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Association of Genetic Variations in X-Ray Repair Cross-Complementing Group 1 and Tourette Syndrome

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Background: X-ray repair cross-complementing group 1 (*XRCC1*) plays a central role in mammalian DNA repair process. The polymorphism rs25487 (Arg>Gln at codon 399) of this gene is common in Han Chinese population. *Objectives:* The objective of this study was to analyze the association between this functional SNP of *XRCC1* and Tourette syndrome (TS) in Han Taiwan Chinese population. *Methods:* Genotyping was performed by using PCR–RFLP method on 73 TS patients and 158 normal controls. *Results:* Our data indicated that genotype frequency of A/G polymorphism at codon 399 of the patients differed from the controls ($P = 0.026$, OR: 2.22, 95% CI: 1.22–4.03). The allele frequency analysis also showed significant differences with higher A allele frequency in patients ($P = 0.015$, OR: 1.70, 95% CI: 1.11–2.62). *Conclusion:* Our study indicates that the functional SNP at codon 399 of *XRCC1* is associated with TS development. J. Clin. Lab. Anal. 26:321–324, 2012.

Key words: X-ray repair cross-complementing group 1; *XRCC1*; Tourette syndrome (TS); polymorphism

INTRODUCTION

Tourette syndrome (TS) is an inherited neuropsychiatric disorder, which is defined as a part of a spectrum of tic disorders that includes transient and chronic tics. The most common, first-presenting tics are eye blinking, facial movements, sniffing, and throat clearing. Previous studies on the tics of TS indicated sensory phenomena as the core symptom of the syndrome, even though they are not included in the diagnostic criteria (1–3). As compared to other movement disorders, e.g., choreas, dystonias, myoclonus, and dyskinesias, the tics of TS are stereotypic, temporarily suppressible, nonrhythmic, and often preceded by an unwanted premonitory urge (4). These abnormal behaviors are believed to result from dysfunction in cortical and subcortical regions in the brain, including the thalamus, basal ganglia, and frontal cortex (5, 6).

Genetic and environmental factors have been proposed to play roles in the etiology of TS, although the exact causes are largely unknown (7). Patients with TS

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syndrome could be found in all ethnic groups, genders, as well as in children and adults. Environmental factors, including smoking, stress, and infections, also have been implicated in the increased incidence of TS development. Family tree analyses have shown that the overwhelming majority of cases of TS are inherited and more efforts are needed to identify the key genes involved in the pathogenesis of this syndrome (8). Due to lack of understanding of the molecular mechanisms, no specific screening test can be used in the diagnosing TS and the medication is usually not effective (9). Functional magnetic resonance imaging (fMRI) studies suggested that TS may be caused by impaired modulation of a neural circuit involved in behavioral inhibition (10–12). Since bioactivity of neuron cells determines the formation and function, genes involved in regulation of neuron death may play certain roles in pathogenesis of TS.

In particular, neurons have been shown to be more vulnerable than other cell types to DNA-damaging conditions such as oxidative stress. Increasing evidence suggests that the accumulation of damaged DNA may contribute to neuronal loss in neurodegenerative disorders (13–15). There are three major mechanisms involved in the repair of DNA damages, the base excision repair (BER), nucleotide excision repair (NER), and double-strand break (DSB) repair by the homologous recombination or nonhomologous end joining pathways. Recent studies further indicate that chromosomal fragility and translocations have been found in some TS patients (16–18), suggesting the crucial roles of genome instability during TS development. X-ray repair cross-complementing group 1 (*XRCC1*) is an important component to regulate BER pathways, because it operates as a scaffold protein to interact with other key proteins, such as DNA ligase III and DNA polymerase β, to synthesize and rejoin the DNA strand break site (19). It is possible that repair capability of *XRCC1* in BER may influence the accumulation of damaged DNA, which results in neuronal death and deficiency of brain function. Therefore, since *XRCC1* is a critical single-strand break repair protein that orchestrates efficient damage repair at DNA break points, the main goal of this study is to know whether genetic variations in *XRCC1* gene determine the susceptibility to TS.

MATERIALS AND METHODS

Patients

The study subjects with a total of 73 TS patients were recruited from China Medical University Hospital in Taiwan. The 158 healthy controls were selected from the general population with similar age profile who had regular health examination at the same hospital. This study was approved by the Institutional Review Board (IRB) at China Medical University Hospital prior to patient enrollment. All the individuals including patients and healthy controls signed a consent form, and blood samples were collected by venipuncture for genomic DNA isolation and preparation.

Genomic DNA Extraction and *XRCC1* Genotyping

Peripheral venous blood samples from patients and healthy controls were collected in ethylenediaminetetraacetic acid (EDTA) tubes. After blood cell separation by centrifugation, genomic DNA was then purified from the buffy coat using Qiagen genomic DNA isolation kit (Valencia, CA). DNA fragments containing the rs25487 polymorphism was amplified by Polymerase chain reaction (PCR) and detected by Restriction fragment length polymorphism (RFLP) by digesting DNA with *MspI* restriction enzyme digestion. The conditions for PCR were initial denaturation at 95°C for 5 min; 40 cycles for DNA amplification by denaturation at 95°C for 10 sec, followed by annealing at 58°C for 10 sec, and extension at 72°C for 20 sec; and then one cycle of final extension at 72°C for 5 min. The sequences of the primers for RFLP analyses were forward 5′-CCCCAAGTACAGCCAGGTC-3′ and reverse 5′-TGTCCCGCTCCTCTCAGTAG-3′.

Statistical Analysis and Clinical Association

Statistical analysis was performed by the Chi-square method using SPSS software (version 10.0) to study the genotype and allelic frequency distributions for this functional polymorphism in both TS patients and controls. A P value less than 0.05 was considered statistically significant. Significance was calculated with the use of 2×2 and 2×3 contingency tables to contingency table to obtain P values, odds ratios (OR), and 95% confidence intervals (95% CI).

RESULTS

Genotype analyses indicated a significant difference between TS patients and the controls in the frequency of A/G at codon 399 of *XRCC1* ($P = 0.026$, OR: 2.22, 95% CI: 1.22–4.03) (Tables 1 and 2). In addition, allele frequency analyses revealed a significant difference at codon 399 between the patients and controls ($P = 0.015$, OR: 1.70, 95% CI: 1.11–2.62) (Tables 1 and 2). TS patients tended to have a higher frequency to carry an A allele at this locus. Interestingly, our data indicate that A/G genotype at codon 399 is very unique in TS patients as compared to other genotypes. Individuals with A/A genotype did not show statistically higher risk of TS development than people with A/G or G/G genotypes (Table 1).

TABLE 1. Distribution of Genotypes among the Tourette Syndrome Patients and Healthy Control Subjects

Tourette syndrome patients *n* (%)		Normal controls *n* (%)	*P* [a]
Genotype			0.026
A/A	8 (10.96)	13 (8.22)	
A/G	34 (46.58)	48 (30.38)	
G/G	31 (42.47)	97 (61.39)	
Allelic frequency			0.015
Allele A	50 (34.25)	74 (23.42)	
Allele G	96 (65.75)	242 (76.58)	

[a] *P* values were calculated by χ^2 test.

TABLE 2. Odds Ratio and 95% Confidence Interval of *XRCC1* Gene 399A/G Polymorphism

Genotype	Odds ratio (95% CI)
A/A	1.93 (0.72–5.15)
A/G	2.22 (1.22–4.03)
G/G	1 (reference)
Per copy of Allele A	1.70 (1.11–2.62)
Per copy of Allele G	1 (reference)

CI = confidence interval.

DISCUSSION

This study is the first assay of the *XRCC1* gene Single-nucleotide polymorphism (SNP) (rs25487) in TS patients. It is demonstrated that *XRCC1* SNP is associated with TS in the Chinese population in Taiwan. The *XRCC1* gene SNP substitution caused Arg to Gln change at codon 399 is one of the most extensively studied SNPs of XRCC1 gene and reported to highly correlate with the progression of some diseases, including cancer (20) and end-stage renal disease (ESRD) (21). Thus, we chose codon 399 to perform an associative study to explore the etiology of TS.

On the basis of mRNA level study, *XRCC1* gene polymorphism at codon 399 did not change the *XRCC1* gene expression (22). However, individuals with A allele rather than G allele seem to have higher risk to development of carcinoma (23), suggesting that G to A substitution of *XRCC1* gene, which causes Arg to Gln amino acid change at codon 399, may alter DNA repair activity of *XRCC1* and thus modulate cancer susceptibility (24). Other studies in DNA repair activity of *XRCC1* showed that individuals with homozygous carriers of A allele for *XRCC1* polymorphism at codon 399 had higher tendency to accumulate DNA adducts after the treatment of DNA damage-inducing agents (25). Lunn et al. also demonstrated that *XRCC1* polymorphism at codon 399 was associated with higher levels of DNA damage and A allele homozygotes showed significant association with higher levels of aflatoxin B1–DNA adducts and glycophorin-A somatic variants, suggesting that codon change might alter the *XRCC1* function and result in deficiency of DNA repair (26). Our data showed that A allele is a risk allele that is highly correlated with having TS and individuals with A/G genotype at codon 399 presented higher risk of TS development than people with A/A or G/G genotypes. One possible explanation for these findings would be that the SNP analyzed in this study is in tight linkage disequilibrium with other unknown allele variants that impart an opposite effect. Therefore, only in the heterozygote A/G individual, the compound phenotype can be observed. Although this interpretation favors our finding, more detailed studies are needed to further investigate the molecular mechanisms controlled by *XRCC1* genetic variants.

In recent years, TS has been defined as one kind of autoimmune diseases induced by a series of immunopathogenic mechanisms (27, 28). Autoantibodies (Abs) against brain self-epitopes could be detected in the sera of TS patients (27,29,30). Notably, it has been known that DNA breaks mixed with nuclear proteins are strong immunogens for eliciting autoreactive Abs (31, 32), suggesting that DNA repair efficiency in cells is a determining factor for the development of autoimmune diseases. Several components involved in DNA repair systems, such as XRCC5 and XRCC4 in recombinational repair, have been found to trigger auto-Abs. This study is the first attempt to understand whether a DNA repair protein such as *XRCC1* could play important roles during TS development. Our data indicated the involvement of genetic variations of *XRCC1* in the susceptibility to TS. It is therefore interesting to further study the associations of other DNA repair proteins and TS development.

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