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Research article

Lack of association between *SLC5A7* polymorphisms and Tourette syndrome in a Chinese Han population

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ABSTRACT

Although Tourette syndrome (TS) is a chronic neuropsychiatric disorder whose pathogenesis remains unclear, genetic factors play an important role in the occurrence and development. A variety of studies have been shown that the candidate genes related to cholinergic neurons may be associated with the onset of TS. To investigate the association between the *SLC5A7* polymorphisms and Tourette syndrome (TS) in the Chinese Han population, the SNP rs1013940, rs2433718, and rs4676169 were genotyped in 401 TS trios and 400 controls. The transmission disequilibrium test (TDT) and haplotype relative risk (HRR) compared genetic distributions of trios, while the chi-square test compared patients and controls. However, no transmission disequilibrium was found between the three *SLC5A7* SNPs and TS. Therefore, we think that this gene may not be the main risk factor on the onset of TS. However, these results should be further validated in different populations.

1. Introduction

Tourette syndrome (TS) is a chronic childhood-onset neurodevelopment illness characterized by involuntary multiple motor or vocal tics lasting for at least 1 year [15]. The prevalence of TS has increased from 1% to 3% year by year, and is more common in males than females which the ratio of the incidence is about 3–4:1 [21]. Most TS individuals have comorbid psychiatric disorders such as attention deficit hyperactivity disorder (ADHD), obsessive–compulsive disorder (OCD), impulsive and self-injurious behaviour [5], which together have severe effects on the patient's family and society. Although some earlier studies indicated that TS may be the result of an interaction between genetics, immunology, the environment, and hormonal factors [6,7,17], the precise disease aetiology and pathogenesis has remained unknown. However, pedigree and twin studies have confirmed that TS is a polygenic disease with a complex inheritance pattern and obvious genetic predisposition [27]. To date, many candidate genes have been studied such as dopamine receptors D1–D4, dopamine β–hydroxylase (*DBH*), monoamine oxidase A (*MAOA*), and serotonin receptor (*5-HT2A*) [19], but most of these candidate gene associations have not been replicated. The manifestation of TS is also associated with the dysfunction of monoaminergic systems, especially increased dopaminergic activity [26].

Several genes related to cholinergic neurons such as choline *O*-acetyltransferase (*CHAT*), solute carrier family 5, member 7 (*SLC5A7*), solute carrier family 18, member 3 (*SLC18A3*), and cholinergic receptor, muscarinic 2 (*CHRM2*) are significantly downregulated in the basal ganglia of TS mouse models [4,18,29]. This affects the synthesis of the neurotransmitter acetylcholine (ACh), which is responsible for the control of autonomic functions, learning, memory, and cognition. *SLC5A7*, also known as the choline transporter 1 gene (*CHT1*), is located on chromosome 2q12 and is divided into nine exons. It is a Na^+ and Cl^--dependent high-affinity presynaptic choline transporter that mediates choline uptake into cholinergic nerve terminals, which is a rate-limiting step for ACh synthesis [11]. Mouse experiments previously demonstrated a compensatory mechanism between *SLC5A7* and Ach, which suggested that *SLC5A7* expression increases to compensate for the dysfunction of other cholinergic neurons to maintain ACh synthesis and to sustain normal cholinergic signalling [3]. Although the symptoms of TS such as yawning, motion sickness, pain, and sensory tics may be related to the cholinergic system [23], this has not been proven. Taken together, we hypothesised that *SLC5A7* may affect the synthesis of ACh and be involved in the pathogenesis of TS.

In the present study, therefore, we investigated the potential association between three *SLC5A7* single nucleotide polymorphisms (SNPs) and TS in a Chinese Han population. We selected the SNP rs1013940

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(located in exon 3), rs2433718 (located in intron 1) and rs4676169 (located in intron 4) in *SLC5A7* which are all important tag SNPs in Chinese Han population. Moreover, they are good representatives for *SLC5A7* because they are independent inheritance from each other (the linkage disequilibrium tests between the three SNPs are 3%, 17% and 0.1%, respectively). In addition, there is little research on the association between these three SNPs and TS. To evaluate the role of *SLC5A7* in TS pathogenesis, we combined a family-based analysis with a case–control study which may supply an authentic interpretation for our hypothesis.

2. Materials

2.1. Subjects

We enrolled 401 TS trios and 400 healthy controls from the Affiliated Hospital of Qingdao University and Linyi People's Hospital, China. Patient diagnoses were made independently by two experienced psychiatrists using criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV). The control group we collected has excluded any symptoms of TS and other neuropsychiatric disorders. Moreover, all matched to the TS patients in relation to gender, geographic region and ethnic origin. As TS is an early onset disorder, age was not taken as a matching factor. All participants or their legal guardians gave their informed written consent, and the Human Ethics Committee of the Affiliated Hospital of Qingdao University approved the study project.

2.2. Genotyping analysis

Blood samples were collected from all participants and stored at −20 °C until analysed. Genomic DNA was extracted from 300 μl peripheral blood leukocytes using a DNA extraction kit (Qiagen, Hilden, Germany). Alleles of *SLC5A7* SNPs rs1013940, rs2433718, and rs4676169 were determined by TaqMan allelic discrimination real-time PCR. Taqman probes and primers were designed by Applied Biosystems of Life Technologies. For rs1013940, forward and reverse primers were 5′-TGGCCTAGCTTGGGCTCAGGCACCA-3′ and 5′-TTGGATATTCT CTTAGTCTGATTTT-3′, respectively; for rs2433718, these were 5′-GAAATGGGTAGAGGGGGCCGAGGAA-3′ and 5′-GGCCGCAGG GGGCCGGGAGAGCATC-3′, respectively; and for rs4676169, these were 5′-AAACTACCTAAACTCCTGAGACAAC-3′ and 5′-GTGTTGCATGTT ATTCTAAAAGCAC-3′, respectively. PCR was conducted in 25 μl reactions mixture, containing 20 × SNP Genotyping Assay 1.25 ul, 2 × PCR Master Mix 12.5 ul, DNA and DNase-free water 11.25 ul. Amplifications were carried out using a C1000TM thermal cycler system with the following conditions: 95 °C for 3 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. All steps were carried out with relevant guidelines and regulations, and the fluorescent signal from VIC- or FAM-labelled probes could be detected in each cycle. The discrimination of genotypes was conducted using Bio-Rad CFX manager 3.0 software.

2.3. Statistical analysis

All analyses were performed by the statistical software package SPSS21.0. Hardy–Weinberg equilibrium (HWE) was examined in control groups using the chi-square test. Case–control and family-based studies were used to detect the association between the three *SLC5A7* SNPs and TS, and all data were tested by means of haplotype relative risk (HRR) and the transmission disequilibrium test (TDT). To increase the efficiency, we enlarged cases by haplotype-based haplotype relative risk (HHRR). Allelic and genotypic distributions of patients and controls were compared using the chi-square test, with P values < 0.05 considered to be significant.

3. Results

3.1. Case–control study

The study group consisted of 401 patients (319 males, 82 females; mean age, 8.87 ± 3.24 years) and 400 controls (310 males, 90 females; mean age, 33.00 ± 8.44 years). The distribution of genotypic frequencies for all three SNPs in control groups was consistent with the Hardy-Weinberg equilibrium, suggesting that the population was genetically balanced and could be used for association studies (for rs1013940, $\chi^2 = 3.457$, $P = 0.063$; for rs2433718, $\chi^2 = 2.171$, $P = 0.141$; for rs4676169, $\chi^2 = 1.012$, $P = 0.314$).

To estimate whether there was a difference between cases and controls, the allelic and genotypic distributions of the two groups were compared by the chi-square test which indicated that there was no significant difference (for rs1013940, genotype: $\chi^2 = 0.622$, $P = 0.733$, allele: $\chi^2 = 0.257$, $P = 0.612$, OR = 1.079, 95% CI = 0.804-1.449; for rs2433718, genotype: $\chi^2 = 3.405$, $P = 0.182$, allele: $\chi^2 = 3.465$, $P = 0.063$, OR = 1.211, 95% CI = 0.990–1.482; for rs4676169, genotype: $\chi^2 = 2.908$, $P = 0.234$, allele: $\chi^2 = 2.918$, $P = 0.088$, OR = 0.830, 95% CI = 0.670–1.028). The results are shown in Table 1.

3.2. Family-based study

A total of 401 trios took part in the family-based study. No significant statistical significance of allele transfer was determined for the three SNPs (for rs1013940, TDT = 0.268, $P = 0.657$, OR = 0.728, 95% CI = 0.366–1.451; HRR = 0.959, $\chi^2 = 0.111$, $P = 0.739$, 95% CI = 0.762–1.466; for rs2433718, TDT = 0.968, $P = 0.351$, OR = 1.120, 95% CI = 0.839–1.496; HRR = 1.089, $\chi^2 = 0.341$, $P = 0.559$, 95% CI = 0.818–1.450; for rs4676169, TDT = 3.440, $P = 0.072$, OR = 1.027, 95% CI = 0.739–1.428; HRR = 1.185, $\chi^2 = 1.441$, $P = 0.230$, 95% CI = 0.898–1.563). Genotypic and allelic frequencies are presented in Tables 2 and 3.

To increase the test efficiency, we enlarged case numbers by HHRR and obtained similar results for these SNPs, which confirmed the absence of transmission disequilibrium in genotypic and allelic frequencies (for rs1013940, HHRR = 1.082, $\chi^2 = 0.276$, $P = 0.599$, 95%

Table 1
The genotypic and allelic frequencies of three genetic loci in two groups.

Group	N	rs1013940					rs2433718					rs4676169				
		CC	CT	TT	C	T	CC	CT	TT	C	T	AA	AG	GG	A	G
Patients	401	9	87	305	105	697	71	184	146	326	476	192	160	49	544	258
Control	400	10	78	312	98	702	59	171	170	289	511	210	154	36	574	226
χ^2		0.622			0.257		3.405			3.465		2.908			2.918	
P-value		0.733			0.612		0.182			0.063		0.234			0.088	
OR					1.079					1.211					0.830	
95%CI					0.804–1.449					0.990–1.482					0.670–1.028	