Pathogenetic model for Tourette syndrome delineates overlap with related neurodevelopmental disorders including Autism

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Tourette syndrome (TS) is a highly heritable neuropsychiatric disorder characterised by motor and vocal tics. Despite decades of research, the aetiology of TS has remained elusive. Recent successes in gene discovery backed by rapidly advancing genomic technologies have given us new insights into the genetic basis of the disorder, but the growing collection of rare and disparate findings have added confusion and complexity to the attempts to translate these findings into neurobiological mechanisms resulting in symptom genesis. In this review, we explore a previously unrecognised genetic link between TS and a competing series of trans-synaptic complexes (neurexins (NRXNs), neuroligins (NLGNs), leucine-rich repeat transmembrane proteins (LRRTMs), leucine rich repeat neuronals (LRRNs) and cerebellin precursor 2 (CBLN2)) that links it with autism spectrum disorder through neurodevelopmental pathways. The emergent neuropathogenetic model integrates all five genes so far found to be uniquely disrupted in TS into a single pathogenetic chain of events described in context with clinical and research implications. Translational Psychiatry (2012) 2, e158; doi:10.1038/tp.2012.75; published online 4 September 2012

Introduction

Tourette syndrome (TS) is characterised by motor and vocal tics, with a pre-pubertal age of onset, a waxing and waning course, and improvement in symptoms in adulthood.1 Clinical and epidemiological studies point to an association with other childhood onset behavioural and developmental disorders such as obsessive-compulsive disorder (OCD), attention-deficit/hyperactivity disorder (ADHD) and to a lesser extent with autism spectrum disorder (ASD) (see Glossary).2 The remarkable complexity, high heritability and intriguing phenotype of TS linking genetics, brain, mind and behaviour has spawned an international research effort to discover the pathogenetic basis of this neurodevelopmental disorder. Although the genetic and pathophysiological basis of the disorder remains unresolved, there is converging evidence to suggest involvement of the cortical striatal–pallidothalamic–cortical circuitry that mediates the integration of movement, sensation, emotion and attention. It is suggested that the improvement with advancing age is the result of compensatory responses that come in line with maturation when the frontal cortices become more efficiently connected to the striatum and to the motor and sensorimotor cortices.1

The familial nature of TS was evident from the time of its original description by Gilles de la Tourette in 1885. Twin studies suggest a monozygotic to dizygotic concordance of 77 to 23%, whereas family studies have consistently shown a 10- to 100-fold increase in the rates of TS in first-degree relatives.3 Furthermore, it is suggested that chronic tics and OCD are manifestations of the same underlying genetic susceptibility as TS.4 ADHD is another significant co-morbidity and more recent studies have highlighted that ASD is over represented in TS, occurring in about 4 to 5% of the TS population.5,6 Furthermore, Kadesjo and Gillberg7 found that while 5% of individuals with TS also had a diagnosis of Asperger’s syndrome, 17% showed three or more autistic symptoms and 65% had deficits relating to the autism spectrum. There is emerging evidence to suggest an overlap between TS and ASD from phenomenological, epidemiological and pathogenetic perspectives.8 TS and ASD are both conditions that begin during childhood and mostly affect males. Clinically, symptoms such as obsessions, compulsive behaviours, involuntary movements (tics in TS and stereotypies in ASD), poor speech control and echolalia are common in both conditions. Genetic epidemiology studies also support the existence of common susceptibility genes in both disorders.8 In 1991, Sverd9 hypothesised that the TS gene in its homozygous form may lead to the co-occurrence of TS and ASD, while in its dominant heterozygous form may lead to poor socialisation or communication. Twenty years on, there has been little progress in understanding the pathogenetic basis of TS except that linkage and candidate gene analyses have now virtually ruled out any likelihood of common dominant mutations of large effect.

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Attractive candidates

To contextualise the significance of the integrated synaptic model for TS presented in this review, it is important to cover with some detail the present state of uncertainty that exists regarding the genetic and molecular aetiology of TS. The depth of this uncertainty is reflected in the many candidate gene studies that populate the recent TS literature. 2, 5, 10 As dopamine antagonists represent the most effective medications for tic suppression, speculative candidate gene studies have largely focussed on those genes implicated within the dopaminergic pathway including the dopamine receptors DRD1-5, dopamine β-hydroxylase, dopamine-associated transporter SLC6A3, as well as the adrenergic receptors α1c, α2c, α2a and β2, various serotonin receptors and others. 3

Sadly, the candidate gene approach has not yielded a single susceptibility gene of large effect for TS. However, together these studies do tend to suggest that if common biochemical pathways involving neurotransmitters such as serotonin, dopamine and glutamate are involved, they are likely positioned downstream of the primary molecular anomalies in TS.

Linkage promises

Two non-parametric linkage analyses sufficient in size to identify common variants of moderate to large effect have now been completed: a genome scan for Tourette disorder in affected-sibling-pair and multigenerational families; 11 and a complete genome screen in sib pairs and multigeneration families by the Tourette Syndrome Association International Consortium (TSAICG). 12 One significant linkage region on 2p23 was identified 11 but results were not reproducible between these two studies and no associated mutations have been identified. 10-12 By comparison, parametric linkage analyses have been applied in the search for rare variants within a conserved region of the 3q27 critical linkage region. 11 dCNTNAP2 disrupted in family without TS. 53

Independent genomic rearrangements

Genomic rearrangements and copy number variations (CNVs) are the most common DNA lesions associated with TS and the most commonly rearranged locus in TS is chromosome 18q22. 2, 5, 11, 24-47 Recent reviews and reports on the genetics of TS 5, 45, 48 have identified at least 28 large independent genomic rearrangements and CNVs with unique breakpoints including deletions, insertions, duplications, inversions and inter-chromosomal translocations. 5, 11, 24-47 Of the rearrangements that have been characterised, approximately one third have directly disrupted genes (Table 1), whereas the remainder have breakpoints within intergenic regions (Table 2). 24-39, 40, 53-55, 64 Intergenic breakpoints are of great interest as they can lead to the dysregulation of a neighbouring gene(s) even when separated by very long distances. 49 Candidate genes (Table 2) located near TS intergenic translocation breakpoints may be similarly affected by long-range dysregulation. For example, the SLITRK1 gene, which encodes a neuronal leucine-rich repeat transmembrane protein (LRRTM) involved in neurite outgrowth, is located immediately adjacent to a large gene desert on chromosome 13q. This gene desert was disrupted by a de novo inter-chromosomal translocation breakpoint in a child with TS and ADHD. 24 Follow-on studies identified two rare functional sequence variants in SLITRK1 in unrelated TS patients. One of these mutations, a single nucleotide variation within a conserved region of the 3’UTR, 24 appears to strengthen the binding of a micro RNA hsa-miR-189 with consequent downregulation of SLITRK1 expression. Unfortunately, a later study that identified the same variants in unaffected individuals 50 indicates that SLITRK1 may be of limited effect in TS. 10

Table 1 The five genes directly disrupted in Tourette syndrome by unique genomic lesions

| Gene       | Locus | Function                  | Co-morbidities | DNA lesions                | Other |
|------------|-------|---------------------------|----------------|---------------------------|-------|
| NRXN4      | 2p21  | Neurexin 1 synapse        | ADHD           | Two truncating deletions   | c,d   |
| CNTPAP2    | 7q35  | Neurexin superfamily      | OCD, MR, SD    | Intragenic insertion      |       |
| LRRN3      | 7q31  | (Neural development)      | MR, SD         | Two exonic deletions      | T, SA, R, Dup |
| LRRTM5     | 10q21 | Neurexin ligand           | OCD, ADHD      | Two intragenic deletions  | SA    |
| NLGN4      | Xq22.33| Neurexin ligand           | ASD            | Truncating deletion       | XM    |

Abbreviations: ADHD, attention deficit/hyperactivity disorder; ASD, autism spectrum disorder; BD, block deletion associated with TS; Dup, duplication in TS; LS, linkage region of interest; MR, mental retardation; OCD, obsessive-compulsive disorder; R, most commonly duplicated locus in ASD; SA, strong polymorphic association with ASD; SCHZ, schizophrenia; SD, speech delay; T, TS translocation breakpoint association; TS, Tourette syndrome; XM, XXX chromosome mosaicism. 53

aRecurrent disruption of this gene in TS. bGene disruption co-segregates with disorder in TS family. c2p21-23 block insertion that disrupted CNTPAP2 harbours a TSAICG critical linkage region. 11 dCNTNAP2 disrupted in family without TS. 53

Bold type indicates protein implicated in Tourette syndrome.
Table 2 Candidate genes located near translocation breakpoints in Tourette syndrome

| Candidate | Locus | Function | Proximity/distance | Co-morbidities | Support data |
|-----------|-------|----------|--------------------|----------------|--------------|
| CBLN2 | 18q22.2/18q21.1 | Neurexin ligand | Adjacent ~ 2.0 Mb | OCD | BD53 |
| CBLN2 | 18q22.2/7q31 | Neurexin ligand | Adjacent ~ 400 kb | OCD | BD53 |
| LRRTM1 | 2p12/18q22.2 | Neurexin ligand | Breakpoint region9 | LS | OCD |
| LRTM1 | 3p21/8q24 | Neuro development | Break point region26 | LS | OCB |
| SLITRK1 | 13q31/13q33 | Neuro development | Adjacent ~ 350 kb24 | LS | OCB |
| SLC6A7 | 8q13/6p23 | Anion transport | Adjacent < 200 kb28 | OCB | T |
| SLC6A7 | 8q13/6q24 | Anion transport | Adjacent < 200 kb28 | ADHD, OCD | T |
| GRIK2 | 6q21/17p11 | Glutamate transporter | Break point region33 | OCD, ADHD | LS, SCZ81 |
| SLC11A1 | 9p23 recurrent del | Glutamate transporter | Within deleted region13,47 | Coprolalia | BD |
| CLIC6 | 21q22 | Neuronal Cl channel | Adjacent to duplication55 | | |
| DGCR2 | 22q11.2 duplication | Dopamine D2, 3 and 4R | Mutated in SCZ81 | | |

| Candidate | Function | Proximity/distance | Co-morbidities | Support data |
|--------------|------------|-----------------|----------------|--------------|
| CBLN2 | Neurexin ligand | Adjacent ~ 2.0 Mb | OCD | BD53 |
| CBLN2 | Neurexin ligand | Adjacent ~ 400 kb | OCD | BD53 |
| LRRTM1 | Neurexin ligand | Breakpoint region | LS | OCD |
| LRTM1 | Neuro development | Break point region | LS | OCB |
| SLITRK1 | Neuro development | Adjacent ~ 350 kb | LS | OCB |
| SLC6A7 | Anion transport | Adjacent < 200 kb | OCB | T |
| SLC6A7 | Anion transport | Adjacent < 200 kb | ADHD, OCD | T |
| GRIK2 | Glutamate transporter | Break point region | OCD, ADHD | LS, SCZ81 |
| SLC11A1 | Glutamate transporter | Within deleted region | Coprolalia | BD |
| CLIC6 | Neuronal Cl channel | Adjacent to duplication | | |
| DGCR2 | Dopamine D2, 3 and 4R | Mutated in SCZ | | |

Bold type indicates protein implicated in Tourette Syndrome.

Gene disruptions

Five genes have been directly disrupted in TS by independent genomic rearrangements and CNVs with unique breakpoints, namely IMMP2L, NRNX1, CTNNA3, NLGN4X and CNTNAP2.6 Viewed in isolation, each of these novel genomic rearrangements can be difficult to interpret in relation to a complex disorder like TS, even when a gene has been disrupted. There is always the concern that the rearrangement may be incidental or conversely that more than one gene may be affected.10,45,48 For example, in the 2011 case report describing the disruption of the IMMP2L gene there were additional genes deleted.39 Together these findings for IMMP2L are most unlikely candidate for TS that has no obvious functional association with the neuropathology of TS. IMMP2L is localised to the mitochondrial membrane where it regulates levels of reactive oxygen species associated with aging, kyphosis, wasting and ataxia.51 Extensive screening of TS patients also failed to identify any coding mutations in IMMP2L.39,52 Together these findings for IMMP2L only heightened uncertainty regarding other rearrangements and CNVs in TS.6,48 For example, there was already a degree of uncertainty regarding the disruption of the neurexin 1 (NRNX1), NLGN4X and CNTNAP2 genes in TS as each of these genes had been previously disrupted and/or mutated in ASD patients without the presentation of tics.53,54

Identification of nestlings hatches new perspective of TS

Nevertheless, this fore mentioned case report implicating IMMP2L was significant in a larger context as it represented the second disruption of the IMMP2L gene described in TS. This now meant that all five of the genes known to be disrupted in TS by unique rearrangements had been disrupted in multiple individuals with the disorder. Three of the five genes (IMMP2L, NRNX1 and CTNNA3) had now undergone recurrent disruption in unrelated individuals with TS and the other two gene disruptions (NLGN4X and CNTNAP2) co-segregate with the disorder within families (Table 1).6,36,39–40,45–46 which greatly strengthens the likelihood of their pathogenicity. The co-morbidities associated with these five gene disruptions also overlap in a representative manner the developmental and behavioural spectrum of disorders reported independently in TS: two of these gene disruptions were associated with OCD; two with ADHD; two with speech delay; and the disruption of NLGN4X was associated with ASD (Table 1). However, this series of co-morbidities does not reflect the full spectrum of phenotypic associations reported for these same five genes from other studies. For example, the IMMP2L, NRNX1, CTNNA3, NLGN4X and CNTNAP2 genes have all shown independent association with ASD (without tics), often through multiple means of enquiry,10,55 thus strengthening the case further for a pathogenic relationship between all the five genes. This overlap also lends a scientific support to the long-standing clinical and epidemiological finding of a phenotypic overlap between TS and ASD. Albeit, the functional nature of this relationship remained obscure until we reviewed it through the lense of a seemingly unrelated ASD association study that implicated two additional genes LRRN3 and LRRTM3.56

Polymorphisms in two structurally related genes LRRN3 and LRRTM3 show strong association with ASD susceptibility.55,56 LRRN3 and LRRTM3 are both neuronal leucine-rich repeat transmembrane protein genes (Box 1) and both are curiously positioned/nested inside other genes (see Glossary).57–61 Herein lies a most revealing twist in the TS story; LRRN3 and LRRTM3 are actually nested within two of
Box 1 Leucine-rich repeat transmembrane protein genes

Leucine-rich repeats (LRRs) are common protein–protein interaction domains found in proteins with diverse structure and function. LRRs are typically 20–29 amino acids in length with a conserved consensus sequence LxxLxxN/CxL (where x can be any amino acid and L can be replaced by V, I or F).67–70,75,76 There are several subgroups of LRR proteins differentiated by the consensus sequence and the inclusion of different combinations of supplementary domains (Figure 1). Among the ~313 LRR coding genes in the human genome, the transmembrane subgroupings are brain enriched and/or highly expressed in the nervous system, with roles in neuronal development and/or synaptogenesis. 64–67,77 The different families of transmembrane LRR proteins include AMIGO, NGL, LINGO, LRIG, FLRT, PAL, SALM, SLITTK, LRRN, LRRTM and LRTM (Figure 1). 54–87 The LRRN1, LRRN3, LRRTM3 and LRTM1 genes implicated in the pathogenetic model for Tourette syndrome (Figure 2) all share the curious structural relationship of being nested in the antisense orientation within an intron of another gene (see ‘Nested genes’ in Glossary). Evidence indicates convergent evolution of LRR gene nesting in different classes of genes.

The LRRTMs (leucine rich repeat transmembrane family) represent a highly conserved four-member gene family, which, with the exception of LRRTM4, are nested in the introns of different 2-catenin genes.69 LRRTMs are enriched in the nervous system, each with a distinct and highly regulated pattern of expression. LRRTMs are synaptic cell adhesion organizing molecules initiating excitatory presynaptic differentiation and mediating post-synaptic specializations. LRRTM1 is located within intron 7 of CTNNA2 (α2-catenin) and is highly expressed within the brain and salivary gland. 78 LRRTM1 appears to be associated with human handedness (relative hand skill), schizophrenia and language. LRRTM3, located within intron 7 of CTNNA3 (α7-catenin), expression is enriched within the cerebellum.

The LRRN (leucine rich neuronal) gene family of four are all brain-enriched type I transmembrane protein genes. LRRN1 is nested within intron 8 of the extended form of the SUMF1 (sulphatase modifying factor 1) gene. LRRN1 regulates boundary formation within the brain. LRRN3 is nested within intron 3 of the IMMP2L (inner mitochondrial membrane peptidase-like) gene. Lrm3 exhibits regulated expression in the developing ganglia and motor neurons of the neural system, and is upregulated during neuronal cortical injury. 54

The LRTM (leucine-rich and transmembrane domains) gene family is a highly conserved two-member family and both members are nested in introns of different CACNA2D (calcium channel, voltage-dependent, alpha 2/delta subunit) genes. LRTM1 is nested within intron 23 of CACNA2D3 and LRTM2 is nested within intron 23 of CACNA2D3.

The genes that have been disrupted in TS, namely IMMP2L and CTNNA3, respectively (NCBI Build 37.2, 2011). Thus, ASD associations have now been reported for both IMMP2L and CTNNA355,62–63 and for both of their nested genes, LRRN3 and LRRTM3, respectively.55,56 that suggests that these separate studies have identified the same functional associations. LRRN3 and LRRTM3 share a very close structure–function relationship with each other as do their larger gene families (Box 1)64–68 that are intimately involved in brain development (discussed below). This close structure–function relationship strongly implies that the original ASD associations reported for IMMP2L and possibly CTNNA3 may represent cryptic association assignments. Nevertheless, as host genes, any change in their transcription has the very real potential of directly impacting the transcription of their nested genes. Two scenarios exist whereby the disruption of the IMMP2L and CTNNA3 genes could alter the transcription of their nested genes: first through the introduction or disruption of a regulatory element(s) that modulates the transcription of the nested gene(s); or second and more consistent with the data is that the disruption of transcription of the host gene directly affects the transcriptional efficiency of the nested gene on the opposing DNA strand. The latter scenario infers the disruption of a pre-existing regulatory relationship between the host gene and nested gene(s). In this context, the nested relationship of the two LRR genes has significant implications for understanding TS: first, it strengthens the case for a genuine functional relationship between TS and ASD and all five genes disrupted in TS; second, given the close structure–function relationships that exist between LRRN3 and LRRTM3 it provides an invaluable starting point to begin to understand the biological function of the IMMP2L-LRRN3 and CTNNA3-LRRTM3 transcription complexes in neurodevelopment as it pertains to TS and its relationship to ASD (Box 1); third, it helps resolve confounding associations like that of IMMP2L; and finally, it provides the basis of a pathogenetic framework on which to connect other seemingly disparate genetic findings for TS.

Expression of LRRN3 and closely related family member LRRN1 are both enriched within the brain.39,56,69 LRRN3 is localised within the genomic region most commonly duplicated in ASD62,74 and it also appears duplicated in TS.55 and LRRN1 is also duplicated in ASD, 71 suggesting that dose increases for these two related molecules maybe pathogenic for ASD and TS. Consistent with the upregulation of LRRN3 in TS is the finding that the LRRN3 gene, nested within IMMP2L, was not disrupted in either of the TS cases where IMMP2L was disrupted.39–40,45 In addition, the relative high-level expression of LRRN3 in the adult brain is discordant with very low-level expression for IMMP2L when compared with other tissues.39 Together these findings suggest the discordant co-regulation of both genes. Such discordant regulation of nested genes being transcribed from opposing DNA strands has been hypothesised to occur via a mechanism of transcriptional interference.59 Discordant regulation of LRRN3 is also suggested by studies in the mouse where modification of the Immp2l transcription unit appears to result in the upregulation of Lrm3 transcription.72 In this context, the discordant regulation of the IMMP2L-LRRN3 transcription complex ceases to be a confounding factor in TS aetiology and provides an all important element of structural and functional integrity to the TS story. Both LRRN3 and its closest relation LRRN1 are involved in brain development. LRRN1 is known to have a key role in regional boundary formation during brain development including an essential role in midbrain-hindbrain boundary (MHB) formation.73 Studies in the chick demonstrate that the MHB is established by the downregulation of Lrn1 by Fgf8 on the posterior side of the future boundary73 by creating a differential cellular affinity between the two compartments. The molecular basis of this
differential affinity appears likely to involve an as yet unspecified extracellular binding partner for LRRN1. During this process, Lrrn1 regulates the expression of another well-known developmental gene, Lunatic Fringe, which modulates Notch signalling to complete MHB formation. Experimental overexpression of Lrrn1 in cells positioned on the hindbrain side of the future MHB results in violation of the boundary and mixing of cells between midbrain and hindbrain compartments.73

The LRRTMs have also been implicated in brain development and disease: LRRTM1 expression levels have been implicated in schizophrenia, left brain asymmetry and handedness.74 The LRRTMs and LRRNs also have important structural similarities: both are neuronal transmembrane proteins; both have short intracellular tails with putative PDZ-binding domains; and both have extracellular LRR domains. The LRR domain of the LRRTMs represents a ligand-binding site for the formation of trans-synaptic complexes with NRXNs1,64–67,75,76 LRRTM1, LRRTM2 and LRRTM3 are all nested within other genes. However, in contrast to the discordant expression of LRRTM3 and IMMP2L, the high-level expression of LRRTM’s in the brain is concordant with the high-level expression of their host genes.77 Here it is interesting to note that the LRRTM1 and LRRTM2 genes have bidirectional promoters that they share with their host genes.77 The co-regulation of LRRTM3 with its host gene CTNNA3 could therefore be a factor in how the recurrent disruption of the CTNNA3 gene could affect and implicate LRRTM3 expression in TS.

The neurexin connection

NRXNs 1–3 (NRXN1, NRXN2 and NRXN3) represent some of the largest genes in the human genome. The NRXN genes have dual promoters (α- and β-) and their transcripts are alternatively spliced into >1000 synaptic proteins. NRXNs are single-pass neuronal transmembrane proteins concentrated on the presynaptic side of the synapse. NRXNs appear to organise synapses by mediating cellular adhesion. The extracellular domain of presynaptic NRXNs binds to postsynaptic ligands (neuroligins (NLGNs), LRRTMs or cerebellin precursors (CBLNs)) to form trans-synaptic cell-adhesion complexes.6,78 The three alpha-NRXNs 1–3 are essential for survival and have a pivotal role in neurodevelopment where their roles partially overlap.78 NRNX1 and NRNX4/CNTNAP2, two of the genes recurrently disrupted in TS, both encode members of the NRXN superfamily.75,76 NLG4X, another of the genes recurrently disrupted in TS, is a member of the NLGN gene family that also encode single-pass neuronal transmembrane proteins. More importantly, NLGN4X functions as a postsynaptic cell-adhesion ligand for the NRXNs. In similar manner, LRRTM3, the gene nested within CTNNA3, is a member of the LRRTM gene family that also encode single-pass neuronal transmembrane proteins that function as postsynaptic cell-adhesion ligands for the NRXNs.8,36,39–40,45–46 Similarly, the LRRN3 gene nested within IMMP2L encodes another neuronal transmembrane protein that has a close structure–function relationship with LRRTM3 (discussed above); however, extracellular binding partners have yet to be identified for the LRRNs and for NRXN4/CNTNAP2.77 As such, all five of the genes uniquely disrupted in TS encode neuronal transmembrane proteins of which at least four are members of the NRXN superfamily or form trans-synaptic connections with the NRXNs. We propose a neuropathogenetic model for TS (Figure 2) where an imbalance in the type and/or level of NRXN trans-synaptic connections triggers changes in the dynamics of synapse assembly, maintenance and function within the brain resulting in abnormalities in the neuronal circuitry.

Using the neuropathogenetic model to interrogate other TS loci

The pathogenetic model for TS outlined in Figure 2 implicates an intersecting series of NRXN trans-synaptic complexes. We therefore searched the other TS translocation loci for genes that function within trans-synaptic signalling pathways. We revisited the locus most commonly rearranged in TS on 18q22.2,10,25,33,44 In 2003, Mathew State and colleagues44 performed mutation analyses for the two genes located on either side of one of the TS translocation breakpoints on 18q22.2, namely CIS4 and GTSCR-1, but no mutations were identified in TS patients.44 With hindsight, this result was not altogether surprising given that CIS4 has since been shown to encode a regulator of T-cell activation and GTSCR-1 has recently been identified as a small pseudogene (–0.6 kb). More revealing is the absence of the GTSCR-1 pseudogene from mouse and rat indicating its relatively recent evolutionary retro-transposition within what is essentially a conserved gene desert (>2 Mb) that harbours numerous strongly conserved non-coding sequences (Vista Plot, NCBI Build 37.2). Unbeknown at the time of the original investigation, this 2-Mb gene desert on 18q22.2 is bordered at its distal end by the CBLN2 gene. CBLN2 was never screened for mutations in TS 44 but we can now clearly locate a second TS translocation breakpoint25,44 within the same gene desert, albeit, much closer to CBLN2.44 The CBLN2 gene has also been deleted in TS.10,25,33,44
CBLN2 is expressed widely in the brain and belongs to the CBLN subfamily (consisting of CBLN1-4) of the C1q/tumour necrosis factor superfamily, which serves diverse roles in intercellular signalling, neuronal cell adhesion, brain development and synaptogenesis. More specifically, CBLN2 encodes another ligand of the NRXNs. The full-length precursor of CBLN2 is secreted intact into the synaptic cleft in a similar manner to its most closely related family member CBLN1. CBLN2 is an important synaptic organiser with similar synaptic connections to that of CBLN1, which forms a tripartite signalling complex with the NRXNs and the postsynaptic glutamate receptors delta 1 or delta 2 (GluD1/GRID1 or GluD2/GRID2). Both CBLNs induce synaptogenesis in cerebellar, hippocampal and cortical neurons in vitro and the tripartite CBLN complex (NRXN-CBLN-GRID) actually competes with synaptogenesis mediated by NLGN1. 

We searched other TS intergenic breakpoint loci for genes that are structurally or functionally related to those within the model (Figure 2). Other LRR-coding genes LRRTM1, LRRTM1 and SLITRK1 are located near breakpoints at 2p12, 3p21 and 13q31, respectively (Table 2). Likewise, we searched critical TS linkage regions and found that the strongest linkage markers identified (D2S139 and D3S1289) from two separate parametric linkage analyses of single multi-generation TS pedigrees were positioned within the genes CTNNA2 and CACNA2D3, respectively (Table 3). Surprisingly, both of these genes harbour nested neuronal LRR transmembrane coding genes, namely LRRTM1 and LRRTM1, respectively. LRRTM1 and LRRTM1 are the same two LRR candidates identified near TS translocation breakpoints (Table 2). 

Expanding the neuropathogenetic model for TS

Another gene of interest, ZnT3, is also worthy of mention here if only to demonstrate the utility of the new model to help interrogate broader aspects of TS pathogenicity (Figure 2). ZnT3 is one of the many genes located under the one and only significant linkage peak identified on chromosome 2p23 in the latest TSAICG non-parametric analysis of sib pairs and multi-generation families (Table 3). In light of our new pathogenic model for TS (Figure 2), ZnT3, a synaptic zinc transporter that controls \( Zn^{2+} \) concentrations within synaptic vesicles, now emerges as a most compelling candidate for TS on 2p23. The concentration of \( Zn^{2+} \) ions within the postsynaptic density (PSD), a specialised intracellular region of the excitatory synapse, is known to affect the recruitment of scaffolding proteins like Shank2 and Shank3, both of which
have been mutated in ASD. The SHANK proteins mediate the attachment of the intracellular PDZ-binding domains of model trans-synaptic receptor/ligand complexes, like NRXN-NLGN and NRXN-LRRTM (Figure 2), to the local actin-based cytoskeleton within dendritic spines. In Purkinje cells, the postsynaptic clustering of SHANK2 with GluD2/GRID2 also appears to be dependent on the integrity of the tripartite NRXN-CBLN1-GRID2 trans-synaptic complex. In contrast, CBLN1 and CBLN2 only bind molecules within the synapse including dystroglycan, GABA\(_\text{A}\)-receptors and the secreted neurexophillins 1 and 3. The LRRNs also have putative extracellular PDZ-binding domains.

A gene of related interest to ZnT3 is synapse-associated protein 97 (SAP97). Linkage analysis of a large Dutch pedigree with TS identified the highest linkage peak using a marker (D3S1311) located within the SAP97 gene (Table 3). A male individual with TS has also been identified with a duplication of the SAP97 gene locus (unpublished data), whereas micro-deletion of 3q inclusive of SAP97 is commonly characterised in schizophrenia. SAPs are thought of as scaffolds that organise the PSD of excitatory synapses with the ability to bind to membrane receptors, signalling molecules and the cytoskeleton. The SAP family consists of four homologues PSD93, PSD95/ SAP90, SAP102 and SAP97/DLG1. All SAPs contain three PDZ domains. SAP family proteins have been found to bind to AMPA, NMDA and kainate receptors at synapses and postsynaptic cell-adhesion molecules including NGLN1. There is also recent evidence that membrane-diffusing AMPARs can be rapidly trapped at PSD95 scaffolds assembled at nascent NRXN/NLGN adhesions, in competition with existing synapses. Overexpression of SAP97 enhances glutamatergic synaptic transmission.

### Synaptic model mechanisms

The synaptic model for TS outlined in Figure 2 had its genesis through the integration of all five of the genes recurrently disrupted in TS by novel breakpoints and is by all accounts an unbiased construction. In so doing, the model integrates two members of the NRXN superfamily (NRXN 7 and NRXN4) and two of the three known NRXN postsynaptic cell-adhesion ligand gene families, namely the NLGNs and LRRTMs. Further interrogation of other TS loci subsequently implicated another NRXN ligand gene CBLN2 located at the distal end of the gene desert most commonly rearranged in TS. As such, the synaptic model for TS (Figure 2) now includes the full complement of known NRXN postsynaptic cell-adhesion ligand gene families (NLGNs, CBLNs-GRIDs and LRRTMs) that regulate both the nature and strength of synaptic signalling, notwithstanding reports of NRXNs binding other molecules within the synapse including dystroglycan, GABA\(_\text{A}\)-receptors and the secreted neurexophillins 1 and 3. In hindsight, this finding should not have been altogether unexpected, given the recurrent disruptions of NRXN1 in TS (Table 1). The resulting haploinsufficiency of NRXN1 would have the potential to impact cell-adhesion through the full range of competitive NRXN postsynaptic ligands including the NLGNs and CBLNs-GRIDs and more particularly the LRRTMs, as the latter have NRXN connections that appear to be restricted to NRXNs1 (Figure 2). This scenario is supported further by the comparable set of comorbidities associated with the disruptions of the NRXN1 gene and LRRTM3 gene in TS, including OCD, ADHD, speech delay, mental retardation and ASD (Table 1).

In the model (Figure 2) different affinities exist between the various ligands and the different NRXN isoforms which may provide important insight into the mechanisms at play in TS and ASD. For example, LRRTMs bind only NRXN1 isoforms that lack the 30 amino-acid insert at splice site 4 (NRXN1S1\(^{34}\)). In contrast, CBLN1 and CBLN2 only bind NRXN isoforms that include this same insert at splice site 4 (NRXN1S1\(^{34}\)) compared with NLGNs, which appear to bind both of these isoform types but with different affinity (Figure 2). These common affinities in turn allow for competition for synaptogenesis, for example, competition
exists between the NRXNs1IS4–NLGN1 trans-synaptic complex and the tripartite NRXNs1IS4–CBLN1–GRID2 complex,79 and likely the NRXNs1IS4–CBLN2–GRID1 complex (Figure 2). There is also clear potential for competition for synaptogenesis between the NRXNs1IS4–NLGN and NRXNs1IS4–LRRTM complexes (Figure 2).26,65,67,75,76 The outstanding question is to what degree do the patterns of expression of the NLGNs overlap with the other ligands. NLGNs are expressed throughout the brain but are differentially targeted to specific synapses.92 NLGN4X induces excitatory synapse formation and NLGN1 is specific for excitatory synapses; NLGN3 appears to be present in both inhibitory and excitatory synapses, whereas NLGN2 is restricted to inhibitory synapses.92 Together these findings suggest that loss of certain excitatory pathways may be pathogenic during development resulting in varying phenotypic presentations. Recent unexpected findings by Ko et al.92 support this interpretation by their demonstration that NLGN1 and NLGN3 act redundantly with the LRRTMs in the maintenance of excitatory synapses.67,79,92 Excitatory synapses can apparently form in the absence of any and all of these ligands but are not maintained. However, any one of these ligands is sufficient to restore excitatory synapses to normal levels.92

From the findings in this review (Figure 2), it is clearly evident that many of the synaptic genes implicated in TS are also associated with ASD. Albeit, the novel association described here between CBLN2 and TS may provide valuable insight into the cellular and molecular features that distinguish TS from ASD. The CBLNs function as synaptic organisers through their binding of NRXNs and the GluD/GRID post-synaptic ligands, respectively.79,93,94 In contrast to the enrichment of CBLN1 and its receptor GRID2 within the cerebellum,95–97 CBLN2 expression patterns in the brain more closely overlap those of its receptor GRID1 including enrichment within the cortex.97,98 This is of particular relevance given the common association of TS with ADHD and OCD and that the GRID1 knockout mouse presents with hyperactivity and aberrant emotional and social behaviours,98 which contrasts markedly with the ataxic presentation of the GRID2 knockout mouse.99 The behavioural phenotype of the CBLN2 knockout mouse100 is eagerly anticipated. At the molecular level GRID1’s preferential role with CBLN2 in the induction of inhibitory presynaptic differentiation101 suggests that reduced inhibitory synaptogenesis may represent a distinguishing molecular feature of TS compared with ASD but this remains to be tested. Such a scenario, however, would be consistent with the fact that NLGN2, which is known to be restricted to inhibitory synapses, is the only NLGN that has not been linked with ASD.

Those regions of the brain where the expression of CBLN2 and GRID1 are enriched are likely to be of particular importance to the pathogenesis of TS. In contrast to the NLGNs, the other molecules within the synaptic model (Figure 2) display both mixed and restricted patterns of expression in different regions of the brain. For example, CBLN2 is widely expressed in the brain but it has a distinctive pattern of expression in cortical laminae II, III, V and VI.90,97 GRID1 is also widely expressed in the brain and cortex, while CBLN1, CBLN3 and GRID2 are more selectively enriched within the cerebellum.79,97 All LRRTMs are expressed within the dentate gyrus while their expression is distinct and complimentary within the different laminae of the cortex.56,65,67 These studies of the adult brain, however, are not informative of expression patterns within the developing brain. As indicated earlier, studies during embryogenesis in the chick indicate a dynamic pattern of expression for Lrrn1 during embryogenesis that is of fundamental importance in establishing regional boundaries within the brain. Likewise, CBLN2 has a particularly dynamic pattern of expression during development in the chick.97 In this context, the synaptic model for TS (Figure 2) presented herein overlaps with a range of neuropsychiatric disorders with and without TS and its various co-morbidities including ASD, ADHD, OCD and to a lesser extent schizophrenia. In this broader context, the synaptic model for TS (Figure 2) provides direction for both the genetic stratification of patients and the elucidation of new avenues for improved treatment.

TS model perspective on brain anatomy, neuronal circuitry and synaptic signalling

This study, the first to implicate LRRNs, LRRTMs and CBLN2 in TS (Figure 2), provides an invaluable window for improved understanding of the molecular basis of higher brain functions affected by changes to brain anatomy, neural circuitry and synaptic signalling in TS.

LRRN regulation of neuronal migration and brain pathology.

The extracellular binding affinity of LRRN1 appears certain to restrict cellular migration between brain compartments as the priori basis for boundary formation in the hindbrain.73 The close familial relationship between LRRN1 and LRRN3 and the similarity between the extracellular LRR ligand-binding domains of the LRRNs and LRRTMs suggests that comparable mechanisms of affinity-regulated cellular migration during embryogenesis may be of broader pathological significance for TS and ASD. Abnormal increases in brain volume are commonly observed early in the postnatal life of individuals with ASD, including the frontal lobes and cerebellar vermis.102 As described earlier, the FGF8 signalling that is so central to boundary formation and regionalisation of the hindbrain and midbrain is mediated through the FGF8-dependent downregulation of LRRN1.73 FGF8 appears to have a similar role in the regionalisation and growth of the frontal cortex.102 Loss of hypomorphic mutations in Fgf8 in the mouse result in small and unpatterned telencephalons, particularly of the dorsomedial frontal cortex—the region that shows the largest increase in size in ASD.102 For ASD, loss of parvalbumin (PV) expressing interneurons has been reported as the hallmark of ASD-like dysfunctions. The physiological formation of synaptic connections between PV-positive interneurons and principal pyramidal neurons has been implicated in functional maturation of the postnatal cerebral cortex, and deficits in this process have been proposed as a pathogenic mechanism of ASD.103 In the case of TS, postmortem basal ganglia tissue from individuals with TS and normal controls has revealed markedly higher total neuron number in the
globus pallidus pars interna (GPI) of TS with a lower neuron number and density in the globus pallidus pars externa and in the caudate. These investigators also observed an increased number and proportion of the GPe neurons positive for the calcium-binding protein PV in tissue from TS subjects, whereas lower densities of PV-positive interneurons were observed in both the caudate and putamen of TS subjects, suggesting abnormal neuronal migration during development. In fact, small caudate volume in childhood, perhaps due to the reduction in the interneurons as described above, is one of the prognostic indicators of TS severity in adulthood. Reduction in the interneurons as described above, is one of the prognostic indicators of TS severity in adulthood. These anatomical changes are consistent with a developmental defect in the tangential migration of some GABAergic neurons. Different anatomical and functional deficits of the GABAergic system have also been discovered in ASD mouse models and CNTNAP2 knockout mice, which present with hyperactivity and repetitive behaviours, display anomalies in neuronal migration and reduced number of interneurons, as well as abnormal neuronal network activity.

**LRRTMs compete for neural circuitry.** The imbalance in striatal- and GPe-inhibitory neuron distribution described above suggests that the functional dynamics of cortico–striato–thalamo–cortical circuitry are fundamentally altered in severe, persistent TS. LRRTM1’s role in left right brain asymmetry and handedness also belies a role for the LRRTMs in regulating important aspects of brain circuitry. The LRRTMs compete with the NLGNs for trans-synaptic NRXN binding as do the CBLNs (Figure 2). LRRTMs have similar ligand-binding sites to those of the LRRTNs, indicating further potential for competition in brain circuitry formation (Figure 2). As described earlier, the downregulation of NLGN1 can alter the balance between excitatory and inhibitory neurotransmission leading to changes in the synapse dynamics affecting synaptic production, organisation and patterning. In TS, it has been proposed that the involvement of the dopaminergic striatal pathways results in tics, whereas that of the serotonergic striatal–limbic minicircuits results in OCD, and the involvement of frontal cortical circuits results in ADHD and socially inappropriate behaviours. When the entire cortical striatal–pallidothalamic–cortical circuitry is involved this results in a number of co-morbidities and psychopathology in addition to tics. Thus, the clinical phenotype and the severity of symptoms, as well as the associated psychopathology, observed in TS may be influenced by the nature and extent of involvement of the above circuitry. Similarly, a dimensional model has been suggested for ASD, with autism on one end of the spectrum and language, social-cognitive and other developmental difficulties including mental retardation on the other end, mediated by the extent of circuitry involvement.

In the competitive and dynamic molecular environment of trans-synaptic cell-adhesion, it is not surprising to find that dose effects associated with disruptions, duplications and dysregulation of the genes/ligands described here can render profound pathogenic yet variable consequences for brain, mind and behaviour (Figure 2). These events may be sufficient but not essential for the pathogenesis of TS. Phenotypic variability may be related to the redundancy described above. In addition, variable expression of the different genes is likely to impact on the penetrance of the different co-morbidities. For example, the sex-specific imprinting of NRXN4/CNTNAP2, CTNNA3 and LRRTM1 is known to have dramatic and variable effects on levels of gene expression and the parent-of-origin phenotypic inheritance patterns. Such variations may also be caused by other modifying genes, perinatal events and other environmental factors. Thus, a particular phenotypic co-morbidity may present based on the type and level of involvement of the different neurotransmitter pathways that in turn may be based on the extent (which may be dose dependent) or the timing of events, as different circuits develop at different time points in neurodevelopment. For example, an early environmental insult could alter the epigenetic programming with consequent changes in neural function. Furthermore, as evident from animal models, phenotypic characterisation can show large modifying effects of genetic background and complex and unpredictable epistatic interactions. Using rodent studies, suggested that environmental signals can activate intracellular pathways leading to epigenetic changes that can result in neural function changes during early development.

Phenotypic variability can also be affected by non-genetic factors, or ‘second hits’ such as prematurity, perinatal trauma, injury, hypoxia, oxidative stress, infections, inflammations and autoimmunity, neural and psychosocial stressors or other modulators including gender. It has been shown that there are sex-specific differences in the topographic segregation and functionality of GABA-A systems in the substantia nigra and that the presence of circulating testosterone is essential for the development of the substantia nigra region in the neonatal period and to a lesser extent in the final maturation in the peripubertal period. In this regard, the role for testosterone in the extreme male brain hypothesis has been suggested in ASD. Similarly, OCD has been proposed as an alternative phenotypic expression of the TS genes with a gender-dependent difference in the expression leading to male members of the family exhibiting more tic behaviours and the female members exhibiting OCD. An imbalance in the excitatory/inhibitory ratio in local and extended neuronal circuits could therefore have a role.

**CBLN inhibitory synaptic signalling model.** The present study is the first to identify a disease association for any of the CBLNs. Loss of CBLN2 is associated with reduced mediation of inhibitory synaptogenesis that appears in opposition with reduced number of excitatory synapses associated with the downregulation of the LRRTMs and NLGN4X (Figure 2), albeit the downregulation/disruption of NRXN1 infers loss of both excitatory and inhibitory synaptic connections. Neuronal circuits utilise a number of homeostatic mechanisms to regulate the strength of excitation, inhibition and intrinsic excitability thereby maintaining synaptic homeostasis. In most networks, small changes in the balance between excitation and inhibition can have a significant impact on the neuronal firing and there is compelling evidence to suggest that the balance between excitation and inhibition is tightly regulated. When the balance
is upset, two distinct mechanisms have been proposed for restoring synaptic homeostasis: one mediated by the strength of excitatory and inhibitory synaptic inputs and the second by the balance of inward and outward voltage-dependent conductances. Thus, the neurons can compensate by using synaptic mechanisms to modify the balance between excitatory and inhibitory inputs or they can use intrinsic mechanisms to modify the balance of inward and outward voltage-dependent current.118 When there is an imbalance in the excitatory/inhibitory ratio in the neuronal circuits, this could in turn affect neuronal development.

NRXNs, NLGNs, LRRTMs and CBLN-GRIDs are neuronal adhesion molecules located at the pre- or postsynaptic region and promote synapse formation and/or maintenance bi-directionally in the glutamatergic and GABA-ergic nerve system that may result in subtle differences in neuronal connectivity and synapse patterning.75–78,79,119 Synapses being specialised intercellular junctions that connect the presynaptic machinery for neurotransmitter release to the postsynaptic machinery for receptor signalling. It has been shown that synapses are formed even when αNRXN1 is deleted from the mouse genome; however, this compromises synaptic function.78 Release of neurotransmitters like glutamate requires the presynaptic co-assembly of Ca2+ channels with the secretory apparatus and this Ca2+ channel function is impaired in αNRXN knockout mice with consequent reductions in neurotransmitter release.78 NRXN-NLGN and NRXN-LRRTM connections, which are sensitive to extracellular Ca2+ concentrations, appear to trigger postsynaptic differentiation and control the balance of inhibitory GABA-ergic and excitatory glutamatergic inputs.79 By comparison, LRRTM1 null mice have altered distribution of the excitatory presynaptic vesicular glutamate transporter VGLUT1.75,76 Glutamate, the main excitatory neurotransmitter in the vertebrate brain, has a major role in cortico–striatal–thalamo–cortical circuits and several lines of evidence support the role of glutamate in TS including: the TS association of glutamate receptors that are localised in the cellular membranes of both neurons and glia; the recognised extensive interaction between glutamate and dopamine systems; results of familial genetic studies (Table 3); and data from neurochemical analyses of post-mortem brain samples. Loss of excitatory synaptic connections resulting in a hypo-glutamatergic state is consistent with a loss in the synaptic weight important for reinforcing circuit strength via repeated stimulation as required by language. However, due to the competition and redundancy for trans-synaptic cell-adhesion outlined in the TS model (Figure 2) and the involvement of both excitatory and inhibitory pathways in TS,101 there remains insufficient data to determine whether TS is definitively associated with a hyper- or hypo-glutamatergic state.

Research perspective

The identification of the ligand(s) for the LRRTMs is eagerly anticipated as is the identification of any comparable restrictions to cellular migration regulated by the other cell-adhesion molecules of the neuropathogenetic model for TS (Figure 2). In this respect, the CBLN2 and GRID1 KO mouse models may prove invaluable for identifying those brain-affected regions that overlap within the NRXN1, NRXN3 and NRXN4/ CNTNAP2 KO mouse models. The same mouse models may also be helpful in determining those brain regions and neuronal circuits most sensitive to the haploinsufficiency of heterozygotes as represented in the TS model. In this respect, patient screening should be expanded to include mutations that regulate levels of expression or loss of function of the relevant NRXNs, NLGNs, LRRTMs, LRRNs and CBLN2 including those mutations that affect the expression levels of harbouring genes including IMMP2L. Stratification of more patients through mutation screening should precede the pre-emption of any pharmacological strategies for the treatment of tics and related comorbidities.

Conflict of interest

The authors declare no conflict of interest.
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Clamp model linking TS and ASD

Neurodevelopmental model linking TS and ASD

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significant distress or dysfunction. It affects 1–3% of the general population.

Attention-deficit hyperactivity disorder (ADHD): A disorder characterized by inattention and/or hyperactivity and impulsivity affecting around 5% of school-aged children and causing impairment in social and academic performance; the symptoms may persist into adult life.

Autism spectrum disorder (ASD): A developmental disorder characterized by abnormalities in social interactions and communication, as well as restricted interests and repetitive behaviours.

Synapse: Synapse formation is the key step in the development of neural networks. Synapses are specialized intercellular junctions in which cell adhesion molecules connect the presynaptic machinery of neurons for neurotransmitter release to the postsynaptic machinery for receptor signalling.

Striatum: A subcortical structure of the brain, which is part of the basal ganglia system and is divided into the caudate nucleus and putamen by a white matter tract called the internal capsule.

Cortices: The outer layer of the cerebral cortex composed of gray matter.

Nested genes: A nested gene is any gene located wholly within another gene. Nested genes are usually located within an intron of the host gene. Nested genes are relatively common within the genome and are most often coded on the complementary strand and transcribed in an antisense direction relative to the host gene. Nested genes often display high levels of tissue-specific expression and overlapping genes more generally are four times more likely to be co-expressed than expected by random probability; however, little is known regarding the mechanism of co-regulation or whether co-regulation and transcriptional interference operate simultaneously, thereby constraining gene expression within the normal range. Two hypotheses have been proposed for interactive expression of nested gene pairs. The functional co-regulation hypothesis predicts a positive correlation between levels of expression of nested genes in different tissues (for example, BMCC1 and PCA3) and the transcriptional collision/interference hypothesis predicts a negative correlation as proposed in this study between LRRN3 and IMMP2L (Table 1). Transcriptional interference between the gene pairs has been investigated in bacteria and might take place by direct competition for the transcription apparatus and/or by formation of double-stranded RNAs.