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Association of Glutathione S-Transferase P1 (*GSTP1*) Polymorphism with Tourette Syndrome in Taiwanese Patients

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The etiology of Tourette syndrome (TS) is multifactorial. TS vulnerability may be associated with genetic and environmental factors. From the genetic point of view, TS is heterogeneous. Previous studies showed that some single-nucleotide polymorphisms (SNPs) of the glutathione-S-transferase P1 (*GSTP1*) gene can affect cellular proliferation and apoptotic activity and TS is a neurodevelopmental disorder. We guessed that there was a relationship between TS and genetic variants of the *GSTP1* gene. Therefore, in this study, we aimed to test the hypothesis that *GSTP1* SNPs were associated with TS. We performed a case–control study. One hundred twenty-one TS children and 105 normal children were included in the study. Polymerase chain reaction was used to identify the *GSTP1* gene polymorphism at position rs6591256 (A/G, promoter polymorphism) in TS patients and normal children. The polymorphism at position rs6591256 in the *GSTP1* gene revealed significant differences in the allele ($p = 0.0135$) and genotype ($p = 0.0159$) distributions between the TS patients and the control group. The A allele was present at a higher frequency than the G allele in the TS patients compared with the control group (odds ratio [OR] = 1.91, 95% confidence interval [CI]: 1.14–3.21). The AA genotype was associated with susceptibility to TS with an OR of 2.38 for the AA versus AG genotype (95% CI: 1.29–4.41). These findings suggest that variants in the *GSTP1* gene may play a role in susceptibility to TS.

Introduction

Tourette syndrome (TS) is a neurologic disorder characterized by involuntary repetitive motor or vocal tics. Children with TS usually have other associated conditions, such as obsessive–compulsive disorder, attention deficit hyperactivity disorder, anxiety disorders, learning difficulties, sleep abnormalities, and depression. To date, the precise etiology of TS is still unknown, but there is evidence demonstrating that the etiology of TS may be associated with genetic (O'Rourke *et al.*, 2009) and environmental (Conelea and Woods, 2008) factors. From the genetic point of view, TS is a heterogeneous familial disorder. However, no gene has been identified as a causative TS susceptibility gene. Therefore, we assumed some other genes might be involved in the pathogenesis of TS.

Glutathione-S-transferase P1 (GSTP1), a member of the glutathione-S-transferase (GST) π class, participates in the detoxification of oxidative stress products (Hayes and McLellan, 1999). It catalyzes the conjugation of glutathione with a variety of toxic electrophilic substances to form nontoxic derivatives (Parl, 2005). GSTP1 can affect cell proliferation and apoptosis through modulating the activity of c-Jun N-terminal kinases (JNKs) (Thevenin *et al.*, 2011). Previous experiments demonstrated that an increase in expression of GSTπ can protect PC12 cells against dopamine-induced apoptosis through suppressing JNK activity (Ishisaki *et al.*, 2001).

TS is a neurodevelopmental disorder, so we guessed that variants of genes modulating neural proliferation or apoptosis might be involved in the etiology of TS. There is some indirect evidence suggesting that *GSTP1* single-nucleotide

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polymorphisms (SNPs) may play a role in TS. The basal ganglia are considered candidate loci of pathology in TS. Brain magnetic resonance (MR) images show that the lenticular and caudate nuclei of basal ganglia are smaller in TS patients than in healthy persons (Peterson *et al.*, 1993, 2003; Singer *et al.*, 1993) and TS patients have structural abnormalities in the substantia nigra (Davila *et al.*, 2010). These data imply that genes modulating cell proliferation or apoptosis may be involved in the pathogenesis of TS. It is already known that GSTP1 not only has neuroprotective effects due to its detoxification ability (Baez *et al.*, 1997), but also affects cell proliferation or/and apoptosis (Thevenin *et al.*, 2011). In addition, previous experiments demonstrated that dopamine neurotransmission is abnormal in TS (Wong *et al.*, 2008) and GSTπ is the only isoenzyme expressed in substantia nigra neurons (Smeyne *et al.*, 2007), which are dopaminergic neurons. In addition, it has been showed that the polymorphism in the *GSTP1* gene (I105V) causes a significantly lower enzyme activity of GSTP1 and less effective capability of detoxification (Hu *et al.*, 1998) and that the V105/V114 haplotype of GST π is a more potent inhibitor of JNK activity than the wild-type haplotype (I105/A114) (Thevenin *et al.*, 2011).

SNPs located at the promoter can affect the expression of a gene. It has been shown that three frequently observed haplotypes of the promoter region of the *GSTP1* gene have different mRNA expression and protein levels of GSTP1 (Cauchi *et al.*, 2006). SNPs located in the open reading frame, such as the SNP I105V, may affect the function of a protein (Hu *et al.*, 1998). Therefore, studying the effects of promoter polymorphisms on the etiology of TS is as important as those of polymorphisms in the open reading frame. So far, nobody knows the effects of promoter polymorphisms, such as SNP rs6591256 (Koutros *et al.*, 2009), on the GSTP1 protein chemical properties, structure, and function. However, the SNPs rs6591256 and rs6591255 are located in the same haplotype block (Tan *et al.*, 2009). Previous studies show that the SNP rs6591256 or rs6591255 is associated with asthma, wheezing, and prostate cancer outcomes (Li *et al.*, 2008; Islam *et al.*, 2009; Koutros *et al.*, 2009). In addition, three frequently observed haplotypes in the promoter region of the *GSTP1* gene have different mRNA expression and protein levels of GSTP1 (Cauchi *et al.*, 2006). These studies imply that the SNP rs6591256 or rs6591255 might affect mRNA expression and protein levels of GSTP1. Therefore, we chose the SNP rs6591256 as a tag SNP to test whether the *GSTP1* SNP had an association with TS.

Materials and Methods

Subjects

All participants enrolled in this study were recruited at the China Medical University Hospital in Taiwan. The institutional ethics committee approved the project, with informed consents obtained from all subjects. One hundred and twenty-one unrelated children with Tourette syndrome and one hundred and five age-matched normal controls were enrolled in this study. The diagnosis of TS was evaluated at the Department of Pediatric Neurology and followed the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition (DSM-IV). These criteria include the presence of multiple motor and at least one vocal tic (not necessarily concurrent); a waxing and waning course, with tics evolving in a progressive manner; the presence of tic symptoms for at least 1 year; the onset of symptoms before 21 years of age; the absence of a precipitating illness (e.g., encephalitis, stroke, or degenerative disease) or medication; the observation of tics by a knowledgeable neurologist; and marked distress or significant impairment in social, occupational, or other important areas of functioning.

Determination of GSTP1 *gene variant by polymerase chain reaction–restriction fragment length polymorphism*

Genomic DNA was extracted from peripheral blood samples by a Genomic DNA extraction kit (Blossom, Taipei, Taiwan). A SNP near the 5′ region (rs6591256) was selected from a public dbSNP database. To identify allele preference for the SNP, polymerase chain reaction (PCR)–restriction fragment length polymorphism was designed for genotype analyses. In brief, PCRs were performed in a total volume of 25 μL, containing 50 ng of genomic DNA and specific primers (forward 5′-TGG ATT TCT GTT CCT GCA TT-3′ and reverse 5′-TGA TTT GCA GAT CTC CAT TTA TTC-3′). PCR amplification protocol was set as 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min, and one last elongation step at 72°C for 7 min. A 310 bp size of PCR product was generated and genotyping was performed by the restriction enzyme *Dra*I (New England Biolabs) in a total volume of 20 μL at 37°C overnight. The digested fragments were ascertained by agarose gel electrophoresis and stained with ethidium bromide.

Statistical analysis

Adherence to Hardy–Weinberg equilibrium was tested to ensure data quality by using the Hardy–Weinberg equilibrium test. The hypotheses that whether the distributions of allele and genotype frequencies in the case and control groups were the same were tested using the chi-square test and the Fisher–Freeman–Halton test, respectively. In addition, we used the chi-square test to analyze whether *GSTP1* SNP rs6591256 (A > G) had an association with TS under the dominant model of inheritance. The Cochran-Armitage trend test was used under the additive model, and the Fisher's exact test was used under the recessive model. Statistical analysis of the odds ratios (OR) and 95% confidence interval (CI) were carried out with SPSS version 10.0 software based on the reference allele and genotype frequencies. Statistical analysis considered only *p*-values below 0.05 as significant. Adherence to Hardy–Weinberg equilibrium constant was tested using the χ^2 test with one degree of freedom.

Results

To ensure data quality, we evaluated whether the distribution of genotypes in the case and control groups was in the Hardy–Weinberg equilibrium. The distribution of all genotypes in case and control groups was consistent with the Hardy–Weinberg equilibrium ($p = 0.1919$ and 0.7589, respectively, Table 1). These results indicated that the data were of good quality and could be used for association studies.

To know whether *GSTP1* SNP rs6591256 (A > G) was associated with TS, the Fisher–Freeman–Halton test was used because the mode of inheritance for this SNP was unknown and this test is a disease model-free approach. The results

Table 1. Genotype and Allele Frequencies of *GSTP1* Polymorphism rs*6591256* (A>G) in Tourette Syndrome Patients and Controls

Test	*Genotype/allele*	*Cases*, n *(%)*	*HWE*[a]	*Controls*, n *(%)*	*HWE*	p-*Value*	*OR (95% CI)*
Genotypic association	AA	96 (79.3)	0.1919[a]	66 (62.9)	0.7589[a]	**0.0159**[b]	2.38 (1.29–4.41)
	AG	22 (18.2)		36 (34.3)			1
	GG	3 (2.5)		3 (2.9)			1.63 (0.30–8.83)
Additive model	AA	96 (79.3)		66 (62.9)		0.0501[c]	
	AG	22 (18.2)		36 (34.3)			
	GG	3 (2.5)		3 (2.9)			
Dominant model	GG + AG	25 (20.7)		39 (37.1)		**0.0061**[d]	
	AA	96 (79.3)		66 (62.9)			
Recessive model	GG	3 (2.5)		3 (2.9)		≈1[e]	
	AA + AG	118 (97.5)		102 (97.1)			
Allelic association (multiplicative model)	A	214 (88.4)		168 (80.0)		**0.0135**[d]	1.91 (1.14–3.21)
	G	28 (11.6)		42 (20.0)			1

[a]HWE: *p*-values of the Hardy–Weinberg equilibrium test.
[b]The *p*-value was calculated using the Fisher–Freeman–Halton test. *p*-Values less than 0.05 are highlighted in bold.
[c]The *p*-value was calculated using the Cochran–Armitage test.
[d]*p*-Values were calculated using the χ^2 test.
[e]*p*-Values were calculated using the Fisher's exact test.
OR, odds ratio; 95% CI, 95% confidence interval.

(Table 1) showed that this SNP had an association ($p = 0.0159$) with TS. In addition, marked differences appeared among the distributions of AA, AG, and GG genotype frequencies ($p = 0.0159$). The AA genotype was associated with susceptibility to TS with an OR of 2.38 for the AA versus AG genotype (95% CI: 1.29–4.41). In addition, there was a statistical difference in the allele frequency distribution ($p = 0.0135$) between cases and controls. OR of the A allele presented 1.91-fold higher risk (95% CI: 1.14–3.21) than the G allele. Next, we tried to ask which mode of inheritance best explained this association. The data showed that the dominant ($p = 0.0061$) or multiplicative ($p = 0.0135$) model might explain this association. Altogether, SNP rs6591256 had an association with TS, and subjects with A allele or with AA genotype in the *GSTP1* gene tended to have a higher incidence of pathogenic TS.

Discussion

This study showed that *GSTP1* SNP (rs6591256, A/G) had a protective effect on the risk of TS (Table 1). The A allele (major allele) or the AA genotype of *GSTP1* SNP (rs6591256) was a risk factor for TS. Although statistical analysis showed that SNP rs6591256 might be associated with TS under the dominant ($p = 0.0061$) or multiplicative ($p = 0.0135$) model, this protective SNP was more likely to follow the dominant mode of inheritance because the ORs of AA, AG, and GG are 2.38 (95% CI: 1.29–4.41), 1, and 1.63 (95% CI: 0.30–8.83), respectively (Table 1). If the genetic model for allele G is dominant (i.e., allele A is recessive.), there is an increase in disease risk for the AA genotype (Clarke *et al.*, 2011).

The data showed that SNP rs6591256 had an association with TS. It implied that the pathophysiology of TS may involve the detoxification system. Previous experiments showed that a genetic variation in the DNA repair gene *XRCC1* (X-ray repair cross-complementing group 1) has an association with TS (Lin *et al.*, 2012) and that XRCC1 is involved in DNA single-strand break and base excision repair to preserve genetic stability (Brem and Hall, 2005) after oxidative DNA damage. However, so far there is little evidence to support the hypothesis that oxidative stress is a risk factor for the development of TS.

Another explanation of the relationship between *GSTP1* SNPs and TS is that *GSTP1* polymorphisms affect cell proliferation or apoptosis during brain development. Brain MR images show that the lenticular and caudate nuclei of basal ganglia are smaller in TS patients (Peterson *et al.*, 1993; Kataoka *et al.*, 2010) The reduced volume may be the result of a faulty inhibitory (GABAergic) circuitry found in some patients. A faulty inhibitory circuitry, possibly caused by a developmental defect in GABAergic neurons, may lead to decreased number of neurons, and hence, the reduced volume in the globus pallidus pars externa and in the caudate (Kalanithi *et al.*, 2005). As the wild-type haplotype (I105/A114) of GSTP1 is a less potent inhibitor of JNK activity than the V105/V114 haplotype (Thevenin *et al.*, 2011), there may be reduced cellular proliferation as a result of the increased JNK activity (Holley *et al.*, 2007). It has been shown that inhibition of GSTP1 can activate JNK and result in cell apoptosis (Bernardini *et al.*, 2000). In our study, we showed that the A allele (major allele) or AA genotype was a risk factor for TS. These genotypes may similarly result in increased JNK activity, hence apoptosis and reduced cell proliferation as the I105/A114 haplotype. In contrast, the A/G haplotype of *GSTP1* (SNP rs6591256, A/G) would presumably decrease the susceptibility of TS because of its effects on JNK kinase activity, cell proliferation, and apoptosis.

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Author Disclosure Statement

No competing financial interests exist.

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