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The *BTBD9* gene polymorphisms in Polish patients with Gilles de la Tourette syndrome

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Gilles de la Tourette syndrome (GTS) is a neurodevelopmental disorder characterized by motor and vocal tics. The etiology of the disorder is unknown, although the predominant role of genetic factors has been established. Variants of the *BTBD9* gene (rs4714156, rs9296249 and rs9357271) have been reported to be associated with GTS in French Canadian and Chinese Han populations. Therefore, we decided to test the association between GTS and polymorphisms of the *BTBD9* gene in Polish patients. Our cohort of GTS cases comprised 162 patients aged 4–54 years (mean age: 19.9±8.7 years; 131 males, 80.9%). The control group consisted of 180 healthy persons aged 14–55 years (mean age: 23.1±2.1 years; 149 males, 82.8%). The rs4714156, rs9296249 and rs9357271 variants of the *BTBD9* gene were genotyped. No significant differences were found in minor allele frequencies (MAFs) of the SNPs tested between the two groups. The frequency of MAFs of the genotyped SNPs was lower in GTS patients with Attention Deficit Hyperactivity Disorder (for rs9357271 and rs9296249, P=0.039 and rs4714156, P=0.040) and higher in GTS patients without comorbidities (for rs9357271 and rs9296249 P=0.021 and rs4714156 P=0.025). There was a trend toward an association between the minor allele of the SNPs and mild tics (P=0.089 for rs9357271 and rs9296249, P=0.057 for rs4714156). Despite limitations of the study, including the small number of cases and analyzed SNPs, our results suggest that the examined *BTBD9* variants are not associated with GTS risk, but may be associated with comorbidity and tic severity in the Polish population.

Key words: Gilles de la Tourette syndrome, tics, *BTBD9*, genetic variants, single nucleotide polymorphism

INTRODUCTION

Gilles de la Tourette syndrome (GTS) is a childhood-onset neuropsychiatric disorder manifested by tics – brief, repetitive, stereotyped and intermittent movements (motor tics) or sounds (vocal tics). For a GTS diagnosis, both multiple motor and one or more vocal tics must be present at some time during the illness. They must be present for at least 12 months without any tic-free period of more than three consecutive months, the onset of tics must be before the age of 18 years, and other causes of tics, such as substance intoxication or general medical condition have to be excluded (American Psychiatric Association 2000). The anatomical location, number, frequency, complexity, and severity of tics change over time. Tics typically begin around the age of 5–6, reach their worst-ever between ages 10 and 12, and then decline in severity throughout adolescence (Leckman et al. 1998). Analysis of adulthood tics has revealed that over one-third of children with GTS are completely tic free as adults, slightly less than half have minimal to mild tics, and less than a quarter have moderate or stronger tics. These results contrast with their worst-ever period where all individuals experience at least moderate tics (Bloch et al. 2006, Spencer et al. 1999).

In addition to tics, many patients with GTS exhibit a variety of mental disorders and behavioral symptoms such as attention deficit hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), poor impulse control, conduct disorder, oppositional defiant disorder, anxiety, depression, temper outbursts, rage attacks, self-injuries, and inappropriate sexual behavior (Janik et al. 2007). Similarly to tics, symptoms of ADHD and

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OCD may fluctuate over time. ADHD usually occurs before the tic onset. Hyperactivity symptoms generally improve during adolescence, whereas inattention symptoms often persist into adulthood (Faraone et al. 2006). OCD has an onset around the time the tics reach their worst-ever intensity, but symptoms may also appear *de novo* during adolescence and early adulthood (Bloch et al. 2006).

The worldwide prevalence of GTS has been reported to be about 1% of all children between the age of 5 and 18 (Robertson 2008) and ten times less in adults due to the natural course of tics (Burd et al. 1986). Many patients with GTS do not require treatment for their tics, since they do not interfere with daily life. Some patients with very mild tics are not even aware of being ill. Indeed, only a minority of individuals with tics seek medical advice (Robertson 2008). Thus, despite the relatively high prevalence of GTS in the general population the disorder is considered rare in the clinical setting.

The etiology of GTS remains unknown, although a significant contribution of genetic factors has been established. The failure of family and candidate gene studies to identify a precise linkage with GTS has led investigators to examine polymorphism associations. Numerous independent studies in different populations have sought to identify GTS-associated single nucleotide polymorphisms (SNPs) in many genes. Recently, variants of the *BTBD9* gene (OMIM 611237*)* have been reported to be associated with restless legs syndrome and GTS (Winkelmann et al. 2007, Riviere et al. 2009, Guo et al. 2012). The *BTBD9* gene, coding for BTB/POZ domain-containing protein, maps on chromosome 6 at 6p21, and is highly expressed in the amygdale, cerebellum, hippocampus, and caudate and subthalamic nuclei, as well as outside the brain, in the heart, kidneys, pancreas, and liver. Eight alternative *BTBD9* transcripts have been described, resulting in seven protein isoforms, 92-612 amino acids long. The BTB/POZ domain is a known protein–protein interaction motif, but the function of the protein remains largely unknown. Other proteins containing the BTB/POZ domain function in transcription repression, cytoskeleton regulation, tetramerization, gating of ion channels, and ubiquitin-dependent protein degradation (Stogios et al. 2005). The molecular nature of the BTBD9 protein and the universal occurrence of the domain in question make the assignment of a specific function difficult at present. The *BTBD9* variants may also influence the metabolism of iron. Some such variants may reduce the iron content in the ventral midbrain (Jones et al. 2003), which is consistent with lower ferritin and serum iron levels in patients with GTS (Gorman et al. 2006).

Riviere and colleagues (2009) have reported three intronic SNPs (rs4714156, rs9296249, rs9357271) within the *BTBD9* gene associated with GTS in the French Canadian population. Another study that examined the association between variants of the *BTBD9* gene and GTS has revealed that only the rs9296249 variant, but not rs4714156 or rs9357271, is associated with GTS in the Chinese Han population (Guo et al. 2012).

The aim of this study was to further analyze three SNPs of the *BTBD9* gene rs4714156, rs9296249, and rs9357271, that showed an association with GTS in the population mentioned, and to determine whether they could be involved in the etiology of GTS in Polish patients.

METHODS

Study participants

The cohort of GTS cases comprised 162 patients aged 4–54 years (mean age: 19.9±8.7 years; 131 males (80.9%), 75 children and 87 adult patients defined as ≥18 years, 53.7%). The family history was positive in 80 (49.7%) patients, and was unknown for one patient. The mean age of tic onset was 7.5±3.2 years. 125 (77.2%) patients had at least one of the following comorbidities: ADHD *n*=63 (38.9%), OCD/OCB *n*=70 (43.2%), Learning Disorder *n*=51 (31.5%), Mood Disorder *n*=25 (15.4%), Anxiety Disorder *n*=34 (21.0%), Conduct Disorder *n*=12 (7.4%), while 37 (22.8%) had no comorbid mental disorders. None of the GTS patients included into the study had symptoms of restless legs syndrome. As a control group we enrolled 180 unrelated, ethnically and gender matched individuals with no diagnosed mental, neurological or general disorder, aged 14–55 years (mean age: 23.1±2.1 years; 149 males, 82.8%)**.** The patients were evaluated for the clinical diagnosis of GTS and comorbid mental disorders according to DSM-IV-TR (American Psychiatric Association 2000). Obsessive Compulsive Behavior (OCB) was diagnosed when obsessions and compulsions were present but did not meet the criteria of DSM-IV-TR.

Table I

Frequencies of haplotypes derived from three *BTBD9* SNPs

Haplotype[a]	Number		Frequency		GTS vs. Control	
	GTS	Control	GTS	Control	Chi[2]	*P*[b]
TTC	262	282	0.81	0.78	0.671	0.413
CCT	59	74	0.18	0.21	0.599	0.439
CCC	3	4	0.01	0.01	0.058	0.810

(GTS) Gilles de la Tourette syndrome; (SNPs) single nucleotide polymorphisms. [a]Haplotype includes 3 loci in order: rs9357271-rs9296249-rs4714156, [b]chi-square test.

Assessment of tic severity

The tic severity was determined retrospectively based on reports from parents or patients and defined descriptively as mild, moderate or intensive. Mild tics were diagnosed if they were not related to physical or mental discomfort, problems in relations with peers, lower-than-expected academic achievements or a need of treatment. Moderate tics generated only minor and temporary restrictions in a patient's daily life (e.g., few-day absence from school). Intensive tics disrupted normal daily activities (e.g., repeating grades, job loss), caused physical discomfort (e.g., neck pain caused by cervical tics) and significant deterioration of the quality of life, and required symptomatic treatment with neuroleptics. The severity of tics, ADHD and OCD was not measured by quantitative scores.

The study was approved by the local Ethics Committee. All participants 18 years of age and older signed informed consent forms, and parents signed it on behalf of individuals under 18.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using standard salting-out method. The rs4714156, rs9296249, rs9357271 polymorphisms were genotyped using pre-designed TaqMan SNP genotyping assay (Applied Biosystems) with the StepOne Plus Real-Time PCR system.

Statistical analysis

Chi-square test was used for both allelic and genotypic association studies. Association of genotypes with age of tic onset was analyzed with Kruskal-Wallis test. Haploview 4.2 program was used for haplotype analysis and Statistica 10 for other calculations. The significance level was set at $P<0.05$.

RESULTS

Genotypic and allelic association analysis

The genotype frequencies of the SNPs tested were in accordance with the Hardy–Weinberg equilibrium both in the control group and in the patient group ($P>0.7$). The SNPs showed a strong linkage disequilibrium ($D=1$, $r^2=0.938–1$). Allele C of rs9296249 was in a total linkage disequilibrium with allele C of rs9357271. Three locus haplotype analyses showed three different haplotypes (Table I). Minor allele frequencies (MAFs), genotype frequency, allelic, genotypic or haplotype association analysis of rs4714156, rs9296249, and rs9357271 variants of the *BTBD9* gene revealed no significant differences between controls and GTS patients (Tables I, II).

Comorbidities

Significant associations were found between variants of the *BTBD9* gene and the clinical phenotype of GTS (Table III). The minor alleles of all three SNPs were found significantly less frequently in patients with ADHD and more frequently in patients with no comorbidities. The allele frequencies in GTS patients with comorbid mental disorders other than ADHD were similar to those in patients without the disorders (Table III).

Table II

Genotypic association analysis of the rs4714156, rs9296249 and rs9357271 variants of *BTBD9* gene in Polish populations of patients with Gilles de la Tourette syndrome and controls

rs9357271 and rs9296249*			rs4714156		
MAF GTS/Control	Allele Major/Minor/Risk		MAF Cases/Controls	Allele Major/Minor/Risk	
0.19/0.22	T/C/T		0.18/0.21	C/T/C	
Allelic association analysis			Allelic association analysis		
Compared alleles	OR (95% CI)	*P* [a]	Compared alleles	OR (95%CI)	*P* [a]
C vs. T	0.86 (0.59–1.24)	0.413	T vs. C	0.86 (0.59–1.26)	0.439
Genotype frequency			Genotype frequency		
Genotype	GTS (*n*=162)	Control (*n*=180)	Genotype	GTS (*n*=162)	Control (*n*=180)
T/T	106 (65.4%)	110 (61.1%)	C/C	109 (67.3%)	114 (63.3%)
C/T	50 (30.9%)	62 (34.4%)	C/T	47 (29.1%)	58 (32.2%)
C/C	6 (3.7%)	8 (4.4%)	T/T	6 (3.7%)	8 (4.4%)
Genotypic association analysis			Genotypic association analysis		
Compared genotypes	OR (95%CI)	*P* [a]	Compared genotypes	OR (95%CI)	*P* [a]
CC+CT vs. TT	0.83 (0.53–1.29)	0.408	TT+CT vs. CC	0.84 (0.54–1.31)	0.444
CC vs. CT+TT	0.83 (0.28–2.44)	0.730	TT vs. CT+CC	0.83 (0.28–2.44)	0.730
CC vs. TT	0.78 (0.26–2.32)	0.652	TT vs. CC	0.78 (0.26–2.33)	0.662
CT vs. TT	0.84 (0.53–1.32)	0.446	CT vs. CC	0.85 (0.53–1.35)	0.486
CC vs. CT	0.93 (0.30–2.86)	0.899	TT vs. CT	0.93 (0.30–2.85)	0.893

(GTS) Gilles de la Tourette syndrome; (MAF) minor allele frequency; (OR) odds ratio; (CI) confidence interval. *Allele and genotype frequencies of rs9357271 and rs9296249 are identical due to complete linkage disequilibrium. [a] chi-square test.

Tic severity

Minor allele frequencies in patient groups with moderate (*n*=97) and intensive tics (*n*=42) were very similar: 17.5% and 17.9% for rs9296249/rs9357271, and 16.5% and 16.7% for rs4714156, respectively. Thus, the two groups were combined as severe tics (*n*=139) and then compared with the patient group with mild tics (*n*=23). The C allele of rs9296249 and rs93572714, and allele T of rs4714156 were less frequent in patients with severe tics at borderline statistical significance (Table III).

No significant differences were found with regard to the MAFs of the three studied variants of *BTBD9* gene between male and female patients with GTS (data not shown). None of the three SNPs was associated with family history or age of tic onset.

Table III

Allele frequencies in patients with Gilles de la Tourette syndrome stratified according to comorbid mental disorders and tic severity

GTS comorbidities (present vs. absent)	rs 9296249 and rs93572714 C vs. T		rs4714156 T vs. C	
	OR (95% CI)	*P* [a]	OR (95% CI)	*P* [a]
Any comorbidity	0.50 (0.27–0.91)	0.021	0.50 (0.27–0.92)	0.025
ADHD	0.53 (0.29–0.98)	0.039	0.52 (0.28–0.98)	0.040
OCD/OCB	0.67 (0.38–1.19)	0.172	0.62 (0.34–1.12)	0.110
Learning Disorders	0.71 (0.38–1.33)	0.285	0.63 (0.33–1.20)	0.156
Mood Disorders	1.07 (0.50–2.27)	0.866	1.15 (0.54–2.45)	0.721
Anxiety Disorders	0.78 (0.38–1.59)	0.485	0.63 (0.29–1.33)	0.231
Conduct Disorder	0.84 (0.28–2.54)	0.749	0.62 (0.18–2.16)	0.451
Tic severity (severe vs. mild)	0.54 (0.27–1.11)	0.089	0.50 (0.25–1.03)	0.056

(ADHD) attention deficit hyperactivity disorder; (OCD/OCB) obsessive-compulsive disorder/behavior. [a] chi-square test.

DISCUSSION

Multiple independent studies in different populations have investigated putative GTS-associated SNPs within various genes, mainly those associated with the metabolism of dopamine and serotonin based on the fact that dopamine antagonists and selective serotonin reuptake inhibitors are the most effective medications for tic suppression and obsessive-compulsive symptom reduction, respectively. Association between genetic polymorphisms and GTS were found for genes encoding the following proteins: dopamine receptor D2 (Devor 1992, Comings et al. 1996, Noble 2000, Lee et al. 2005), dopamine receptor D3 (Comings et al. 1993, Hebebrand et al. 1993), dopamine receptor D4 (Cruz et al. 1997, Diaz-Anzaldua et al. 2004), dopamine active transporter (Rowe et al. 1998, Tarnok et al. 2007, Yoon et al. 2007), monoamine oxidase A (Gade et al. 1998), 5- Hydroxytryptamine 2a receptor (Huang et al. 2001, Dickel et al. 2007), 5- Hydroxytryptamine 3a receptor (Niesler et al. 2005) and tryptophan 2, 3- dioxygenase (Comings et al. 1996). However, those positive results remain unreplicated in most cases. A variety of other genes, coding for dopamine receptor D1 (Chou et al. 2004), dopamine receptor D5 (Brett et al. 1995, Barr et al. 1997), tyrosine hydroxylase (Brett et al. 1995, Barr et al. 1996), dopamine β-hydroxylase (Brett et al. 1995, Ozbay et al. 2006), cathechol-O-methyltransferase (Tarnok et al. 2007), 5- hydroxytryptamine 1a receptor (Brett et al. 1995, Erdmann et al. 1995), 5-hydroxytryptamine 3b receptor (Niesler et al. 2005), 5-hydroxytryptamine 7 receptor (Gelernter et al. 1995), serotonin transporter (Cavallini et al. 2000), cannabinoid receptor 1 (Gadzicki et al. 2004), histidine decarboxylase (Lei et al. 2012), interleukin 1 receptor antagonist (Chou et al. 2010), major histocompatibility complex class II DR beta 1 (Schoenian et al. 2003), myelin oligodendrocyte glycoprotein (Huang et al. 2004) and epsilon-sarcoglycan (de Carvalho et al. 2004) have been screened for significant SNPs, but no association with GTS has been found.

The rs9296249 SNP polymorphism in the *BTBD9* gene has been shown to confer a risk of GTS in two distant ethnic groups: the French Canadian population (Riviere et al. 2009) and in the Chinese Han population (Guo et al. 2012), suggesting that it could be an universal GTS risk factor. However, the rs9357271and rs4714156 variants have been found to be associated with GTS only in the French Canadian patients but not in the Chinese Han population. We decided to investigate the association of those three SNPs (rs4714156, rs9296249, and rs9357271) in a group of Polish patients with GTS, compared to healthy controls. Both the Canadian and the Chinese study compared only the

Table IV

Allele distribution of the *BTBD9* variants in different populations

dbSNP ID	Polish population (GTS, *n*=162 Control, *n*=180 present study)	French Canadian population (GTS, *n*=322 Control, *n*=290 Riviere et al. 2009)	Chinese Han population (GTS, *n*=110 Control, *n*=440 Guo et al. 2012)
	Minor allele frequencies, GTS / Control		
rs4714156	0.18/0.21	0.17/0.23	0.17/0.19
rs9296249	0.19/0.22	0.16/0.22	0.54/0.44
rs9357271	0.19/0.22	0.17/0.23	0.11/0.11
	Alleles, major/minor		
rs4714156	C/T	C/T	T/C
rs9296249	T/C	T/C	C/T
rs9357271	T/C	T/C	C/T

(GTS) Gilles de la Tourette syndrome; (dbSNP) single nucleotide polymorphism database – http://www.ncbi.nlm.nih.gov/SNP/; (*n*) number of persons included into the study

allele frequencies between groups without performing a genotypic or haplotype association analysis. In order to study the polymorphisms of the *BTBD9* gene in more detail, we additionally performed a genotypic and haplotype analysis.

No significant differences were found for allele, haplotype or genotype frequencies between the GTS and control groups (Tables I, II), therefore our results do not confirm the significant associations between GTS and variants of the *BTBD9* gene reported in the two previous papers (Riviere et al. 2009, Guo et al. 2012). However, it should be noted that the odds ratios for the association of allele T of rs9357271 and allele C of rs4714156 with GTS in French Canadian patients calculated from the data presented in the report (Riviere et al. 2009) was 0.686 for both polymorphisms and it was within the 95% confidence intervals calculated for the Polish population (Table II). Thus, the results obtained for these two Caucasian populations are actually not inconsistent, since the difference in the odds ratio values could have occurred by chance. An alternative explanation of the differences reported would be ethnic differences between the two populations. This, however, seems unlikely since the Polish population of GTS patients had a similar linkage disequilibrium structure and MAFs of all three studied SNPs as the French Canadian population. These findings suggest that our Polish cohort is genetically comparable with regard to *BTBD9* gene to French Canadian population, although we analyzed only three SNPs compared to fourteen variants tested by Riviere and coworkers (2009). In turn, the Chinese Han population seems to be genetically different from either Caucasian populations regarding the *BTBD9* gene because of differences in MAFs and the type of minor allele of the three SNPs studied (Table IV).

In the French Canadian cohort two subgroups, GTS without comorbid OCD and GTS with neither OCD nor ADHD, displayed the most significant allelic association results when compared to controls. The authors concluded that variants of the *BTBD9* gene predispose to GTS without comorbidities. In contrast to the Canadian study we found significant negative associations of the minor alleles of genotyped SNPs with comorbidity. The MAFs correlated inversely with ADHD and any comorbidity, and in the patient group with no comorbidities the minor alleles of all three SNPs tested were found significantly more frequently than in patients with at least one mental disorder. The Canadian study did not analyze associations between variants of the *BTBD9* gene and comorbid mental disorders other than OCD and ADHD. We investigated

the associations of other mental disorders, frequently seen in GTS, such as learning disorders, mood disorders, anxiety disorders and a conduct disorder with three SNPs of the *BTBD9* gene in the Polish cohort with a GTS. However, we did not find allelic or genotypic associations between studied SNPs and comorbid mental disorders except ADHD (Table III).

We also analyzed associations between the SNPs and tic severity. The minor alleles were less frequent in the patient group with severe tics compared to those with mild tics, although the difference did not reach statistical significance (Table III). It should be stressed that the assessment of tic severity was retrospective, based on reports from parents or patients. We decided not to use the Yale Global Tic Severity Scale, which is a quantitative scale commonly used to measure tic severity within the last two weeks, since the tic severity at the time of evaluation does not necessarily reflect lifetime tic severity. In our study, 53.7% of GTS patients were adults of the mean age of almost 20 years at the time of evaluation, and some of them had mild tics at the time of examination but had had severe tics in the childhood. Therefore, to avoid the disease being classified as mild if the patient was evaluated in a period of remission, we defined tic severity as the worst-ever tic severity during the patient's life. The tic severity was therefore defined descriptively, not quantitatively, but an advantage of the indicator of tic severity used here was the possibility of assessing tic intensity over a period of time which is particularly important in the case of adult patients. Nevertheless, retrospective assessment of tic severity subject to patients' recall bias, and not using the Yale Global Tic Severity Scale should be considered as limitation of the study.

We are aware that our study has several limits, including the small number of cases and controls, and the fact that only a fragment of the *BTBD9* gene has been analyzed. Thus, the relationship between variants of the *BTBD9* gene and the risk of developing GTS cannot be excluded and larger-scale studies are needed. An another important question is, how representative for the Polish population is the sample included into the study. As the group comprised only patients referred to a specialist (neurologist) it could be speculated that milder cases of GTS were under-represented. This could cause the observed disproportion between the number of patients with tics rated mild compared to severe in our study.

CONCLUSION

In summary, our results indicate that the studied variants of the *BTBD9* gene do not significantly influence the GTS risk in the Polish population. They may predispose to milder clinical phenotype, as the minor alleles of all the SNPs examined are associated with less frequent comorbidity, especially ADHD, and less frequent severe tics. Further studies are warranted to identify an association between genetic variants and the risk of developing GTS in the Polish population.

REFERENCES

American Psychiatric Association (2000) Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. American Psychiatric Association, Arlington, VA.

Barr CL, Wigg KG, Zovko E, Sandor P, Tsui LC (1996) 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 67: 301–305.

Barr CL, Wigg KG, Zovko E, Sandor P, Tsui LC (1997) Linkage study of the dopamine D5 receptor gene and Gilles de la Tourette syndrome. Am J Med Genet 74: 58–61.

Bloch MH, Peterson BS, Scahill L, Otka J, Katsovich L, Zhang H, Leckman JF (2006) Adulthood outcome of tic and obsessive-compulsive symptom severity in children with Tourette syndrome. Arch Pediatr Adolesc Med 160: 65–69.

Brett PM, Curtis D, Robertson MM, Gurling HM (1995) The genetic susceptibility to Gilles de la Tourette syndrome in a large multiple affected British kindred: linkage analysis excludes a role for the genes coding for dopamine D1, D2, D3, D4, D5 receptors, dopamine beta hydroxylase, tyrosinase, and tyrosine hydroxylase. Biol Psychiatry 37: 533–540.

Brett PM, Curtis D, Robertson MM, Gurling HM (1995) Exclusion of the 5-HT1A serotonin neuroreceptor and tryptophan oxygenase genes in a large British kindred multiply affected with Tourette's syndrome, chronic motor tics, and obsessive-compulsive behavior. Am J Psychiatry 152: 437–440.

Burd L, Kerbeshian J, Wikenheiser M, Fisher W (1986) Prevalence of Gilles de la Tourette's syndrome in North Dakota adults. Am J Psychiatry 143: 787–788.

Cavallini MC, Di Bella D, Catalano M, Bellodi L (2000) An association study between 5-HTTLPR polymorphism, COMT polymorphism, and Tourette's syndrome. Psychiatry Res 97: 93–100.

Chou IC, Tsai CH, Lee CC, Kuo HT, Hsu YA, Li CI, Tsai FJ (2004) Association analysis between Tourette's syndrome and dopamine D1 receptor gene in Taiwanese children. Psychiatr Genet 14: 219–221.

Chou IC, Lin HC, Wang CH, Lin WD, Lee CC, Tsai CH, Tsai FJ (2010) Polymorphisms of interleukin 1 gene IL1RN are associated with Tourette syndrome. Pediatr Neurol 42: 320–324.

Comings DE, Muhleman D, Dietz G, Dino M, LeGro R, Gade R (1993) Association between Tourette's syndrome and homozygosity at the dopamine D3 receptor gene. Lancet 341: 906.

Comings DE, Gade R, Muhleman D, Chiu C, Wu S, To M, Spence M, Dietz G, Winn-Deen E, Rosenthal RJ, Lesieur HR, Rugle L, Sverd J, Ferry L, Johnson JP, MacMurray JP (1996) Exon and intron variants in the human tryptophan 2, 3- dioxygenase gene: potential association with Tourette syndrome, substance abuse and other disorders. Pharmacogenetics 6: 307–318.

Comings DE, Wu S, Chiu C, Ring RH, Gade R, Ahn C, MacMurray JP, Dietz G, Muhleman D (1996) Polygenic inheritance of Tourette syndrome, stuttering, attention deficit hyperactivity, conduct and oppositional defiant disorder: the additive and subtractive effect of the three dopaminergic genes. Am J Med Genet 67: 264–288.

Cruz C, Camarena B, King N, Páez F, Sidenberg D, de la Fuente JR, Nicolini H (1997) Increased prevalence of the seven-repeat variant of the dopamine D4 receptor gene in patients with obsessive-compulsive disorders with tics. Neurosci Lett 231: 1–4.

de Carvalho Aguiar P, Fazzari M, Jankovic J, Ozelius LJ (2004) Examination of the SGCE gene in Tourette syndrome patients with obsessive-compulsive disorder. Mov Disord 19: 1237–1238.

Devor EJ (1992) The D2 dopamine receptor and Tourette's syndrome. JAMA 267: 651.

Díaz-Anzaldúa A, Joober R, Rivière JB, Dion Y, Lespérance P, Richer F, Chouinard S, Rouleau GA; Montreal Tourette Syndrome Study Group (2004) Tourette syndrome and dopaminergic genes: a family-based association study in the French Canadian founder population. Mol Psychiatry 9: 272–277.

Dickel DE, Veenstra-VanderWeele J, Bivens NC, Wu X, Fischer DJ, Van Etten-Lee M, Himle JA, Leventhal BL, Cook EH Jr, Hanna GL (2007) Association studies of serotonin system candidate genes in early-onset obsessive-compulsive disorder. Biol Psychiatry 61: 322–329.

Erdmann J, Shimron-Abarbanell D, Cichon S, Albus M, Maier W, Lichtermann D, Minges J, Reuner U, Franzek E, Ertl MA (1995) Systematic screening for mutations in the promoter and the coding region of the 5-HT1A gene. Am J Med Genet 60: 393–399.

Faraone SV, Biederman J, Mick E (2006) The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. Psychol Med 36: 159–165.

Gade R, Muhleman D, Blake H, MacMurray J, Johnson P, Verde R, Saucier G, Comings DE (1998) Correlation of length of VNTR alleles at the X-linked MAOA gene and phenotypic effect in Tourette syndrome and drug abuse. Mol Psychiatry 3: 50–60.

Gadzicki D, Müller-Vahl KR, Heller D, Ossege S, Nöthen MM, Hebebrand J, Stuhrmann M (2004) Tourette syndrome is not caused by mutations in the central cannabinoid receptor (CNR1) gene. Am J Med Genet B Neuropsychiatr Genet 127B: 97–103.

Gelernter J, Rao PA, Pauls DL, Hamblin MW, Sibley DR, Kidd KK (1995) Assignment of the 5HT7 receptor gene (HTR7) to chromosome 10q and exclusion of genetic linkage with Tourette syndrome. Genomics 26: 207–209.

Gorman DA, Zhu H, Anderson GM, Davies M, Peterson BS (2006) Ferritin levels and their association with regional brain volumes in Tourette's syndrome. Am J Psychiatry 163: 1264–1272.

Guo Y, Su L, Zhang J, Lei J, Deng X, Xu H,Yang Z, Kuang S, Tang J, Luo Z, Deng H (2012) Analysis of the *BTBD9* and HTR2C variants in Chinese Han patients with Tourette syndrome. Psychiatr Genet 22: 300–303.

Hebebrand J, Nöthen MM, Lehmkuhl G, Poustka F, Schmidt M, Propping P, Remschmidt H (1993) Tourette's syndrome and homozygosity for the dopamine D3 receptor gene. Lancet 342: 1483–1484.

Huang Y, Liu X, Li T, Guo L, Sun X, Xiao X, Ma X, Wang Y, Collier DA (2001) Cases-control association study and transmission disequilibrium test of T102C polymorphism in 5HT2A and Tourette syndrome (in Chinese). Zhonghua Yi Xue Yi Chuan Xue Za Zhi 18: 11–13.

Huang Y, Li T, Wang Y, Ansar J, Lanting G, Liu X, Zhao JH, Hu X, Sham PC, Collier D (2004) Linkage disequilibrium analysis of polymorphisms in the gene for myelin oligodendrocyte glycoprotein in Tourette's syndrome patients from a Chinese sample. Am J Med Genet B Neuropsychiatr Genet 124B: 76–80.

Janik P, Kalbarczyk A, Sitek M (2007) Clinical analysis of Gilles de la Tourette syndrome based on 126 cases. Neurol Neurochir Pol 41: 381–387.

Jones BC, Reed CL, Hitzemann R,Wiesinger JA, McCarthy KA, Buwen JP, Beard JL (2003) Quantitative genetic analysis of ventral midbrain and liver iron in BXD recombinant inbred mice. Nutr Neurosci 6: 369–377.

Leckman JF, Zhang H, Vitale A, Lahnin F, Lynch K, Bondi C, Kim YS, Peterson BS (1998) Course of tic severity in Tourette's syndrome: The first two decades. Pediatrics 102: 234–245.

Lee CC, Chou IC, Tsai CH, Wang TR, Li TC, Tsai FJ (2005) Dopamine receptor D2 gene polymorphisms are associated in Taiwanese children with Tourette syndrome. Pediatr Neurol 33: 272–276.

Lei J, Deng X, Zhang J, Su L, Xu H, Liang H, Huang X, Song Z, Deng H (2012) Mutation screening of the HDC gene in Chinese Han patients with Tourette syndrome. Am J Med Genet Part B 159B: 72–76.

Niesler B, Frank B, Hebebrand J, Rappold G (2005) Serotonin receptor genes HTR3A and HTR3B are not involved in Gilles de la Tourette syndrome. Psychiatr Genet 15: 303–304.

Noble EP (2000) The DRD2 gene in psychiatric and neurological disorders and its phenotypes. Pharmacogenomics 1: 309–333.

Ozbay F, Wigg KG, Turanli ET, Asherson P, Yazgan Y, Sandor P, Barr CL (2006) Analysis of the dopamine beta hydroxylase gene in Gilles de la Tourette syndrome. Am J Med Genet B Neuropsychiatr Genet 141B: 673–677.

Riviere JB, Xiong L, Levchenko A, St-Onge J, Gaspar C, Dion Y, Lesperance P, Tellier G, Richer F, Chouinard S, Rouleau GA, Montreal Tourette Study Group (2009) Association of intronic variants of the *BTBD9* gene with Tourette syndrome. Arch Neurol 66: 1267–1272.

Robertson MM (2008) The prevalence and epidemiology of Gilles de la Tourette syndrome. Part 1: the epidemiological and prevalence studies. J Psychosom Res 65: 473–486.

Rowe DC, Stever C, Gard JM, Cleveland HH, Sanders ML, Abramowitz A, Kozol ST, Mohr JH, Sherman SL, Waldman ID (1998) The relation of the dopamine transporter gene (DAT1) to symptoms of internalizing disorders in children. Behav Genet 28: 215–225.

Schoenian S, Konig I, Oertel W, Remschmidt H, Ziegler A, Hebebrand J, Bandmann O (2003) HLA-DRB genotyping in Gilles de la Tourette patients and their parents. Am J Med Genet B Neuropsychiatr Genet 119B: 60–64.

Spencer T, Biederman M, Coffey B, Geller D, Wilens T, Faraone S (1999) The 4-year course of tic disorders in boys with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 56: 842–847.

Stogios PJ, Downs GS, Jauhal JJ, Nandra SK, Prive GG (2005) Sequence and structural analysis of BTB domain proteins. Genome Biol 6: R82.

Tarnok Z, Ronai Z, Gervai J, Kereszturi E, Gadoros J, Sasvari-Szekely M, Nemoda Z (2007) Dopaminergic candidate genes in Tourette syndrome: association between tic severity and 3'UTR polymorphism of dopamine transporter gene. Am J Med Genet B Neuropsychiatr Genet 144B: 900–905.

Winkelmann J, Schormair B, Lichtner P, Ripke S, Xiong L, Jalilzadeh S, Fulda S, Pütz B, Eckstein G, Hauk S, Trenkwalder C, Zimprich A, Stiasny-Kolster K, Oertel W, Bachmann CG, Paulus W, Peglau I, Eisensehr I, Montplaisir J, Turecki G, Rouleau G, Gieger C, Illig T, Wichmann HE, Holsboer F, Müller-Myhsok B, Meitinger T (2007) Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nat Genet 39: 1000–1006.

Yoon DY, Rippel CA, Kobets AJ, Morris CM, Lee JE, Williams PN, Bridges DD, Vandenbergh DJ, Shugart YY, Singer HS (2007) Dopaminergic polymorphisms in Tourette syndrome: association with the DAT gene (SLC6A3). Am J Med Genet B Neuropsychiatr Genet 144B: 605–610.