopamine transporter 1 (DAT1) gene, the three D2-like dopamine receptor (DRD2,

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Address for correspondence: Prof. Chuan-Yue Wang, Department of Psychiatry, Beijing Anding Hospital, Capital Medical University, Beijing 100088, China; Center of Schizophrenia, Beijing Institute for Brain Disorders, Beijing 100088, China
E-Mail: wang.cy@vip.tom.com

DRD3, and DRD4) genes and the monoamine oxidase-A gene.[8] Still, a lack of replicated and consistent results was observed, a single nucleotide polymorphisms (SNPs) in these genes have a poor reproducibility.[9] Moreover, existing studies have focused on DRD2, DRD4, and DAT1,[10-14] while little is known about DRD3. In the past 10 years, we had focused on the study of exploring the association between the gene DRD2, DRD4, and DAT between TS in a Han Chinese population.

There has not been any report about the association study of TS and DRD3 gene in Han Chinese population. In this study, we investigated the presence of the DRD3 rs6280 polymorphism in TS patients, their unaffected parents, and normal controls, compared genotype and allele frequencies between TS patients and controls, and observed transmission disequilibrium between TS patients and their unaffected parents.

Methods

Subjects

A total of 160 TS patients was recruited from the Department of Pediatrics, Beijing Anding Hospital, Capital Medical University, China between December 2009 and December 2012, including 101 nuclear pedigrees. Basic clinical information in the pedigree was collected using a self-report questionnaire. Psychiatric diagnoses were made by a psychiatrist according to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). A total of 90 healthy children served as controls.

The following inclusion criteria were used for the TS group: (1) Meeting the diagnostic criteria of DSM-IV for TS; (2) an age of 6–18 years; (3) having no other central nervous system diseases or serious physical illness; (4) a Chinese Han; (5) having no tic disorder or other mental disorder patients in three generations of two pedigrees; (6) being able to complete all diagnostic tests; (7) providing informed consent.

The following inclusion criteria were used for the control group: (1) A healthy child or adolescent; (2) an age of 6–18 years; (3) having no central nervous system disease or severe physical illness, and no drug abuse or dependence; (4) having no mental disorder, including attempted suicide, or family history of mental disorder; (5) a Chinese Han individual unrelated to other controls or TS patients; (6) a similar gender ratio to TS patients; (7) being able to complete all diagnostic tests; (8) providing informed consent.

The following inclusion criteria were used for the parents of TS patients: (1) Biological parents of the TS patients; (2) healthy individuals with no history of mental disorder and not meeting the DSM-IV diagnostic criteria; (3) providing informed consent.

This experiment was approved by the Ethics Committee, Beijing Anding Hospital, Capital Medical University, China. The study was explained to all subjects prior to experimentation, and informed consent was obtained from all patients and their families. The consent forms were signed by the guardians of subjects <7 years old, and by both guardians and subjects ≥7 years old. The work in this paper has been carried out in accordance with The Code of Ethics of the World Medical Association (*Declaration of Helsinki*).

DNA extraction

Fasting peripheral blood samples (5 ml) were collected in EDTA tubes, and genomic DNA was extracted using the centrifugation column method with the whole blood genomic DNA extraction kit (Promega; Madison, WI, USA) according to the manufacturer's instructions. The concentration and purity of all DNA samples were determined.

Single nucleotide polymorphism screening

Based on Chinese Han population genetics data from the database of SNPs (http://www.ncbi.nlm.nih.gov/SNP/; the US National Center for Biotechnology Information), the International HapMap Project (http://www.hapmap.org/), and previous studies,[15,16] the DRD3 SNP rs6280 locus was selected for gene polymorphism analysis.

Genotyping

Dopamine D3 receptor SNP rs6280 genotyping was carried out using a TaqMan SNP genotyping probe. Polymerase chain reaction (PCR) reactions (5 μL) contained 10 ng DNA template, 2.5 μL × 2 TaqMan® Universal PCR Master Mix (ABI), and 0.25 μL × 20 SNP assay (Applied Biosystems, Bedford, MA, USA; including primers and FAM/VIC probe). The reaction conditions were 95°C for 10 min followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. PCR signals and fluorescence signals were read on an ABI7900HT sequence detector (384-well plates), and then analyzed using the SDS2.4 image analysis software (ABI Prism®, ABI Corp) allelic discrimination program. FAM and VIC fluorescence intensities of different alleles were analyzed to determine whether samples were homozygous wild-type, heterozygous or homozygous mutated.

Statistical analysis

SPSS 17.0 software (IBM SPSS Statistics for Windows, IBM Corp.,Armonk, NY, USA) was used for data analysis. Genotype and allele frequencies were expressed as percentages. The fitness of the genotype and allele was tested using the Hardy–Weinberg equilibrium (HWE) test, and polymorphism differences between the TS patient and control groups were analyzed using the Chi-squared (χ^2) test. The transmission disequilibrium test (TDT) of nuclear pedigrees was performed using Plink1.07 software (Shaun Purcell, USA), $\alpha = 0.05$.

Results

Case–controlled study

Comparison of general information

A total of 160 sporadic TS patients (including 101 TS patients) involved in pedigree studies and 59 unrelated

cases underwent DRD3 SNP rs6280 analysis. There were 128 males and 32 females, at a ratio of 4:1. The mean age was 11.62 ± 3.33 years. Of the 90 normal controls, 73 were male and 17 were female, at a ratio of 4.3:1. The mean age was 11.42 ± 2.37 years. There was no significant difference in the gender ratio (P = 0.164) or mean age (P = 0.927) between the two groups.

Fitness of Hardy–Weinberg equilibrium test

Dopamine D3 receptor rs6280 genotype frequencies achieved HWE in the control group (P = 0.074) and TS patient group (P = 0.582), as shown by the χ^2-test. This indicated that the data are reliable and representative for genetic association analysis [Table 1].

Comparison of dopamine D3 receptor 3 gene rs6280 genotype and allele frequencies

The frequencies of DRD3 rs6280 genotypes C/C, C/T, and T/T were 10.6, 46.9, and 42.5%, respectively, in the TS patient group, and 13.3%, 34.4%, and 52.2%, respectively, in the control group. The frequencies of C and T alleles were 34.1% and 65.9%, respectively, in the TS patient group, and 30.6% and 69.4%, respectively, in the control group. There were no significant differences in genotype or allele frequencies between the TS patient and control groups (P = 0.161 and 0.423, respectively; Table 2).

Nuclear pedigree study

Comparison of general information

The nuclear pedigree study included TS patients (n = 101) and their biological parents (n = 202). Of the TS patients, 80 were male, and 21 were female, at a ratio of 3.8:1. The mean age of TS patients was 11.26 ± 3.46 years.

Fitness of Hardy–Weinberg equilibrium test

Dopamine D3 receptor rs6280 genotype frequencies achieved HWE in both the TS patient group (P = 0.805) and parents group (P = 0.727), as shown by the χ^2-test [Table 3].

Comparison of genotype and allele frequencies in the Tourette syndrome patient and parents groups

The frequencies of genotypes C/C, C/T, and T/T were 10.9%, 42.6%, and 46.5%, respectively, in the TS patient group, and 23.8%, 43.6%, and 32.7%, respectively, in the parents' group. The C allele frequency was 32.2% and the T allele frequency 67.8% in both groups [Table 4].

Transmission disequilibrium test analysis

Transmission disequilibrium test analysis revealed no transmission disequilibrium in the DRD3 rs6280 SNP among TS nuclear pedigrees, indicating that this locus is not associated with TS (P = 1; Table 5).

Table 1: DRD3 rs6280 Hardy-Weinberg equilibrium test in the control and TS patient groups

Group	Genotype	Practical value	Expected value	χ^2	P
Control	C/C	12	8.40	3.195	0.074
	C/T	31	38.19		
	T/T	47	43.40		
TS patients	C/C	17	18.56	0.302	0.582
	C/T	75	71.87		
	T/T	68	69.56		

DRD3: Dopamine D3 receptor; TS: Tourette syndrome.

Table 2: Comparison of genotype and allele frequencies between the TS patient and control groups

Group	n	Genotype (%)			Allele (%)	
		C/C	C/T	T/T	C	T
Control	90	12 (13.3)	31 (34.4)	47 (52.2)	55 (30.6)	125 (69.4)
TS patients	160	17 (10.6)	75 (46.9)	68 (42.5)	109 (34.1)	211 (65.9)
χ^2		3.647			0.643	
P		0.161			0.423	

TS: Tourette syndrome.

Table 3: DRD3 rs6280 Hardy-Weinberg equilibrium test in the TS patient and parents groups

Group	Genotype	Practical value	Expected value	χ^2	P
TS patients	C/C	11	10.46	0.237	0.805
	C/T	43	44.08		
	T/T	47	46.46		
Parents	C/C	22	20.92	0.122	0.727
	C/T	86	88.17		
	T/T	94	92.92		

TS: Tourette syndrome.

Table 4: Comparison of genotype and allele frequencies in the TS patient and parents groups

Group	n	Genotype (%)			Allele (%)	
		C/C	C/T	T/T	C	T
TS patients	101	11 (10.9)	43 (42.6)	47 (46.5)	65 (32.2)	137 (67.8)
Parents	202	22 (23.8)	86 (43.6)	94 (32.7)	130 (32.2)	274 (67.8)

The number in brackets refers to the frequency (%).
TS: Tourette syndrome.

Discussion

Current molecular genetics methods for the detection of susceptible genes in complex diseases include linkage analyses and association studies,[16,17] the latter comprising either case–control or pedigree studies. Case–control studies compare differences in genotype and allele frequencies at candidate loci between cases and controls, so analyze the data of all subjects. However, they are limited in that the association analysis is affected by population genetic stratification and other factors.[18-20] Therefore, many association studies are difficult to repeat in different population. For this reason, pedigree studies are often preferred. These involve internal controls such as parents, as in TDT studies.[21,22] Pedigree studies effectively avoid the interference of population stratification;[23,24] however, the efficiency of TDT studies has been shown to be lower than the case–control studies.[25] The two types of analyses can

Table 5: TDT analysis in TS pedigree

Locus	Secondary allele	Main allele	Transmission	Nontransmission	*OR* (95% *CI*)	χ^2	*P*
rs6280	C	T	43	43	1 (0.723-1.345)	0	1

OR: Odds ratio; *CI*: Confidence interval; TS: Tourette syndrome; TDT: Transmission disequilibrium test.

complement each other, enabling the same genetic marker to be detected by both methods with more reliable results.

Dopamine D3 receptor is mainly located in the region of the brain associated with cognition and affection, and the DRD3 receptor is highly expressed in the mesolimbic dopamine system including the extended amygdala. The DRD3 Ser9Gly polymorphism (rs6280) involves a serine to glycine substitution that may affect the D3 receptor affinity. It has been widely used in studies addressing the genetic causes of neuropsychiatric disorders, including an investigation of susceptible loci correlations with psychiatric disease.[26]

Comings *et al*. previously identified an Association between TS and homozygosity at the DRD3 gene,[15] but this was not replicated in two studies published in the same year.[27,28] Brett *et al*. did not find the linkage and increased homozygosity for the DRD3 gene in TS. Hebebrand *et al*. had the same result like Brett *et al*. Devor *et al*. demonstrated linkage disequilibrium in DRD3 polymorphism loci BalI and MspI, which was not associated with TS in 16 pedigrees.[29] Díaz-Anzaldúa *et al*. found no association between TS and DRD3 in 110 nuclear pedigrees by TDT analysis,[30] while Kowalska *et al*. reviewed TS-susceptible genes and found no association between DRD3 and TS.[31] There has not been any report about the association study of TS and DRD3 gene polymorphisms in Han Chinese population.

This study aimed to explore the association between the DRD3 gene rs6280 and TS using both case–controlled study and nuclear pedigrees TDT analysis. The normal controls were well-matched with TS patients with respect to race, region, gender, and age, thus minimizing the influence of race, geography, and population stratification on experimental results, and increasing the reliability of our findings. We found no significant differences in genotype or allele frequencies between healthy controls and TS patients, while TDT analysis also revealed no transmission disequilibrium in nuclear pedigrees.

Few efforts have directly focused on the correlation between TS and DRD3, and this study was the first to do so in a Chinese Han population. Our combination of a case–control study and nuclear pedigrees TDT analysis produced a more reliable result, which suggests that the DRD3 SNP rs6280 may be not associated with TS in a Han Chinese population. The cause of inconsistent findings is complicated. The main is inconsistent with research method and different study populations. At the same time, TS is a heterogeneous disorder, often comorbidity with attention deficit hyperactivity disorder and obsessive-compulsive disorder. This is also an important reason for leading to inconsistent findings.

Our findings provide no evidence for an association between DRD3 gene rs6280 and TS in the Han Chinese population. It should be verified by making larger samples and making further efforts to subtype TS in future study.

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