Tourette Syndrome: Bridging the Gap between Genetics and Biology

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Key Words

Common variant · Copy number variation · Dopamine · Genetics · Genome-wide association study · Glutamate · Histamine · Linkage analysis · Neurotransmitters · Rare variant

Abstract

Tourette syndrome is a childhood neuropsychiatric disorder, which presents with disruptive motor and vocal tics. The disease also has a high comorbidity with obsessive-compulsive disorder and attention deficit hyperactivity disorder, which may further increase the distress experienced by patients. Current treatments act with varying efficacies in alleviating symptoms, as the underlying biology of the disease is not fully understood to provide precise therapeutic targets. Moreover, the genetic complexity of the disorder presents a substantial challenge to the identification of genetic alterations that contribute to the Tourette phenotype. Nevertheless, genetic studies have suggested involvement of dopaminergic, serotonergic, glutamatergic, and histaminergic pathways in the pathophysiology of at least some cases. In addition, genetic overlaps with other neuropsychiatric disorders may point toward a shared biology. The findings that

are emerging from genetic studies will allow researchers to piece together the underlying components of the disease in the hopes that a deeper understanding of Tourette syndrome can lead to improved treatments for those affected by it.

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Introduction

Tourette syndrome (TS) is a neuropsychiatric disorder characterized by combinations of motor and vocal tics that persist for longer than 1 year [1]. The presentation of motor tics can be categorized as either simple or complex, with simple tics generally involving only one muscle group, such as blinking or twitching of the nose [2, 3]. Complex tics tend to require multiple muscle groups and can be a sequence of movements or a combination of simple tics [2]. Examples include grimacing combined with twisting of the head, kicking, or spinning around [3]. Vocal tics often arise after the onset of motor tics and can also be divided into simple and complex [3]. Simple vocal tics are the production of various sounds and noises, such as throat clearing, coughing, or sniffing [3]. Complex vo-

cal tics, on the other hand, can be linguistically meaningful, including phenomena such as echolalia, palilalia, and coprolalia [2].

Many patients report a premonitory urge that is present before the occurrence of a tic, followed by a sense of relief as soon as the tic is performed; however, this is usually temporary [2, 4]. Moreover, tics will present in a waxing and waning fashion, exacerbated in periods of anxiety, stress, excitement, or anger, and decreasing or ceasing during sleep, rest, or concentration [2]. Age also appears to be a determinant of severity, since tic prevalence tends to decrease from childhood to adolescence and early adulthood [5].

In the general population, 0.5–0.7% of people are affected by TS at some point in their lives, with a greater prevalence in males than females (~3:1) [6, 7]. The median age of onset among children is 6 years, and tics reach the peak of their severity at approximately 10.6 ± 2.6 years of age [5, 8]. Twin studies have found a concordance rate of 53% in monozygotic and 8% in dizygotic twins, indicating a substantial genetic component to the disease [9]. In addition to tics, people with TS can also be affected by other comorbidities [8]. The lifetime prevalence of at least one other psychiatric disorder with TS is near 85%, with obsessive-compulsive disorder (OCD) and attention deficit hyperactivity disorder being the most common [8]. There is evidence that these conditions are genetically correlated, since about 20% of patients with TS also suffer from OCD or attention deficit hyperactivity disorder, rates that are much higher than the population prevalence [8]. The percentage of individuals with all three disorders is even higher, at around 30% [8]. While tics themselves may be sufficient to impact quality of life, comorbid conditions can further increase strain on relationships, academic achievement, self-esteem, as well as social interactions [4].

Current treatment options for TS vary in effectiveness among individuals and include behavioral therapy and various medications for controlling disruptive tics [10, 11]. Behavioral therapy has been demonstrated to be effective in decreasing tic severity in both the presence and absence of medications [12]. In Comprehensive Behavioral Intervention for Tics therapy, awareness training is utilized to teach patients how to recognize premonitory urges that precede tics [10]. Competing-response training instructs patients to complete a different voluntary action in the place of the tic, while relaxation training is used to address stress and anxiety, which may have an impact on the frequency and intensity of tic occurrence [10]. Available drugs include typical (e.g. haloperidol)

and atypical (e.g. risperidone) antipsychotics and α_2 adrenergic agonists (e.g. clonidine and guanfacine) [3]. These medications act with varying efficacies, and many have detrimental side effects such as sedation, weight gain, dyskinesia, or cognitive blunting [3, 13–19]. The remaining questions about TS pathophysiology and a need for new and improved treatment options has prompted the study of genetic causes that underlie the disease in the hopes of identifying novel therapeutic targets and increasing our understanding of this complex disorder.

Progression of Genetic Studies

It is now widely accepted that TS has a heterogeneous genetic architecture, where multiple genes likely contribute to disease development and pathogenesis; however, the assumption that a single gene controls the tic phenotype was a pitfall of many early genetic studies [20]. Linkage studies were among the first to investigate the genetic cause of TS by identifying and studying families where the disease was present in multiple members, in the hopes of pinpointing certain alleles that may be linked to the symptoms [21]. Parametric linkage studies were conducted on the premise that TS is inherited in an autosomal dominant fashion, where mutations in a specific gene result in the development of the disease [22]. Numerous studies examined TS-affected families spanning multiple generations; however, no specific locus was confirmed, and the findings demonstrated that TS is a genetically complex disorder [23–29].

Nonparametric linkage studies take into account the heterogeneity of TS and the possible interplay of environmental risk factors [30, 31]. Several studies have used this approach and have identified multiple potential loci [32–38]. However, there has not been overlap in a specific genomic region, indicating that different approaches and larger sample sizes may be required to identify risk genes [39].

Thus far, one causative mutation has been found using the single-pedigree analysis method, where Ercan-Sencicek et al. [40] performed linkage analysis in a family where the father and all 8 children were diagnosed with TS, suggesting a dominant mode of transmission and high penetrance. All affected family members had current or past motor and vocal tics, and 8 had current or past vocal tics; all shared the same allele on chromosome 15, harboring a heterozygous nonsense point mutation (W317X) at nucleotide position 951 in exon 9 of the gene *HDC* (*histidine decarboxylase*), which generated a maxi-

imum theoretical logarithm of the odds score [40]. The W317X abnormality resulted in a mutant form of the histidine decarboxylase (HDC) enzyme, lacking an essential portion of its active domain, which is responsible for catalyzing the synthesis of histamine from histidine [40]. To date, this mutation has not been found in other TS-affected or -unaffected individuals outside of this family. However, its discovery, for the first time, added histaminergic neurotransmission to the list of possible mechanisms involved in the biology of TS and prompted a clinical trial of an H3 receptor antagonist, AZD5213, in adolescents with TS (ClinicalTrials.gov ID: NCT01904773); subsequent support for a 'histamine hypothesis' came from a European family-based association study, a study of copy number variation (CNV) in TS [41], and animal models [42–44].

Candidate gene association studies have tested TS cases and control subjects for single nucleotide polymorphisms (SNPs) in candidate genes that are chosen based on hypotheses of plausible biological pathways [39, 45]. These include dopamine (DA) receptors, transporters, and catabolizing genes, serotonergic genes, and noradrenergic transcripts [46–57]. Although the low cost of examining only a few specific variants is appealing, the a priori probability that these variants will play a role in conferring risk for the disease is low, and sample sizes have generally been inadequate. Consequently, studies to date have produced varying results with nonsignificant or nonreproducible findings [39, 45].

Chromosomal abnormalities have been studied to determine which regions of the genome may have alterations as a result of deletions, duplications, translocations, or inversions. Areas affected by these variations can highlight candidate regions and genes to be examined in the context of TS. *IMMP2L* was implicated in a Danish TS cohort of 188 patients, where Bertelsen et al. [58] identified an increase in small intergenic deletions. Three separate studies also found abnormalities in chromosome 18q22, where breakpoints near the *IMMP2L* gene were observed [59–61]. The *CNTNAP2* gene was also discovered to have rearrangements in one TS family, while another family had a translocation affecting the gene, but with no symptoms [62, 63]. *CNTNAP2* has also been implicated in autism spectrum disorders (ASD), schizophrenia, and intellectual disability, and it is believed to be involved in ion channel distribution along the axon as well as in interactions between neurons and glial cells [62, 64]. A small, transmitted exonic deletion was observed in *NLGN4X*, also involved in ASD, in a family with TS, motor tics, autism, learning problems, anxiety, and depres-

sion [65–67]. Prontera et al. [68] identified a disrupted locus of *DPP6* in a son and father with TS. The gene has also been connected to ASD pathogenesis and neuroleptic-induced dyskinesia.

Lastly, the study of a family with 1 case of TS highlighted a de novo inversion on chromosome 13, which caused a breakpoint at 13q31, 350 kb away from the gene *SLITRK1* (*Slit and Trk-like, Family Member 1*) [69]. This same study examined 174 unrelated TS patients and found a frameshift mutation, resulting in a truncated protein in 1 TS and 1 trichotillomania patient [69]. Furthermore, this study identified a rare single base pair change, var321, at a regulatory micro-RNA-binding site in the *SLITRK1* 3' UTR. This finding had a significant association with TS ($p = 0.0056$) [70]. Several subsequent studies have examined the association of *SLITRK1* with TS by resequencing probands but did not locate additional pathogenic coding frame mutations in the gene [71–73] or did not find an association for the specific var321 [74, 75]. On the other hand, significant associations have been reported between var321 and TS [76, 77] or trichotillomania [78]. These results must be interpreted with caution, as studies examining the association between variants in *SLITRK1* and TS to date have been relatively small and generally lacking sufficient power to conclusively confirm or refute an association. Further studies involving much larger TS cohorts and employing the latest genomic technologies will be needed to clarify the contribution of variation in and around *SLITRK1* toward TS disease risk.

Little is known about its function; however, *SLITRK1* is believed to enhance expression during development to promote axon path finding, while it is also highly expressed in cholinergic neurons of the striatum throughout the lifespan [79]. In vitro studies have determined that *SLITRK1* promotes dendritic growth, while dendritic regression results from TS-associated alleles [69]. A *SLITRK1* knockout (KO) mouse model presented with anxiety as well as increased noradrenergic activity; however, no tic-like movements were observed [80]. Technological advances and a decline in genotyping costs have allowed studies to better investigate genetic susceptibility to TS across the genome [81]. In contrast to candidate gene association studies, genome-wide association studies (GWAS) do not require preselection of genomic regions, performing individual comparisons to analyze the combined effect size of SNPs on disease susceptibility [45, 82]. However, there is a high genome-wide statistical significance threshold ($p < 5 \times 10^{-8}$) to confirm a disease locus due to the large number of comparisons [83]. With

the modest effect sizes seen for common variants in complex genetic disorders (~ 1.1), large sample sizes are generally required to achieve a statistically significant signal. The one TS GWAS published to date (1,496 cases and 5,249 controls) has not pinpointed a statistically significant TS locus [83]. However, some sub-threshold GWAS peaks may still signal the involvement of variants fundamental to TS pathophysiology [84]. In the TS GWAS, Scharf et al. [83] found *COL27A1* on chromosome 9q32 to have a strong association with TS ($p = 1.85 \times 10^{-6}$). Furthermore, Paschou et al. [84] examined a top region from this GWAS in 609 cases and 610 controls and identified a single intergenic SNP (rs2060546) near the gene *NTN4* that was associated with TS; this finding remained statistically significant after Bonferroni correction. *NTN4* is believed to code for an axon guidance molecule in the developing striatum, an area implicated in TS; however, larger cohorts are required to confirm these findings [84].

The study of CNV has been made practical through the advent of affordable and high-resolution microarray technology, which can detect structural variants in DNA [85]. While CNVs occur in the general population, studies have examined whether the burden of rare and de novo CNVs is greater in individuals with complex disorders, like TS [86, 87]. Although no significant difference between cases and controls has been reported in TS thus far, the identified CNVs may implicate certain genes in TS. A study conducted by Sundaram et al. [88] examined 111 cases and 73 controls, finding rare recurrent CNVs in *NRXN1* and *CTNNA3*, which are thought to play a role in ASD and schizophrenia. Large CNVs found by Nag et al. [89] in a study of 179 TS patients and 234 controls also highlighted *NRXN1* as a possible TS gene, along with *COL8A1*. Moreover, Fernandez et al. [90] reported disruptions in histamine signaling and GABA receptor genes during an investigation of CNVs in 460 TS and 1,131 control subjects. In addition, a study examining 1,086 TS, 1,613 OCD, and 1,789 control subjects detected large deletions at 16p13.11 for 5 participants; however, the evidence for association was stronger for OCD than TS [91].

More recently, whole-exome and whole-genome next-generation sequencing have become increasingly popular options for the study of complex disorders, especially as declining costs allow greater availability for their use in research. Exome sequencing of a 3-generation pedigree with 7 TS-affected members revealed single nucleotide variants in three genes, *MRPL3*, *DNAJC13*, and *OFCC1*, which have not yet been implicated in other studies [92]. Hooper et al. [93] performed whole-genome sequencing on a single individual with TS and comorbid OCD, dis-

covering a translocation at (6;22)(q16;p13). This alteration caused a 400-kb deletion in 6q16, affecting *GPR64*, *NDUFA4*, and *KLHL32* [93]. While these 2 published studies have applied next-generation sequencing in only a small number of patients to date, larger-scale exome sequencing studies in parent-child trios are currently underway. If such studies reveal an increase in de novo mutation burden, similar to findings in autism and schizophrenia, this approach will be very likely to lead toward gene discovery in TS. Such studies can provide insight into how changes in protein-coding and even regulatory regions have an effect on TS risk, leading us toward the ultimate goal of gaining a deeper understanding of the underlying biology to inform interventions with greater efficacies. Thus far, genetic studies have shown some overlap with findings from other research approaches (e.g. brain imaging and pharmacological studies), pointing us toward the involvement of certain neurotransmitter systems and implicating brain regions suspected to be involved in TS pathology.

Pathophysiology

The cortico-striato-thalamo-cortical circuit has been implicated in the biology of TS due to its involvement in movement initiation and inhibition [94]. The striatum has been a particular region of interest in research, with studies indicating that deficits of striatal pathways contribute to TS etiology [43, 95–100]. A PET study examining 18 TS patients and 16 controls found that the ventral striatum was significantly affected, since the activation of the ventral striatum corresponded with motor cortical area activation in TS patients [100]. On the other hand, healthy individuals demonstrated a negative correlation in activity between the two areas [100]. The aforementioned cortico-striato-thalamo-cortical pathways require various neurotransmitters (glutamate, DA, GABA, and serotonin) for signal transduction, and previous studies have reported abnormalities in these systems in TS [95–98, 101–105]. Most recently, the histaminergic pathway has also been added to the list of potentially malfunctioning systems in the disorder [40, 90].

Genetic studies in TS are beginning to show agreement with imaging, animal models, and pharmacological findings, and the hope is that these convergences will provide a more complete understanding of TS pathophysiology. The dopaminergic system has been an area of interest since dopamine antagonists (e.g. risperidone, pimozide, and haloperidol) have been used to attenuate

tics [11]. Singer et al. [97] found an upregulation of DA receptors in the postmortem striatum of TS patients compared to unaffected controls, indicating a decrease in extracellular DA. In addition, studies have detected an association between TS and certain SNPs in the gene encoding the dopamine receptor D2 (DRD2); however, the results are based on small cohorts [49, 103, 106]. Other studies have not been able to find connections between the DA receptor and TS [107, 108]. Nonetheless, some research examining the dopamine transporter 1 (DAT1) gene (*SLC6A3*) converge to support its involvement in TS biology, strengthening the view that the dopaminergic system plays a substantial role in the disease [47, 50, 109].

In the serotonergic pathway, tryptophan hydroxylase (TPH2) has been an interest of study, since it acts as the rate-limiting enzyme in serotonin synthesis [110]. To date, Mössner et al. [56] have identified intronic SNPs (rs4565946, rs4570625) in the TPH2 gene during a study of 98 TS patients and 178 controls. On the other hand, serotonin receptor genes have not been able to provide significant findings and require more extensive study [111, 112]. Similarly, research focusing on the serotonin transporter (SERT) gene (*SLC6A4*) has provided varying results [53, 113], although a recent finding by Moya et al. [114] highlights the association of a high-expression variant and a rare gain-of-function variant of the gene with TS.

Recently, the glutamatergic pathway has also garnered attention in the understanding of TS biology due to its role in OCD, where malfunctioning neurotransmission of glutamate has been studied [115]. Due to the high comorbidity of TS with OCD, indications for a shared genetic and pathophysiological background have been examined. *SLC1A3* (*Solute Carrier, Family 1, Member 3*), a gene related to a supported OCD candidate gene (*SLC1A1*), was investigated by Adamczyk et al. [116] in the context of TS. Although a functional missense variant was overrepresented in affected individuals, it was not statistically significantly different from controls [116]. In addition, the SAP90/PSD95-associated protein 3 (SAPAP3/DLGAP3), involved in synaptic scaffolding, has been a candidate area of research, as it is present in the glutamatergic synapses of the striatum [117]. As a result, Crane et al. [117] studied 289 TS trios and determined a nominally significant genetic association between *DLGAP3* and TS. Moreover, *DLGAP3*/SAPAP3 KO mice have demonstrated OCD-like behaviors, such as compulsive grooming and anxiety, along with excitatory synaptic dysfunction in the striatum [118, 119].

The findings of Ercan-Sencicek et al. [40] on HDC shed light on the involvement of the histaminergic pathway in TS etiology and its connection to the disease phenotype. Histamine is normally involved in smooth muscle contractions and neurotransmission; however, disturbances in its synthesis by HDC can have detrimental effects [120]. Ohtsu et al. [121] examined *HDC* KO mice and found that they exhibited a 50% decrease in brain histamine levels, reinforcing the enzyme's role in histamine synthesis. A more recent *HDC* KO mouse model also observed a decrease in histamine, but also discerned an increase in DA, along with elevated D2 and D3 receptor density in the substantia nigra [42]. Additionally, increased motor stereotypies were observed with the KO mice, which could be diminished through the administration of haloperidol, a D2 agonist [42]. Individuals affected with the W317X mutation, which causes the production of an abnormal HDC, also present with an increase in D2 and D3 receptors in the same region [42]. These new findings suggest novel therapeutic approaches to treat the disorder, while reinforcing the importance of genetic studies in elucidating the underlying mechanisms that characterize TS.

Discussion and Future Directions

The intricate phenotype and biological mechanisms underlying TS, along with the need for treatments with greater efficacy, have been an impetus for understanding the genetic basis of the disease. In retrospect, early studies did not adequately account for the genetic complexity of TS, and its heterogeneity is becoming evident as research in the field progresses. Nevertheless, some studies are beginning to strengthen support for the involvement of dopaminergic, glutamatergic, and serotonergic systems in the pathophysiology of TS. Most recently, the histaminergic pathway has been included as a new target of research and, possibly, treatment. The genetic overlap between TS and other neuropsychiatric disorders, most notably ASD, may also shed light on shared mechanisms amongst the diseases [88, 90, 91]. For the most part, many genetic findings in TS to date have not reached statistical significance or lack reproducibility. Studies now underway seek to utilize larger cohorts and high-throughput sequencing to garner support for specific genes and biological pathways in TS [122–124]. There is great hope that these new studies will accelerate progress in understanding TS so that we can improve the lives of those suffering from this debilitating disorder.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

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