Genetic Susceptibility and Neurotransmitters in Tourette Syndrome

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Abstract

Family studies have consistently shown that Tourette syndrome (TS) is a familial disorder and twin studies have clearly indicated a genetic contribution in the etiology of TS. Whereas early segregation studies of TS suggested a single-gene autosomal dominant disorder, later studies have pointed to more complex models including additive and multifactorial inheritance and likely interaction with genetic factors. While the exact cellular and molecular base of TS is as yet elusive, neuroanatomical and neurophysiological studies have pointed to the involvement of cortico-striato-thalamocortical circuits and abnormalities in dopamine, glutamate, gamma-aminobutyric acid, and serotonin neurotransmitter systems, with the most consistent evidence being available for involvement of dopamine-related abnormalities, that is, a reduction in tonic extracellular dopamine levels along with hyperresponsive spike-dependent dopamine release, following stimulation. Genetic and gene expression findings are very much supportive of involvement of these neurotransmitter systems. Moreover, intriguingly, genetic work on a two-generation pedigree has opened new research pointing to a role for histamine, a so far rather neglected neurotransmitter, with the potential of the development of new treatment options. Future studies should be aimed at directly linking neurotransmitter-related genetic and gene expression findings to imaging studies (imaging genetics), which enables a better understanding of the pathways and mechanisms through which the dynamic interplay of genes, brain, and environment shapes the TS phenotype.

1. INTRODUCTION

Relatives of Tourette syndrome (TS) probands have between 10 and 100% higher rates of TS and 7 to 22% higher rates of other chronic tic disorders than the general population or relatives of controls (for a review, see O'Rourke, Scharf, Yu, & Pauls, 2009). Twin studies

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have clearly indicated a genetic contribution in the etiology of TS and other tic disorders. Initial, small twin studies on TS and other tic disorders supported a genetic etiologic hypothesis with 53–56% concordance for TS and about 77% for chronic motor tics in monozygotic compared to 8% and 23% in dizygotic twins, respectively (Hyde, Aaronson, Randolph, Rickler, & Weinberger, 1992; Price, Kidd, Cohen, Pauls, & Leckman, 1985). Larger twin studies on tic disorders confirmed this significant heritability with estimated heritability of 50–56% (Bolton, Rijsdijk, O'Connor, Perrin, & Eley, 2007; Lichtenstein, Carlstrom, Rastam, Gillberg, & Anckarsater, 2010).

Early segregation studies of TS suggested a single-gene autosomal dominant disorder. Initial linkage studies of large pedigrees in TS, however, have not converged on a single region or led to the identification of mutations altering the structure or function of transcripts mapping within putative linkage intervals, generally considered a requisite of successful linkage efforts. Later studies have pointed to more complex models including additive and multifactorial inheritance (Pauls & Leckman, 1986; Seuchter et al., 2000; Walkup et al., 1996), with likely interaction of several or many genes with potential environmental factors. The complex, heterogeneous genetic architecture of TS has considerably complicated the identification of causative genetic variants and risk alleles (Bloch, State, & Pittenger, 2011; State, 2011).

Initially, family studies focused on whether or not TS and chronic tic disorders was familial. However, it became apparent that other neuropsychiatric disorders including obsessive–compulsive disorder (OCD), attention-deficit/hyperactivity disorder (ADHD), and autism spectrum disorder (ASD) were also increased in the relatives suggesting a shared genetic relationship could exist (Pauls et al., 1986). Studies indicated that OCD might be etiologically related to TS irrespective if the proband was ascertained for TS or OCD (Pauls, Alsobrook, Goodman, Rasmussen, & Leckman, 1995; Pauls et al., 1986; Pauls, Raymond, Stevenson, & Leckman, 1991; Walkup et al., 1996). Additionally, twin studies have supported the relationship between TS or tic disorder and OCD or obsessive–compulsive symptoms (Bolton et al., 2007; Price et al., 1985). The relationship between TS and ADHD is more complex with some studies supporting and other studies not supporting the shared etiology hypothesis (Debes, Hjalgrim, & Skov, 2010; Mathews & Grados, 2011; O'Rourke et al., 2011; Pauls et al., 1986; Stewart et al., 2006). Recent family studies have indicated a complex neurobiological relationship between TS and ADHD in the context of TS comorbid with OCD suggesting the genetic relationship between ADHD and OCD may partially explain the relationship between TS and ADHD (Debes et al., 2010; Mathews & Grados, 2011; O'Rourke et al., 2011; Stewart et al., 2006).

The rate of comorbidity between TS and ASD exceeds that expected by chance (Canitano & Vivanti, 2007) and family studies suggest a biological relationship irrespective if the proband was ascertained on TS or ASD (Burd, Li, Kerbeshian, Klug, & Freeman, 2009; Canitano & Vivanti, 2007). While no twin study has specifically assessed the relationship between TS and ADHD or ASD, results of a population-based twin study of ASD in Sweden showed high genetic correlations between ASD and tic disorders, tic disorders and ADHD, and between ASD and ADHD (Lichtenstein et al., 2010). The authors concluded that tic

disorders, ADHD, and ASD each exhibit unique genetic influences and that tic disorders and ADHD have separate genetic influences in common with ASD.

2. NEUROTRANSMITTER ABNORMALITIES ASSOCIATED WITH TS

While the exact cellular and molecular base of TS is as yet elusive, neuroanatomical and neurophysiological studies have pointed to the involvement of cortico-striato-thalamocortical circuits, which link specific regions in the frontal cortex to subcortical structures (Wang et al., 2011). Within these circuits, transmission of messages is regulated through various neurotransmitters, including dopamine, glutamate, gamma-aminobutyric acid (GABA), and serotonin. Evidence of potential neurotransmitter abnormalities associated with TS may stem from several areas, such as response of tics to certain classes of medication; investigation of cerebrospinal fluid, blood, and urine; analysis of postmortem brain tissue; and position emission tomography (PET) and single photon emission tomography (SPECT) studies.

The most consistent evidence is available for involvement of dopamine-related abnormalities based on the response of tics to dopamine blocking agents such as risperidone, pimozide, and haloperidol and supported by SPECT/PET and postmortem studies. Available studies have pointed to a reduction in tonic extracellular dopamine levels (as suggested by an upregulation of postsynaptic dopamine receptors; Singer, Hahn, & Moran, 1991) along with hyperresponsive spike-dependent dopamine release, following stimulation (Singer et al., 2002).

Most likely, dopamine is not the only neurotransmitter involved in TS pathogenesis. A role for serotonin is suggested by a reduction in cerebrospinal fluid of 5-hydroxyindoleacetic acid (Leckman et al., 1995), the principal metabolite of serotonin and by a reduction of plasma tryptophan, whole blood serotonin, and the 24-h urine secretion of serotonin in some patients with TS (Comings, 1990). Moreover, a PET study suggested decreased serotonin transporter (SERT) binding potential (Wong et al., 2008). Involvement of the primary excitatory neurotransmitter glutamate is suggested by reduced glutamate levels in postmortem TS brains (Anderson et al., 1992). Findings of reduced numbers of parvalbumin (PV)-containing interneurons in postmortem TS caudate and putamen form indirect evidence for a role for GABA (Kataoka et al., 2010), given the GABAergic nature of such interneurons. GABA involvement is also suggested by a recent PET study, showing decreased binding of GABA receptors in TS patients in the ventral striatum and globus pallidus as compared to healthy controls (HCs) (Lerner et al., 2012).

In the next sections, we will provide a comprehensive review of the TS literature with regard to (1) neurotransmitter-related genetic variants and (2) neurotransmitter-related RNA expression studies of blood.

3. NEUROTRANSMITTER-RELATED GENETIC FINDINGS

Genes in the dopaminergic and serotonergic pathways have been traditionally included among the prime suspects for investigation in relation to TS etiology. For instance, genes encoding the dopamine receptors were among the first to be investigated by multiple studies,

often with inconsistent results. Early in the 1990s, the *dopamine receptor D2* (*DRD2*) Taq1 A polymorphism (SNP rs1800497) was reported to be associated with TS (Comings et al., 1991). Lee et al. (2005) later replicated this finding in a Taiwanese population and most recently, Herzberg et al. (2010) showed positive association with three tagging single nucleotide polymorphisms (SNPs) and a five-SNP haplotype across the *DRD2* gene. It should be noted that each of these studies only analyzed a small sample and multiple negative results for *DRD2* and TS have also been reported in the literature (Diaz-Anzaldua et al., 2004; Gelernter, Pauls, Leckman, Kidd, & Kurlan, 1994; Nothen et al., 1994). In *DRD4*, a 48-bp variable number of tandem repeats (VNTRs) polymorphism has been associated with TS in a sample of 110 trios of French–Canadian origin (Diaz-Anzaldua et al., 2004), as well as multigenerational pedigrees (Grice et al., 1996). Again, others could not replicate this result (e.g., Barr, Wigg, Zovko, Sandor, & Tsui, 1996; Hebebrand et al., 1997), pointing to the need of studies with much larger sample sizes and greater statistical power. Studies of the *DRD1* and *DRD3* genes have not yielded any positive findings.

On the other hand, indications of association of TS to the *dopamine transporter DAT1* (SLC6A3) gene have been much more consistent, and the 10-repeat allele of a common 40-bp VNTR in the 3′ untranslated region of the *DAT1* gene has been repeatedly implicated in TS etiology. The 10/10 genotype has been reported to be more frequent in TS patients (Comings et al., 1996), and tendency for preferential transmission of the 10-repeat allele was observed in a family-based study (Diaz-Anzaldua et al., 2004). The *DAT1* 40-bp VNTR has also been found to be associated with increased tic severity in a family-based study using a dimensional approach (Tarnok et al., 2007). Studying a sample of 266 individuals with TS and 236 controls, another *DAT1* polymorphism (DdeI site) was also reported to be associated with TS (Yoon et al., 2007). It should be noted that the 10-repeat *DAT1* allele has also been suggested as a genetic risk factor for ADHD (Faraone et al., 2005), and internalizing disorders (Rowe et al., 1998), whereas externalizing behavior problems were linked to the 9-repeat allele (Young et al., 2002).

The *DBH* gene encoding dopamine beta-hydroxylase, the enzyme which catalyzes the conversion of dopamine to norepinephrine, influences both the dopaminergic and adrenergic systems. Thus, the *DBH* gene has also been studied in relation to the TS phenotype, albeit with inconclusive findings. Following a case–control design, Comings et al. (1996) have reported an association to TS while Ozbay et al. (2006) could not find an association in a sample of Canadian and Turkish trios. Again, the difference in methodology used and small sample sizes in each of the studies should be highlighted.

Moving to the serotonergic pathway, multiple candidate genes have also been studied in relation to TS. *TPH2* encodes the isozyme of tryptophan hydroxylase which is found in the serotonergic neurons of the brain and is the rate-limiting enzyme in the synthesis of serotonin (5-hydroxytryptamine, or 5HT) (Walther et al., 2003). Studying 98 individuals with TS and 178 controls Mossner, Muller-Vahl, Doring, and Stuhrmann (2007) showed an association to an intronic *TPH2* SNP (rs4565946) as well as a haplotype of this intronic SNP and SNP rs4570625 (located in the transcriptional control region of TPH2). Previously, this same haplotype was also found to be over-transmitted to individuals with OCD (71 trios; Mossner et al., 2006).

Genetic association studies of TS to the serotonin (5HT) receptor genes are very limited and only a very small number of polymorphisms in each of these genes have been studied in relation to TS. The *HTR2B* gene was screened in Chinese Han and European ancestry TS patients (128 and 132, respectively), and identified polymorphisms were compared to ethnically matched controls (248 Chinese Han and 138 European), revealing no significant association (Guo et al., 2012). In a similar fashion, studying SNPs along the *HTR1A* and *HTR2A* receptor genes in a sample of 87 TS patients and 311 HCs, Dehning et al. (2010) could not find a significant association. However, they reported a nominal association to two polymorphisms in the promoter region of *HTR2C* (C-759T, G-697C) in males (Dehning et al., 2010).

The *SERT* gene *solute carrier family 6—neurotransmitter transporter, serotonin, member 4* (*SLC6A4*) has been implicated in OCD etiology (Lesch et al., 1996; Voyiaziakis et al., 2011) but so far, its study has provided inconclusive results in the TS literature. Particular emphasis has been placed on the study of a functional insertion–deletion variant in the SERT-linked polymorphic region (*5-HTTLPR*). This polymorphism was not found to be associated to TS in a small study of 52 TS patients and 63 controls (Cavallini, Di Bella, Catalano, & Bellodi, 2000) or a sample of 108 TS trios (Liu et al., 2011). Dehning et al. (2010) also studied an *SLC6A4* SNP (rs63749047) and could not report any association. However, most recently, studying a sample of 151 individuals with TS and 858 controls, Moya et al. (2013) found that high expression variants of *SLC6A4* as well as a rare *SLC6A4* gain-of-function variant (I425V) were significantly associated to TS.

On the other hand, the *monoamine oxidase-A* gene (*MAO-A*) plays a vital role in the inactivation of both dopamine and serotonin and its role in TS etiology has been supported by two independent studies. Originally, *MAO-A* was implicated in TS etiology by Gade et al. (1998), who studied a VNTR of *MAO-A* in a sample of 229 individuals with TS, 57 controls, and 90 affected and unaffected relatives of TS probands. Subsequently, Diaz-Anzaldua et al. (2004) studied a sample of 110 trios with TS and found a different VNTR (located in the promoter) as well as a haplotype of this VNTR with two adjacent SNPs to be significantly associated with the disorder. *MAO-A* has also been proposed as a susceptibility gene for ADHD (Xu et al., 2007).

Investigation of the role of glutamatergic pathway genes in TS etiology is regaining interest based on growing evidence that disrupted neurotransmission of glutamate plays a role in the etiology of OCD (Wu, Hanna, Rosenberg, & Arnold, 2012). In fact, the glutamate transporter *Solute Carrier, Family 1, Member 1* (*SLC1A1* gene) represents one of the most well-supported candidate genes for OCD (Wu et al., 2012). Adamczyk et al. (2011) recently studied the role of *SLC1A3*, another member of this family of genes, in relation to TS and found a functional missense variant involving a highly conserved residue (E219D) to be nominally overrepresented in TS patients, although the comparison was not statistically significant (Adamczyk et al., 2011). The SAP90/PSD95-associated protein 3 (SAPAP3/ DLGAP3) is a postsynaptic scaffolding protein which is highly expressed in striatal glutamatergic synapses and its gene is also been investigated in association to TS yielding nominally significant associations in a sample of 289 TS trios (Crane et al., 2011). *DLGAP3* knockout mice display OCD-like behavior, consisting of compulsive grooming behavior

leading to facial hair loss and skin lesions, as well as anxiety-like phenotypes (Welch et al., 2007).

The *CNTNAP2* gene (*contactin-associated protein-like 2—Caspr2*) is a member of the neurexin superfamily that has been found disrupted in patients with TS and is also associated with a broad range of phenotypes including ASD, schizophrenia, intellectual disability, dyslexia, and language impairment (Rodenas-Cuadrado, Ho, & Vernes, in press). Neurexins and neuroligins play a pivotal role in the development and modulation of synaptic connectivity and represent central organizing molecules for excitatory glutamatergic and inhibitory GABAergic synapses in the mammalian brain (Craig & Kang, 2007). Verkerk et al. (2003) first reported on a TS family where the affected father and two affected children shared a chromosome 2p21–p23 insertion on chromosome 7q35–q36, thereby interrupting the *CNTNAP2* gene. Disruption of introns 8–13 of the CNTNAP2 gene was also reported in a boy with mild facial dysmorphisms, speech delay, and ASD, along with features of TS such as violent outbursts and obsessive and self-directed behavior (Poot et al., 2010).

Recently, a deletion involving exons 4, 5, and 6 of the gene *NLGN4* (*neuroligin 4*) was associated with TS and identified in a family with affected individuals represented by a boy affected with autism with a motor tic; his brother with TS and ADHD; and their carrier mother with learning disorder, anxiety, and depression (Lawson-Yuen, Saldivar, Sommer, & Picker, 2008). Finally, another member of the *neurexin family neurexin 1* (*NRXN1*) gene was also recently implicated in TS by a study of copy number variation in populations from Antioquia, Colombia, and of the Central Valley of Costa Rica (Nag et al., 2013). Their genome-wide study of 179 TS patients and 234 controls identified the largest patient restricted rearrangements in the *NRXN1* gene (two ~400 kb deletions) encompassing exons 1–3. Analyzing a total of 232 individuals with TS and 234 Antioquian controls, they found four TS patients carrying rearrangements in *NRXN1*, while studied controls did not carry *NRXN1* rearrangements (Nag et al., 2013). Further investigation of the role or rare variants in TS etiology will require the study of large sample sizes and collaborative efforts.

4. HISTAMINE-RELATED GENETIC FINDINGS

One approach to gaining a foothold on genetic mechanisms and the underlying biology of TS is by studying the contribution of rare variants in the disorder. Rather than investigating TS under the assumption that it is a genetically homogeneous entity, recent rare variant studies have conceptualized TS as a highly heterogeneous disorder. One pursues the hypothesis that rare variants carrying large effects may account for only a small fraction of the total population risk for TS but have the potential to highlight important biological mechanisms that can lead to novel treatments, as has been shown in a variety of other medical conditions (e.g., Ji et al., 2008; Johansen et al., 2010). In other words, the rationale for studying so-called outlier families is not to identify a gene which would explain the etiology of the large majority of TS cases; rather, this approach seeks to identify rare variants which can help to elucidate the underlying biology of TS. Several such studies have reached or approached statistical significance (Breedveld, Fabbrini, Oostra, Berardelli, & Bonifati, 2010; Curtis et al., 2004; Knight et al., 2010; Laurin, Wigg, Feng, Sandor, & Barr,

2009; Merette et al., 2000; Verkerk et al., 2006), but nearly all have not yet identified a rare mutation within the linkage intervals accounting for the statistical results.

The one exception is the report by Ercan-Sencicek et al. (2010) that utilized an outlier approach of investigating an individual interesting family. The authors described a two-generation family with nine affected members with TS. Diagnoses were confirmed using the Structured Clinical Interview for DSM-IV (First, Spitzer, Gibbon, & Williams, 1996). All patients had current or past motor tics, and eight also had current or past phonic tics. Given a model of dominant transmission in the pedigree, a single region on chromosome 15 reached the maximum theoretical LOD score in linkage analysis. Within this region, gene sequencing revealed a heterozygous G-to-A transition at nucleotide position 951 in exon 9 of the *Histidine Decarboxylase* (*HDC*), resulting in a W317X substitution that was predicted to result in a truncated protein lacking key segments of the active domain (Ercan-Sencicek et al., 2010). HDC is the rate-limiting enzyme in histamine (HA) biosynthesis, suggesting that histaminergic neurotransmission may be involved in the pathobiology of TS in this family. A loss of function for the enzyme carrying the mutation was confirmed biochemically. *In vitro* studies in *Escherichia coli* indicated that the mutant protein might act in a dominant-negative manner, altering not only the mutation carrying protein, but the function of the remaining normal allele as well. However, studies in mice that carry a highly similar version of the protein indicate that heterozygous *HDC* knockouts have approximately a 50% reduction in brain HA (Ohtsu et al., 2001).

This linkage finding suggested for the first time that reduced HA biosynthesis can lead to symptoms of TS. Subsequently, an analysis of rare copy number variations in 460 TS subjects versus controls identified an overrepresentation of variants in histaminergic signaling genes (Fernandez et al., 2012). More recently, a study of tagging SNPs across the *HDC* gene in 520 European families with TS found significant overtransmission of alleles at two SNPs and significantly associated haplotypes (Karagiannidis et al., 2013). Together, these studies suggest that abnormalities in histaminergic signaling may contribute to TS in a larger subset of cases, beyond this original unique family.

The causal connection between a reduction in HDC activity and the symptoms of TS remains unclear, but a convergence of evidence from earlier *in vitro* work, recent animal models, and research with members of this index family is making a strong case for causality, providing clues about the underlying neurobiology of TS, and bringing into focus opportunities for novel TS treatments.

HA signaling in the central nervous system is mediated by four G protein-coupled receptors, located both presynaptically (predominantly H3 as well as H4) and postsynaptically (H1–H3). Presynaptic HA receptors regulate not only the release of HA, but also a variety of other neurotransmitters, including dopamine. Several lines of evidence suggest that HA acts in a counter-regulatory fashion, with increased HA resulting in decreased dopamine signaling and vice versa (Ferrada et al., 2008; Munzar, Tanda, Justinova, & Goldberg, 2004). H2 and H3 receptors are enriched in the striatum and cortex, regions of the brain implicated in TS (Haas, Sergeeva, & Selbach, 2008), and studies of rodents with decreased brain HA show increased sensitivity to stereotypies when administered dopamine agonists

(Kubota et al., 2002). Furthermore, *in vitro* studies of mouse brain demonstrate that HA modulates many aspects of functional connectivity within the striatum. Histaminergic activity during increased attention and wakefulness affects the flow of excitatory inputs to the striatum and intrastriatal processing of those inputs, such that the net effect on striatal functioning is feedforward inhibition and a suppressed excitatory drive (Ellender, Huerta-Ocampo, Deisseroth, Capogna, & Bolam, 2011).

Recent studies of *HDC* haploinsufficient and knockout mice have further strengthened evidence for the causality of mutations in this gene. *HDC* gene dosage (i.e., the number of copies of the gene) in these models correlated directly with HA concentrations in the central nervous system and inversely with the induction of tic-like stereotypies following administration of a stimulant (DA agonist) medication. There is an inverse relationship between *HDC* gene dosage and deficits in auditory prepulse inhibition (PPI), a well-established measure of sensory processing that has been found to be abnormal in TS (Swerdlow et al., 2001). Furthermore, *HDC* haploinsufficient and knockout mice show elevated dopamine receptor densities in the substantia nigra and DA dysregulation via microdialysis. Finally, stereotypies in *HDC* knockout mice are reduced by administration of the D2 antagonist haloperidol, an effective treatment for TS. These mouse model findings are congruent with recent findings in members of the index family described by Ercan-Sencicek et al. (2010). PET imaging showed increased D2 and D3 receptor density as well as PPI abnormalities in subjects carrying the *HDC* mutation (Christopher Pittenger, personal communication).

The studies described earlier, beginning with the identification of an outlier family with TS, all converge on a previously unsuspected association of abnormalities in HA neurotransmission with TS. In addition to demonstrating the great potential of rare variant studies to elucidate underlying biological mechanisms, the HA evidence points to potential novel therapeutics for TS. As H3R antagonists and inverse agonists are in late-stage clinical development and being considered for other neuropsychiatric indications (Brioni, Esbenshade, Garrison, Bitner, & Cowart, 2011; Lebois, Jones, & Lindsley, 2011), there may well be near-term opportunities to translate a deeper understanding of the relationship of HA and tics into novel treatment approaches.

5. NEUROTRANSMITTER-RELATED GENE EXPRESSION STUDIES OF BLOOD

Traditional genetic approaches have not been particularly informative for complex genetic disorders such as TS and do not address any role of the environment on phenotype. In contrast, RNA expression studies of tissues including blood and brain are affected by genetics and environment (Sharp et al., 2011; Tang et al., 2004). Thus, a series of studies has been performed that have begun to examine RNA expression in blood of patients with TS, with the expectation that RNA expression will give clues as to genetic and environmental underpinnings of TS.

In all of the studies to be described in this section, blood was obtained from patients and controls in PAXgene tubes which lyse all cells in blood and immediately stabilize the RNA.

The RNA obtained represents that from leukocytes—including neutrophils, monocytes, and lymphocytes—and from immature red blood cells and platelets. The RNA was isolated from the whole blood and processed on whole-genome microarrays that can assess the expression of all known RNAs (genes). The expression of each gene can be measured using the arrays and expression of controls and patients compared. The studies suffer from multiple comparisons with likely many false positives because 20,000 genes are being compared in very small samples (Sharp et al., 2011). Nevertheless, these studies are beginning to provide proof of principle that changes of RNA can be observed and that these changes will need to be confirmed in larger sample sets in future studies.

In one of the first studies, 16 familial TS patients and 16 control TS patients were investigated (Lit, Gilbert, Walker, & Sharp, 2007). It was found that 14 genes, primarily Natural Killer (NK) Cell genes, discriminated between TS and all controls. Five probesets (four genes) resided in chromosomal regions previously linked to familial TS or OCD. Granzyme B and Natural Killer Cell Group 7 (NKG7) were confirmed using RT-PCR. Granzyme B is a cytotoxic serum protease protein that participates in inducing apoptosis of target cells for NK Cells and cytotoxic CD8+ lymphocytes which are part of the innate immune system. NKG7 is a membrane protein expressed by NK Cells and CD8+ cytotoxic lymphocytes and is induced with transplant rejection. Using the 14 genes, a principal components analysis as well as a cluster analysis identified a TS subgroup ($n = 10/16$) that overexpressed the NK genes. 7/10 subjects within this subgroup were diagnosed with an ADHD, suggesting that this expression profile might be associated with familial TS and comorbid ADHD (Lit et al., 2007). These data were among the first to suggest that RNA expression in familial TS might differ from controls, and that specific TS phenotypes might be associated with specific RNA changes in blood. In addition, the data implicated possible differences in the innate immune system in familial TS versus controls (Lit et al., 2007).

Because infection and immune responses have been implicated in the pathogenesis of TS, we hypothesized that children with TS would have altered gene expression in blood compared to controls. In addition, because TS symptoms in childhood vary with age, it was tested whether gene expression changes that occur with age in TS differ from normal control children. Whole blood was obtained from 30 children and adolescents with TS and 28 healthy children and adolescents matched for age, race, and gender (Lit, Enstrom, Sharp, & Gilbert, 2009). Gene expression (RNA) was assessed using whole-genome Affymetrix microarrays. Age was analyzed as a continuous covariate and also stratified into three groups: 5–9 (common age for tic onset), 10–12 (when tics often peak), and 13–16 (tics may begin to wane). Expression of many genes and multiple pathways differed between TS and controls within each age group (5–9, 10–12, and 13–16), including genes involved in the immune synapse, and proteasome- and ubiquitin-mediated proteolysis pathways. Notably, across age strata, expression of interferon response, viral processing, NK, and cytotoxic T-lymphocyte cell genes differed. These findings suggested age-related interferon, immune, and protein degradation gene expression differences between TS and controls. The data also emphasized the need to factor age into any TS study where the immune system and disease status were considered.

Next the relationship of tic severity to RNA expression in blood was examined, and possible confounding effects of medications were also considered (Liao, Corbett, Gilbert, Bunge, & Sharp, 2010). RNA was isolated from the peripheral blood of 20 medicated TS subjects (MED) and 23 unmedicated TS subjects (UNMED), and quantified using whole-genome Affymetrix microarrays. A large number of genes correlated with tic severity in MED and UNMED groups. A total of 116 genes were shared between the two groups, and among these 53 were correlated in the same direction in MED and UNMED, and 63 genes correlated in opposite directions in MED and UNMED. Examples of some of the genes are provided in Table 6.1. *D2 dopamine receptor* expression correlated positively with tic severity in MED but not UNMED. D2 dopamine receptor blockers reliably decrease tic severity in TS patients. GABA(A) receptor epsilon subunit expression negatively correlated with tic severity in UNMED but not MED. This GABA receptor is expressed in leukocytes and in the substantia nigra in brain. The expression of phenylethanolamine *N*-methyltransferase, the enzyme that synthesizes epinephrine from norepinephrine, positively correlated with tic severity in UNMED but not MED. *NPAS4*, a gene that regulates whether progenitor cells become GABAergic inhibitory neurons in brain, positively correlated with tic severity in MED and UNMED subjects (Liao et al., 2010).

These data are intriguing because they are among the first to demonstrate that RNA expression in peripheral leukocytes correlates with a cardinal symptom of TS: tics. Moreover, they demonstrate that it is crucial to ensure that patients are medication free when assessing blood markers since medications can have a profound effect on the gene expression and must be controlled for in all such studies (Liao et al., 2010).

In a subsequent study of the unmedicated TS patients ($n = 26$), RNA expression on Affymetrix exon expression arrays versus tic severity was examined (Gunther et al., 2012). Tic severity was measured using the Yale Global Tic Severity Scale. Among the regulated genes included: *DRD2*, *Histamine receptor H3*, *Monoamine oxidase B*, *Brain-derived neurotrophic factor*, *Synaptosomal-associated protein, 25 kDa*, *SLC6A4*, and *SLC22A3* (*Solute carrier family 22 (extraneuronal monoamine transporter), member 3*), and s*olute carrier family 18 (vesicular monoamine), member 1*. These genes are highly associated with TS and have also been implicated in other movement disorders, ADHD, and OCD (Gunther et al., 2012). Correlation of gene expression in peripheral blood with tic severity may allow inferences about catecholamine pathway dysfunction in TS subjects (Gunther et al., 2012).

Given the altered numbers of GABAergic-PV and cholinergic interneurons observed in the basal ganglia of individuals with TS, it may be postulated that GABA- and acetylcholine (ACh)-related genes might be associated with the pathophysiology of TS (Tian, Gunther, et al., 2011). Total RNA isolated from whole blood of 26 unmedicated TS subjects and 23 HCs was therefore processed on Affymetrix Human Exon 1.0 ST arrays. Data were analyzed to identify genes whose expression correlated with tic severity in TS and to identify genes differentially spliced in TS compared to HC subjects. Many genes (3627) correlated with tic severity in TS ($p < 0.05$) among which GABA- ($p = 2.1 \times 10^{(-)}(3)$) and ACh($p = 4.25 \times 10^{(-)}(8)$) related genes were significantly overrepresented. Moreover, several GABA- and ACh-related genes were predicted to be alternatively spliced in TS compared to HC including GABA receptors GABRA4 and GABRG1, the nicotinic ACh receptor CHRNA4,

and cholinergic differentiation factor. This pilot study suggests that at least some of these GABA- and ACh-related genes observed in blood that correlate with tics or are alternatively spliced are involved in the pathophysiology of TS and tics (Tian, Gunther, et al., 2011).

As noted, alternative splicing of RNA in leukocytes of TS patients has been explored (Tian, Liao, et al., 2011). Alternative splicing is the process by which a single gene (stretch of DNA) gives rise to several different RNA species that code for similar but not identical proteins. To study alternative splicing, RNA was isolated from the blood of 26 unmedicated TS subjects and 23 HCs. Each sample was run on Affymetrix Human Exon 1.0 ST arrays and on 3′ biased U133 Plus 2.0 (HuU133) arrays. To investigate the differentially expressed exons and transcripts, analysis of covariance (ANCOVA) was performed, controlling for age, gender, and batch. Differential alternative splicing patterns between TS and HC were identified using analyses of variance models. Three hundred and 76 exon probe sets were differentially expressed between TS and HC (raw P <0.005, fold change >|1.2|) that separated TS and HC subjects using hierarchical clustering and principal components analysis. The probe sets predicted TS compared to HC with a >90% sensitivity and specificity using a 10-fold cross-validation. Ninety genes (transcripts) had differential expression of a single exon (raw P <0.005) and were predicted to be alternatively spliced (raw P <0.05) in TS compared to HC. These preliminary findings might provide insight into the pathophysiology of TS and potentially provide prognostic and diagnostic biomarkers. These are exciting findings given the possibility that differential exon expression and/or alternative splicing of peripheral blood genes might be useful for the diagnosis of TS (Tian, Liao, et al., 2011). These results need to be replicated in a larger, independent cohort.

Next other symptoms of TS were explored as to whether they might also be related to gene expression in peripheral blood (Tian et al., 2012). Inattentiveness, impulsivity, and hyperactivity are the primary symptoms associated with ADHD. TS is associated with comorbid inattention (IA) and hyperactivity/impulsivity (HI) symptoms in over 50% of cases. A next study determined if gene expression in blood correlated significantly with IA and/or HI rating scale scores in participants with TS. RNA was isolated from the blood of 21 participants with TS and gene expression measured on Affymetrix Human U133 Plus 2.0 arrays. To identify the genes that correlated with Conners' Parents Ratings of IA and HI ratings of symptoms, an ANCOVA was performed, controlling for age, gender, and batch. There were 1201 gene probe sets that correlated with IA scales, 1625 that correlated with HI scales, and 262 that correlated with both IA and HI scale scores (P <0.05, |partial correlation ($r(p)$)|>0.4). Immune, catecholamine, and other neurotransmitter pathways were associated with IA and HI behaviors. A number of the identified genes ($n = 27$) have previously been reported in ADHD genetic studies. Many more genes correlated with either IA or HI scales alone compared to those that correlated with both IA and HI scales. These findings support the concept that the pathophysiology of ADHD and/or its subtypes in TS may involve the interaction of multiple genes. These preliminary data also suggest gene expression may be useful for studying IA and HI symptoms that relate to ADHD in TS and perhaps non-TS participants. These results will need to be confirmed in future studies.

The above studies point out a novel approach to studying TS. Although the relation of blood to brain is obviously not a direct one, the findings suggest that elements of the molecular

pathophysiology of TS brain may be recapitulated to some degree in peripheral leukocytes. This may relate in part to constant communication between the immune system and the brain, to neurotransmitters being expressed in leukocytes as well as in cells in brain, and to the fact that peripheral systemic factors may affect both the blood and the brain.

6. CONCLUSION AND FUTURE STUDIES

TS is a complex disorder with an as yet poorly understood pathogenesis. While family studies have clearly indicated a major genetic component, the search for TS genes is complicated by the likely involvement of a multitude of genetic variants, both common and rare, the interaction with environmental factors, and the genetically heterogeneous background across involved families. PET/SPECT, psychopharmacological, and postmortem studies have pointed to the involvement of multiple neurotransmitter systems in the etiology of TS, including dopamine, serotonin, GABA, and glutamate. Genetic and gene expression findings are very much supportive of involvement of these neurotransmitter systems. Moreover, intriguingly, genetic work on a two-generation pedigree has opened new research pointing to a role for HA, a so far rather neglected neurotransmitter, with the potential of the development of new treatment options.

Future studies should be aimed at directly linking neurotransmitter-related genetic and gene expression findings to imaging studies. The field of imaging genetics uses research approach in which genetic information and structural and functional imaging data in the same subjects are combined to define neuromechanisms linked to genetic variation. Imaging genetic studies have the potential to provide a more complex and nuanced understanding of the pathways and mechanisms through which the dynamic interplay of genes, brain, and environment shapes variability in behavior (Muñoz, Hyde, & Hariri, 2009). Imaging genetics has found widespread application in other neurodevelopmental disorders such as ADHD and schizophrenia but has so far been neglected in the field of TS.

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Table 6.1

Example genes that correlated with tic severity in Tourette syndrome patients

Symbol	Probeset ID	Gene title	MED *r*	UNMED *r*
DRD2	206590_x_at	*Dopamine receptor D2*	0.58[*]	−0.028
GABRE	204537_s_at	*GABAA receptor e*	0.097	−0.71[*]
PNMT	206793_at	*Phenylethanolamine N-methyltransferase*	−0.24	0.52[**]
NPAS4	1554299_at	*Neuronal PAS domain protein 4*	0.51[**]	0.45[**]

[*] *p* 0.001,

[**] *p* 0.05.

MED *r*, correlation coefficient for medicated subjects; UNMED *r*, correlation coefficient for unmedicated subjects.