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Research article

Association of the *AADAC* gene and Tourette syndrome in a Han Chinese cohort

Lamei Yuan[a,b], Wen Zheng[b], Zuocheng Yang[c], Xiong Deng[a], Zhi Song[b], Hao Deng[a,b,*]

[a] Center for Experimental Medicine, The Third Xiangya Hospital, Central South University, Changsha, China
[b] Department of Neurology, The Third Xiangya Hospital, Central South University, Changsha, China
[c] Department of Pediatrics, The Third Xiangya Hospital, Central South University, Changsha, China

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ABSTRACT

Tourette syndrome (TS) is a complex neuropsychiatric disorder with chronic motor and vocal tics. Though the etiology is elusive, strong evidence for a genetic contribution to TS has been established. To date, various chromosomal or genetic alterations have been implicated in its pathogenesis. Recently, the deletion in the arylacetamide deacetylase gene (*AADAC*) was reported to be associated with TS. To investigate the association between the *AADAC* gene variants and TS, we conducted genetic analysis of the *AADAC* gene in 200 Han Chinese patients and 300 ethnicity-matched normal controls. Two variants, including a heterozygous splice-site variant, c.361 + 1G > A (rs762169706), and a missense variant, c.744A > T (p.R248S, rs186388618), were identified in two unrelated patients. The c.361 + 1G > A variant, absent in 300 ethnicity-matched controls, led to the deletion of exon 2 in *AADAC* mRNA, probably associated with development of TS. The c.744A > T variant, predicted to be damaging, was identified in two normal controls. The findings indicate that the *AADAC* gene c.361 + 1G > A variant may be a potential candidate factor for TS development, though further investigations are warranted.

1. Introduction

Tourette syndrome (TS, MIM 137580) is a complex developmental, neuropsychiatric disorder characterized by the presence of chronic motor and vocal tics, frequently comorbid with a variety of behavioral abnormalities, such as obsessive-compulsive disorder, attention-deficit-hyperactivity disorder and impulse control disorders [1–5]. Often arising in childhood, tics may remit with age in some cases [[1–5],6]. The estimated prevalence of TS ranges from 0.4% to 3.8% [2]. The disorder is sex-related, which affects boys 3–4 times more frequently than girls [7,[1–5]]. It is a complex, heterogeneous disorder related to the combined effects of monogenic, multigenic and environmental causes, and genetic contribution to TS has been established with strong evidence [[1–5],[1–5],9]. To date, many chromosomal abnormalities, genetic loci, and genes have been implicated in TS pathogenesis, though subsequent studies have failed to fully replicate them, confounded by heterogeneity, comorbidity, and the complex multigenic inheritance of the disorder, environmental and epigenetic factors, and gene-environment interaction [[1–5],8–10]. Multiple investigations in neuroimaging, neuroanatomy or neurophysiology have revealed the involvement of cortico-striato-pallido-thalamo-cortical circuits and abnormalities of neurotransmitter systems in TS etiology [11–13]. In two previous studies, deletions in the 5′ region or the full region of the arylacetamide deacetylase gene (*AADAC*) were present in TS patients [[11–13],6]. Recently, an extended study confirmed a significant association between the *AADAC* gene deletion and TS in a large European cohort [10], which aroused our interest in evaluating the association between the *AADAC* gene and TS in Han Chinese population.

2. Material and methods

A total of 500 unrelated Han Chinese individuals residing in mainland China were recruited for the study. In the cohort, 200 unrelated Han Chinese patients, including 161 males and 39 females (age: 10.8 ± 5.8 years, age at onset: 8.1 ± 4.5 years, familial/sporadic cases: 49/151) and 300 gender-, age-, ethnicity-matched normal persons (male/female: 242/58, age: 10.9 ± 5.9 years) were included. The patients with TS were enrolled from the Department of Pediatrics and Department of Neurology, the Third Xiangya Hospital of Central South University, Changsha, China. The diagnosis was made by two

Abbreviations: *AADAC*, the arylacetamide deacetylase gene; ExAC, Exome Aggregation Consortium; *HDC*, the histidine decarboxylase gene; *HTR2B*, the 5-hydroxytryptamine receptor 2B gene; PCR, polymerase chain reaction; TS, Tourette syndrome

* Corresponding author at: Center for Experimental Medicine, The Third Xiangya Hospital, Central South University, 138 Tongzipo Road, Changsha, Hunan 410013, China.
E-mail address: hdeng008@yahoo.com (H. Deng).

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Table 1
Primer sequences for the *AADAC* gene.

Exon	Forward primer (5′→3′ on plus strand)	Reverse primer (5′→3′ on minus strand)	Product size (bp)
1	TTCAAAGCTTTTGGGTGGAG	TCTCTGGGTCAAATGGTGGT	307
2	CCATAGATCTCTTTGCCATTTG	AGTAATGTCCATGCCGAAGG	383
3	GTGCAGCAGGATTTTTGTGA	CTGGGTGACAGGGTGAGACT	264
4	TGTGCACCTCCAGCATGTAT	AACAGCGTAGAAACGCTATTTTT	352
5	TTGTGCAAATCATCTTGCTTC	GTCAGGGGTAAGCCACGTAA	426
5	TCCAGGGTTCCTAGATGTGAG	TGCAAATTTTCTGAGGTTTTCA	328

AADAC, the arylacetamide deacetylase gene.

independent pediatricians and neurologists with expertise in TS, according to common clinical diagnostic criteria [1,14,15]. Fifty percent (100/200) of the patients were previously excluded for coding mutations in the histidine decarboxylase gene (*HDC*) and the 5-hydroxytryptamine receptor 2B gene (*HTR2B*) [15,16]. The controls were individuals presenting for routine health checkups or volunteers without similar symptoms and any positive family history of neurological disorders. All protocols for the study were approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University, Changsha, China, following the Declaration of Helsinki guidelines. Informed written consent was obtained from the participants or their legal guardians, and then peripheral blood was drawn and processed. All methods were performed in accordance with the approved guidelines.

Genomic DNA samples were isolated from peripheral blood of all participants, via the standard phenol-chloroform extraction procedures [17]. Primers covering the coding region and exon-intron boundaries of the *AADAC* gene (NM_001086.2) were designed using the online Primer3 program (http://primer3.ut.ee/) and synthesized as required (Table 1) [18]. Polymerase chain reaction (PCR) and direct Sanger sequencing were performed in all the patients as described [15,19]. The pathogenicity of the identified variants was predicted by three bioinformatics prediction tools, including Sorting Intolerant from Tolerant (http://sift.jcvi.org/), Polymorphism Phenotyping version 2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster (http://www.mutationtaster.org/) [20,21]. For the identified variants around the exon-intron boundaries, the potential to change splicing was predicted by the online tool (http://www.fruitfly.org/seq_tools/splice.html). The *AADAC* mRNA was extracted from peripheral blood lymphocytes of the patient. Reverse transcription PCR and Sanger sequencing of PCR products were subsequently conducted for the variant with a potential to affect splicing [16,17]. Locus-specific primers were shown as follows: 5′-TACGCCTCTCCCAGATAACG-3′ and 5′-TTGGTTGATACGACGAC AGC-3′. For gene variants identified in the patient group, normal control subjects were further analyzed.

3. Results

Sequencing of the coding region of the *AADAC* gene in patients with TS revealed two variants. A heterozygous splice-site variant, c.361 + 1G > A (rs762169706), was identified in a 9-year-old male patient with an onset age of 7, who responded well to low-dose haloperidol and was remitted from tics after the administration of half a year. The c.361 + 1G > A variant was predicted to change splicing and produce the loss of the entire exon 2 in mRNA transcripts, which may cause an alteration in the reading frame, p.A47Lfs*2. The deletion of exon 2 was supported by sequencing PCR products from complementary DNA. This variant was also identified in his affected father, but absent in the unaffected mother and elder sister, which seems to cosegregate with the tics in this small family (Fig. 1). Additionally, it was absent in 300 ethnicity-matched Han Chinese control samples. A known heterozygous missense variant, c.744A > T (p.R248S, rs186388618), was observed in a 4-year-old boy with sporadic TS, which was predicted to be “damaging”, “possibly damaging”, and “disease causing” by three bioinformatics prediction tools. However, the variant was also identified in two normal controls (2/600) with the allele frequency of 0.333% in our study.

4. Discussion

TS is a childhood-onset neuropsychiatric disorder, marked by multiple motor and vocal tics for longer than one year, and associated with behavioral comorbidities [2,22,23]. Besides the gender difference in TS prevalence, male patients may have an earlier age of onset of the phenotype than females [7]. Twin, family and association studies consistently provided strong evidence for a substantial genetic contribution to pathogenesis [8,10]. Multiple candidate genes and chromosomal regions have been reported to be implicated in TS etiology [2,8,24]. Recently, a specific association between the *AADAC* deletion and development of TS has been revealed [10].

The *AADAC* gene, mapped to chromosome 3q25.1 and spanning about 14.5 kb, contains five exons and encodes a protein of 399 amino acids [25,26]. The protein is a glycoprotein expressed in the liver, adrenal glands, small intestine, and pancreas. It is perceived as an enzyme involved in drug metabolism by deacetylation and hydrolysis [26,27]. Previous studies showed AADAC expression throughout the brain, including regions previously implicated in TS pathology, with particularly elevated expression in the Purkinje cell layer of cerebellum [10,24]. AADAC expression data indicates a peak in the striatum between birth and adolescence, which is consistent with the typical clinical time course of tic onset in a brain region [24]. However, the exact AADAC function in the brain and pathogenic mechanism of the protein related to TS are still unclear.

In this study, two heterozygous variants, a splice-site variant c.361 + 1G > A (rs762169706) and a missense variant c.744A > T (p.R248S, rs186388618), were observed in two boys with TS. The splice-site variant c.361 + 1G > A, predicted to change splicing and produce the loss of the entire exon 2 in mRNA transcripts, may cause an alteration in the reading frame and result in a truncated protein, p.A47Lfs*2. Additionally, the c.361 + 1G > A variant was absent in the tested controls, and has a global allele frequency of 0.008% (10/121166) and an allele frequency of 0.116% (10/8628) in East Asians, accessed in the public ExAC (Exome Aggregation Consortium) database (http://exac.broadinstitute.org/). Mutations in a critical domain of a gene may potentially cause a monogenic disorder or a severe phenotype, whereas variants in a noncritical region may enhance disease susceptibility or give rise to a less severe form of disorder [28]. Given the presence of c.361 + 1G > A variant in the patient, the absence in our limited controls, and the distribution bias of *AADAC* deletions reported between TS and control [3,6,10], this variant and deletions in the *AADAC* gene probably increase susceptibility or play a disease-causing role in TS, partly associated with incomplete penetrance. The variant, c.744A > T (p.R248S), was identified in two normal controls, with the allele frequency of 0.333% (2/600) in our control group, 0.033% (40/120936) in the global population, and 0.451% (39/8648) in East Asians in the ExAC database. The p.R248 residue of AADAC is highly conserved across vertebrates from zebrafish to humans (http://www.ncbi.nlm.nih.gov/homologene). Though the variant was