Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.e-biomedicine.com

Original article

Association analysis between Tourette's syndrome and two dopamine genes (DAT1, DBH) in Taiwanese children

I-Ching Chou [a,b], Wei-De Lin [c,d], Chung-Hsing Wang [a], Yu-Tzu Chang [a], Zheng-Nan Chin [a], Chang-Hai Tsai [a,e], Fuu-Jen Tsai [a,c,*]

[a] Department of Pediatrics, Children's Medical Center, China Medical University Hospital, Taichung, Taiwan
[b] Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan
[c] Department of Medical Research, China Medical University and Hospital, Taichung, Taiwan
[d] School of Post Baccalaureate Chinese Medicine, China Medical University, Taichung, Taiwan
[e] Department of Healthcare Administration, Asia University, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 22 February 2013
Received in revised form
24 February 2013
Accepted 25 February 2013
Available online 19 April 2013

Keywords:
dopamine beta hydroxylase gene
dopamine transporter gene
polymorphism
Tourette's syndrome

ABSTRACT

Background: Recent research suggests that Tourette's syndrome (TS) may result from a defect in the dopamine system. Several candidate gene polymorphisms have been implicated in attention deficit hyperactivity disorder, including the dopamine transporter (*DAT1*) and dopamine β-hydroxylase (*DBH*) genes. A high rate of comorbidity between attention deficit hyperactivity disorder and TS indicates that they may share the same pathophysiology.
Purpose: We aimed to test the hypothesis that the dopamine gene might play a role in TS.
Methods: An association study, using an independent sample of patients from the midland region of Taiwan, was performed to investigate whether *DAT1* and *DBH* gene polymorphisms can be used as markers of susceptibility to TS. A total of 160 children with TS and 83 normal control participants were included in the study. Polymerase chain reaction was used to identify polymorphisms in the *DAT1* (40 bp VNTR) and *DBH* (TaqI A2) genes. Genotypes and allelic frequencies for the *DAT1* and *DBH* gene polymorphisms in both groups were compared.
Results: The results showed that genotypes and allelic frequencies in both groups were not significantly different. The most common genotype for *DAT1* (40 bp VNTR) was the 10,10 homozygote in both groups. The most common genotype for *DBH* (TaqI A2) was the T homozygote in both groups.
Conclusion: These data suggest that the *DAT1* and *DBH* genes may not be useful markers to predict susceptibility to TS.

1. Introduction

Gilles de la Tourette syndrome (TS) is a neuropsychiatric disorder characterized by both motor and vocal tics. In addition, affected individuals frequently display symptoms such as attention deficit hyperactivity disorder (ADHD) and/or obsessive–compulsive disorder. In the 1970s, investigators first demonstrated that TS has a familial concentration [1]. TS was

* *Corresponding author*. Departments of Pediatrics and Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, Taiwan.
E-mail address: d0704@mail.cmuh.org.tw (F.-J. Tsai).
2211-8020/$ – see front matter
http://dx.doi.org/10.1016/j.biomed.2013.02.003

then shown to be transmitted vertically from generation to generation, and studies of twin pairs confirmed a genetic influence [2,3]. To date, the gene search in TS has been unsuccessful [4], which is illustrative of the many factors that can complicate genetic analysis of complex human traits [5].

The pathogenesis of TS remains obscure. Current evidence suggests that TS may result from a defect in the dopamine system [6–10]. Studies have focused mainly on the dopamine transporter gene [*DAT1* 40 bp variable tandem nucleotide repeat (VNTR)], and the dopamine beta hydroxylase gene (*DBH TaqI* A2) in ADHD [11–13]. ADHD is common in TS probands and is reported to affect about 50–70% of referred TS cases [14–16]. These observations led us to test the polygenic hypothesis by examining the potential effect of *DAT1* and *DBH* in TS. We previously used single nucleotide polymorphisms (SNPs) as a tool in genetic studies of polygenic disorders [17–21]. SNPs are markers that may provide a new way to identify complex gene-associated diseases such as TS. In this study, we tested the hypothesis that genetic variation in the *DAT1* (40 bp VNTR) and *DBH* (*TaqI* A2) genes confers susceptibility to TS. Two SNP markers have been identified in these genes, allowing researchers to detect disease-causing gene associations [22].

2. Materials and methods

The study included Taiwanese children with TS ($n = 100$ in the *DAT1* group and $n = 160$ in the *DBH* group, respectively) and normal control participants ($n = 83$). This study was approved by the Ethics Committee of the China Medical University Hospital, Taichung, Taiwan. All parents signed informed consent before blood tests were performed. TS patients and the controls were both recruited from the midland regions of Taiwan. Diagnosis of TS followed the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [23]. The criteria for TS are as follows: the presence of multiple motor and at least one vocal tic (not necessarily concurrently); 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. A pediatric neurologist (I-C.C.) examined the children and made sure that all cases were unrelated. The 83 controls were healthy volunteers with no history of psychiatric treatment.

All children underwent peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood by mean of a DNA extractor kit (Genomaker DNA extraction kit; Blossom, Taipei, Taiwan). A total of 50 ng of genomic DNA was mixed with 20 pmol of each polymerase chain reaction (PCR) primer in a total volume of 25 μL containing 10 mM Tris-hydrochloride, pH 8.3; 50 mM potassium chloride; 2.0 mM magnesium chloride; 0.2 mM each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin Elmer, Foster City, CA, USA). Four PCR primers were used to amplify the correlated gene. The sequences of these primers were as following (from the 5′ to 3′ end): *DBH* (444 g/a): upstream, CCTGGAGCCCAGTGCTTGTC; downstream, ACGCCCTCCTGGGTACTCGC; and *DAT1*: upstream, TGTGGTGTAGGGAACGGCCTGAGA; downstream, AAATTCCAGTGGGGTCCCTTCCTG. The PCR conditions were as follows: 35 cycles of: 95°C for 30 seconds, 60°C for *DBH* (444 g/a) or 66.5°C for *DAT1* for 30 seconds, and 72°C for 45 seconds, followed by 72°C for 7 minutes, and then held at 4°C. The polymorphisms were analyzed by PCR amplification followed by restriction analysis with *Eco*NI for *DBH* (444 g/a). The PCR products were directly analyzed on 3% agarose gel by electrophoresis, and each allele was identified according to its size.

Allelic frequencies were expressed as a percentage of the total number of alleles. The genotypes and allelic frequencies for *DAT1* and *DBH* polymorphisms in both groups were compared. Using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) with the χ^2 test was used for statistical analyses. A p value of <0.05 was considered statistically significant.

3. Results

Genotype proportions and allele frequencies for *DAT1* and *DBH* were not significantly different between the groups (Tables 1 and 2). The most common genotype for *DAT1* was the 10,10 homozygote in both groups. The allele 10 frequency for *DAT1* in TS patients was 87% and in controls it was 88.6% (Table 1).

The most common genotype for *DBH* was the T homozygote in both groups. The proportions of T homozygotes, T/C heterozygotes, and C homozygotes for *DBH* were: in TS patients, 75.6%, 22.5%, and 1.9%, respectively; and in controls,

Table 1 – Genotypes for DAT1 (40 bp VNTR) polymorphisms in children with Tourette's syndrome and in normal individuals.

	Tourette patients, n (%) ($n = 100$)	Controls, n (%) ($n = 83$)	p
Genotype			
11,11	1 (1)	0	0.795
10,13	0	1 (1.2)	
10,11	2 (2)	2 (2.4)	
10,10	75 (75)	66 (79.5)	
10,9	19 (19)	11 (13.3)	
10,8	1 (1)	0	
10,7	1 (1)	1 (1.2)	
10,6	1 (1)	0	
9,9	0	1 (1.2)	
9,7	0	1 (1.2)	
Allelic frequency			
Allele 13	0	1 (0.6)	0.858
Allele 11	4 (2)	2 (1.2)	
Allele 10	174 (87)	147 (88.6)	
Allele 9	19 (9.5)	14 (8.4)	
Allele 8	1 (0.5)	0	
Allele 7	1 (0.5)	2 (1.2)	
Allele 6	1 (0.5)	0	

The p-values were calculated using the χ^2 test.

Table 2 – Genotypes and allele frequencies for DBH (TaqI A2) polymorphisms in children with Tourette's syndrome and in normal individuals.

	Tourette patients, *n* (%) (*n* = 160)	Controls, *n* (%) (*n* = 83)	*p*
Genotype			
T/T	121 (75.6)	65 (78.3)	0.900
C/T	36 (22.5)	17 (20.5)	
C/C	3 (1.9)	1 (1.2)	
Allelic frequency			
Allele T	278 (86.9)	147 (88.6)	0.700
Allele C	42 (13.1)	19 (11.4)	

The *p*-values were calculated using the χ^2 test.

78.3%, 20.5%, and 1.2%, respectively. The allele T and C frequencies for DBH in TS patients were 86.9% and 13.1%, respectively; and in controls, 88.6% and 11.4%, respectively (Table 2).

4. Discussion

Dopamine transport was first described 40 years ago [24]. *DAT* was itself identified and its molecular structure described more than 20 years later [25]. The human *DAT* gene is localized on chromosome 5p15.3 [26]. A genetic polymorphism of a 40 bp VNTR polymorphic sequence in the 3′ untranslated region of exon 15 of the gene has been described [27]. This VNTR of exon 15 is repeated 3–11 times, most typically 10 times. The 10-repeat shows ethnic heterogeneity with a frequency of 0.7 among Caucasians and Hispanics in the USA, 0.54 in African Americans, and 0.9 in Asians [28–30]. *DATs* are expressed in a small number of neurons in the brain, mainly in the striatum and nucleus accumbens, but also in the globus pallidus, cingulate cortex, olfactory tubercle, amygdala, and midbrain [31]. *DAT*, like the transporters for norepinephrine and serotonin, is a Na^+/Cl^- dependent transmembrane transport protein [32] which regulates the concentration of dopamine in the synaptic cleft.

DBH appears to be a strong candidate for investigation in TS, because it catalyzes the conversion of dopamine to norepinephrine and therefore influences both the dopaminergic and adrenergic systems. Serum *DBH* levels are under strong genetic control and show large interindividual variation [30]. Alleles of several polymorphisms at the DBH locus have been found to be associated with serum *DBH* levels.

In the present study we did not find significant evidence for association in our TS samples. The role of the dopaminergic system in the pathogenesis of TS is still not known. Preliminary studies have suggested that the pathogenesis of tics involves neuronal activity within subcortical neuronal circuits [33]. Therefore, this raises the possibility that classic neurotransmitters, dopamine and serotonin, may be involved in the pathobiology of TS. However, other investigators have emphasized that abnormalities of dopamine fail to explain many clinical and laboratory observations, including the description of unchanged tics in four adults who developed parkinsonism and received treatment with L-dopa [34].

Our review of the literature found that recent linkage studies have not provided any positive results regarding: dopamine D1-5 receptors [35–37], glycine α-1 subunit, GABAA receptor α-1, α-6, and γ-2 subunits (GABRA1, GABRA6, GABRG2), GABAA receptor β-1 and α-2 subunits (GABRB1, GABRA2), glutamate receptor GLUR1, the α-adrenergic receptor ADRA1, the β-adrenergic receptor ADRB1, and the glucocorticoid receptor GRL [38]; norepinephrine transporter gene [39]; or catechol-o-methyltransferase [40]. Other investigators have sought to identify associations between TS and other movement disorders [41]. Further studies will be required to confirm these assertions.

The etiology of TS is therefore unknown. In fact, TS in children may involve a complex interaction between environmental influences, especially infection, autoimmune contributions, epigenetic factors, and genetic factors. Our study suggests that the *DBH* and *DAT1* genes may not contribute to the etiology of TS. Further studies could focus on the analysis of other dopaminergic genes in TS patients. Our results could provide the database for a further survey of *DBH* and *DAT1* gene polymorphisms.

Acknowledgments

This study was supported in part by the China Medical University Hospital (grant number DMR-102-038).

REFERENCES

[1] Eldridge R, Sweet R, Lake R, Ziegler M, Shapiro AK. Gilles de la Tourette's syndrome: clinical, genetic, psychologic, and biochemical aspects in 21 selected families. Neurology 1977;27:115–24.
[2] Price RA, Kidd KK, Cohen DJ, Pauls DL, Leckman JF. A twin study of Tourette syndrome. Arch Gen Psychiatry 1985;42:815–20.
[3] Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's syndrome and associated behaviors. Evidence for autosomal dominant transmission. N Engl J Med 1986;315:993–7.
[4] Heutink P, Breedveld GJ, Niermeijer MF, et al. Progress in gene localization. In: Kurlan R, editor. Handbook of Tourette's syndrome and related tic and behavioral disorders. New York: Marcel Dekker; 1993. p. 317–35.
[5] Comings DE. Tourette's syndrome and psychiatric disorders. Br J Psychiatry 1995;166:399.
[6] Leckman JF, Riddle MA, Cohen DJ. Pathobiology of Tourette's syndrome. In: Cohen DJ, Bruun RD, Leckman JF, editors. Tourette's syndrome and tic disorder: clinical understanding and treatment. New York: Wiley & Sons; 1992. p. 103–16.
[7] Shapiro E, Shapiro AK, Fulop G, Hubbard M, Mandeli J, Nordlie J, et al. Controlled study of haloperidol, pimozide and placebo for the treatment of Gilles de la Tourette's syndrome. Arch Gen Psychiatry 1989;46:722–30.
[8] Jankovic J, Glaze DG, Frost JD. Effect of tetrabenazine on tics and sleep of Gilles de la Tourette's syndrome. Neurology 1984;34:688–92.
[9] Sweet RD, Bruun R, Shapiro E, Shapiro AK. Presynaptic catecholamine antagonists as treatment for Tourette syndrome: effects of alpha methyl para tyrosine and tetrabenazine. Arch Gen Psychiatry 1974;31:857–61.

[10] Golden GS. The use of stimulants in the treatment of Tourette's syndrome. In: Cohen DJ, Bruun RD, Leckman JF, editors. Tourette's syndrome and tic disorder: Clinical understanding and treatment. New York: Wiley & Sons; 1992. p. 317–25.
[11] Cook Jr EH, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE, et al. Association of attention-deficit disorder and the dopamine transporter gene. Am J Hum Genet 1995;56:993–8.
[12] Daly G, Hawi Z, Fitzgerald M, Gill M. Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. Mol Psychiatry 1999;4:192–6.
[13] Smith KM, Daly M, Fischer M, Yiannoutsos CT, Bauer L, Barkley R, et al. Association of the dopamine beta hydroxylase gene with attention deficit hyperactivity disorder: genetic analysis of the Milwaukee longitudinal study. Am J Med Genet B Neuropsychiatr Genet 2003;119B:77–85.
[14] Comings DE, Comings BG. Tourette syndrome: clinical and psychological aspects of 250 cases. Am J Hum Genet 1985;37:435–50.
[15] Comings DE, Gade R, Wu S, Chiu C, Dietz G, Muhleman D, et al. Studies of the potential role of the dopamine D1 receptor gene in addictive behaviors. Mol Psychiatry 1997;2:44–56.
[16] Comings DE, Comings BG. Tourette's syndrome and attention deficit disorder with hyperactivity. Arch Gen Psychiatry 1987;44:1023–6.
[17] Sun SS, Chou IC, Lai YH, Kao CH. 99mTc-ECD SPECT image in children with Gilles de la Tourette's syndrome: a preliminary report. Ann Nucl Med Sci 2001;14:149–53.
[18] Chen WY, Lin CY, Chou IC, Tsai FJ, Sun SS. The role of Tc-ECD brain SPECT in differentiating Tourette's syndrome from chronic tic disorder. Ann Nucl Med Sci 2003;16:59–63.
[19] Chou IC, Tsai CH, Lee CC, Kuo HT, Hsu YA, Li CI, et al. Association analysis between Tourette's syndrome and dopamine D1 receptor gene in Taiwanese children. Psychiatr Genet 2004;14:219–21.
[20] Chou IC, Tsai CH, Wan L, Tsai FJ. Association study between Tourette's syndrome and polymorphisms of noradrenergic genes (ADRA2A, ADRA2C). Psychiatr Genet 2007;17:359.
[21] Chang YT, Li YF, Muo CH, Chen SC, Chen CN, Kuo HT, et al. Correlation of Tourette's syndrome and allergic disease: nationwide population based case-control study. J Dev Behav Pediatr 2011;2:98–102.
[22] NCBI. dbSNP Short Genetic Variations. Available at: http://www.ncbi.nlm.nih.gov/SNP [accessed 08.03.13].
[23] Comings DE. DSM-IV criteria for Tourette's. J Am Acad Child Adolesc Psychiatry 1995;34:401–2.
[24] Iversen LL. Role of transmitter uptake mechanisms in synaptic neurotransmission. Br J Pharmacol 1971;41:571–91.
[25] Giros B, el Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T, et al. Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. Mol Pharmacol 1992;42:383–90.
[26] Donovan DM, Vandenbergh DJ, Perry MP, Bird GS, Ingersoll R, Nanthakumar E, et al. Human and mouse dopamine transporter genes: conservation of 5′-flanking sequence elements and gene structures. Brain Res Mol Brain Res 1995;30:327–35.
[27] Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, et al. Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics 1992;14:1104–6.
[28] Byerley W, Hoff M, Holik J, Caron MG, Giros B. VNTR polymorphism for the human dopamine transporter gene (DAT1). Hum Mol Genet 1993;2:335.
[29] Sano A, Kondoh K, Kakimoto Y, Kondo I. A 40-nucleotide repeat polymorphism in the human dopamine transporter gene. Hum Genet 1993;91:405–6.
[30] Doucette-Stamm LA, Blakely DJ, Tian J, Mockus S, Mao JI. Population genetic study of the human dopamine transporter gene (DAT1). Genet Epidemiol 1995;12:303–8.
[31] Leighton PW, Le Couteur DG, Pang CC, McCann SJ, Chan D, Law LK, et al. The dopamine transporter gene and Parkinson's disease in a Chinese population. Neurology 1997;49:1577–9.
[32] Nakatome M, Honda K, Tun Z, Kato Y, Harihara S, Omoto K, et al. Genetic polymorphism of the 3′ VNTR region of the human dopaminergic function gene DAT1 (human dopamine transporter gene) in the Mongolian population. Hum Biol 1996;68:509–15.
[33] Peterson BS, Skudlarski P, Anderson AW, Zhang H, Gatenby JC, Lacadie CM, et al. A functional magnetic resonance imaging study of tic suppression in Tourette syndrome. Arch Gen Psychiatry 1998;55:326–33.
[34] Kumar R, Lang AE. Coexistence of tics and parkinsonism: evidence for non-dopaminergic mechanisms in tic pathogenesis. Neurology 1997;49:1699–701.
[35] Barr CL, Wigg KG, Zovko E, Sandor P, Tsui LC. No evidence for a major gene effect of the dopamine D4 receptor gene in the susceptibility to Gilles de la Tourette syndrome in five Canadian families. Am J Med Genet 1996;67:301–5.
[36] Barr CL, Wigg KG, Zovko E, Sandor P, Tsui LC. Linkage study of the dopamine D5 receptor gene and Gilles de la Tourette syndrome. Am J Med Genet 1997;74:58–61.
[37] Devor EJ, Dill-Devor RM, Magee HJ. The Bal I and Msp I polymorphisms in the dopamine D3 receptor gene display linkage disequilibrium with each other but no association with Tourette syndrome. Psychiatr Genet 1998;8:49–52.
[38] Brett PM, Curtis D, Robertson MM, Gurling HM. Neuroreceptor subunit genes and the genetic susceptibility to Gilles de la Tourette syndrome. Biol Psychiatry 1997;42:941–7.
[39] Stöber G, Hebebrand J, Cichon S, Brüss M, Bönisch H, Lehmkuhl G, et al. Tourette syndrome and the norepinephrine transporter gene: results of a systematic mutation screening. Am J Med Genet 1999;88:158–63.
[40] Barr CL, Wigg KG, Sandor P. Catechol-O-methyltransferase and Gilles de la Tourette syndrome. Mol Psychiatry 1999;4:492–5.
[41] Nemeth AH, Mills KR, Elston JS, Williams A, Dunne E, Hyman NM. Do the same genes predispose to Gilles de la Tourette syndrome and dystonia? Report of a new family and review of the literature. Mov Disord 1999;14:826–31.