

# 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 neuronal (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.**  
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## 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.<sup>1</sup> 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).<sup>2</sup> 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.<sup>1</sup>

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.<sup>3</sup> Furthermore, it is suggested that chronic tics and OCD are manifestations of the same underlying genetic susceptibility as TS.<sup>4</sup> 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.<sup>5–6</sup> Furthermore, Kadesjo and Gillberg<sup>7</sup> 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.<sup>8</sup> 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.<sup>8</sup> In 1991, Sverd<sup>9</sup> 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.<sup>3,10</sup> 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  $\beta$ -hydroxylase, dopamine-associated transporter SLC6A3, as well as the adrenergic receptors  $\alpha_{1c}$ ,  $\alpha_{2c}$ ,  $\alpha_{2a}$  and  $\beta_2$ , various serotonin receptors and others.<sup>3</sup> 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;<sup>11</sup> and a complete genome screen in sib pairs and multigeneration families by the Tourette Syndrome Association International Consortium for Genetics (TSAICG).<sup>12</sup> One significant linkage region on 2p23 was identified<sup>11</sup> but results were not reproducible between these two studies and no associated mutations have been identified.<sup>10–12</sup> By comparison, parametric linkage analyses have been applied in the search for rare variants of large effect within single multigeneration pedigrees and isolated populations.<sup>11,13–22</sup> These latter studies have identified a number of impressive linkage regions within single pedigrees including peaks on 3q and 14q.<sup>13,17</sup> These two peaks overlap/replicate regions of interest identified in the 2007 TSAICG analysis of sib pairs,<sup>11</sup> but no mutations have yet been reported from these loci either. The first and as yet the only linkage locus to yield a mutation of interest is 15q21.<sup>16</sup> Subsequent sequence analysis within the critical linkage region on 15q identified a non-sense mutation in the

*L-histidine decarboxylase (HDC)* gene that encodes the rate-limiting enzyme in histamine biosynthesis.<sup>16</sup> This non-sense mutation co-segregates with the disorder in the original family composed of a father and his eight children with TS and OCD. Additional investigations suggest that dominant negative effects on histaminergic neurotransmission may be in play here with possible implications for the dysregulation of dopaminergic pathways.<sup>8,23</sup> Albeit, HDC mutations were absent from a large TS cohort screened by State *et al.*<sup>8</sup> suggesting that the HDC association may be limited to this singular family.

### 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.<sup>6,11,24–47</sup> Recent reviews and reports on the genetics of TS<sup>3,45,48</sup> have identified at least 28 large independent genomic rearrangements and CNVs with unique breakpoints including deletions, insertions, duplications, inversions and inter-chromosomal translocations.<sup>6,11,24–47</sup> 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).<sup>24–26,28–29,31–32,37,44–45</sup> 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.<sup>49</sup> 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.<sup>24</sup> 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,<sup>24</sup> 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<sup>50</sup> indicates that *SLITRK1* may be of limited effect in TS.<sup>10</sup>

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

Gene	Locus	Function	Co-morbidities	DNA lesions	Other
<b><i>NRXN1</i><sup>a</sup></b>	2p21	Neurexin 1 synapse	ADHD	Two truncating deletions <sup>45</sup>	T
<b><i>NRXN4/CNTNAP2</i><sup>b</sup></b>	7q35	Neurexin superfamily	OCD, MR, SD	Intragenic insertion <sup>46</sup>	c,d
<b><i>IMMP2L3 (LRRN3)</i></b>	7q31	(Neural development)	MR, SD	Two exonic deletions <sup>39,40</sup>	T, SA, R, Dup <sup>35</sup>
<b><i>CTNNA3 (LRRTM3)</i></b>	10q21	(Neurexin ligand)	OCD, ADHD	Two intragenic deletions <sup>45</sup>	SA
<b><i>NLGN4X</i><sup>b</sup></b>	Xp22.33	Neurexin ligand	ASD	Truncating deletion <sup>36</sup>	XM <sup>43</sup>

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.<sup>43</sup>

<sup>a</sup>Recurrent disruption of this gene in TS. <sup>b</sup>Gene disruption co-segregates with disorder in TS family. <sup>c</sup>2p21-23 block insertion that disrupted *CNTNAP2* harbours a TSAICG critical linkage region. <sup>d</sup>*CNTNAP2* disrupted in family without TS.<sup>53</sup> Bold type indicates protein implicated in Tourette syndrome.

**Table 2** Candidate genes located near translocation breakpoints in Tourette syndrome

<b>Candidate</b>	<b>Locus</b>	<b>Function</b>	<b>Proximity/distance</b>	<b>Co-morbidities Support data</b>
<b>CBLN2</b>	<b>18q22.2/18q21.1</b>	Neurexin ligand	Adjacent ~ 2.0 Mb <sup>44</sup>	OCD BD <sup>33</sup>
<b>CBLN2</b>	<b>18q22.2/7q31</b>	Neurexin ligand	Adjacent ~ 400 kb <sup>25</sup>	OCD BD <sup>33</sup>
<b>LRRTM1</b>	<b>2p12/18q22.2</b>	Neurexin ligand Leucine-rich repeat	Breakpoint region <sup>29</sup>	OCD LS
<b>LRTM1</b>	<b>3p21/8q24</b>	Neural development Leucine-rich-repeat	Break point region <sup>26</sup>	OCB LS
<b>SLITRK1</b>	<b>13q31/13q33</b>	Neural development Leucine-rich repeat	Adjacent ~ 350 kb <sup>24</sup>	ADHD
<b>SLCO5A1</b>	<b>8q13/6p23</b>	Anion transport	Adjacent < 200 kb <sup>28</sup>	OCB T
<b>SLCO5A1</b>	<b>8q13/6q24</b>	Anion transport	Adjacent < 200 kb <sup>28</sup>	ADHD, OCD T
<b>SLC26A7</b>	<b>8q22.1/1q21.1</b>	Cl/HCO <sub>3</sub> exchange	Adjacent ~ 550 kb <sup>32,37</sup>	OCD, ADHD LS, SCZ <sup>81</sup>
<b>GRIK2</b>	<b>6q21/17p11</b>	Glutamate transporter	Break point region <sup>33</sup>	Coprolalia BD
<b>SLC1A1</b>	9p23 recurrent del	Glutamate transporter	Within deleted region <sup>43,47</sup>	BD <sup>43</sup>
<b>CLIC6</b>	21q22	Neuronal Cl <sup>-</sup> channel	Adjacent to duplication <sup>45</sup>	
<b>DGCR2</b>	22q11.2 duplication	Dopamine D2, 3 and 4R Mutated in SCZ <sup>81</sup>	Adjacent inside boundary <sup>27</sup>	Stereotypies BD <sup>41</sup> SCZ <sup>81</sup>

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*.<sup>36,39–40,45–46</sup> 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.<sup>10,45,48</sup> For example, in the 2011 case report describing the disruption of the *IMMP2L* gene there were additional genes deleted.<sup>39</sup> To complicate this finding further, *IMMP2L* is a 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.<sup>51</sup> Extensive screening of TS patients also failed to identify any coding mutations in *IMMP2L*.<sup>39,52</sup> Together these findings for *IMMP2L* only heightened uncertainty regarding other rearrangements and CNVs in TS.<sup>8,48</sup> For example, there was already a degree of uncertainty regarding the disruption of the neurexin 1 (*NRXN1*), *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.<sup>53,54</sup>

### 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),<sup>36,39–40,45–46</sup> 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<sup>10,55</sup> 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*.<sup>56</sup>

Polymorphisms in two structurally related genes *LRRN3* and *LRRTM3* show strong association with ASD susceptibility.<sup>55,56</sup> *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).<sup>57–61</sup> 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 LxxLxLxxN/CxL (where x can be any amino acid and L can be replaced by V, I or F).<sup>67–70,75,76</sup> 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 subgroups are brain enriched and/or highly expressed in the nervous system, with roles in neuronal development and/or synaptogenesis.<sup>64–67,77</sup> The different families of transmembrane LRR proteins include AMIGO, NGL, LINGO, LRIG, FLRT, PAL, SALM, SLITRK, LRRN, LRRTM and LRTM (Figure 1).<sup>64–67</sup> The *LRRN1*, *LRRN3*, *LRRTM1*, *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  $\alpha$ -catenin genes.<sup>65</sup> *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* ( $\alpha 2$ -catenin) and is highly expressed within the brain and salivary gland.<sup>65</sup> *LRRTM1* appears to be associated with human handedness (relative hand skill), schizophrenia and language. *LRRTM3*, located within intron 7 of *CTNNA3* ( $\alpha T$ -catenin), expression is enriched within the cerebellum.

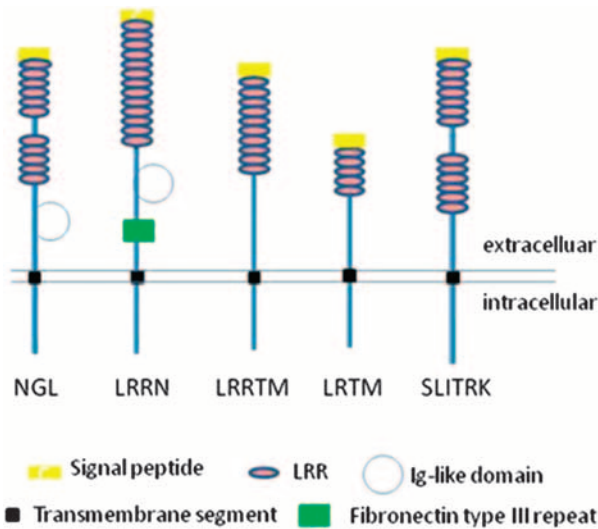
The *LRRN* (*leucine rich repeat neuronal*) gene family of four are all brain-enriched type I transmembrane protein genes. *LRRN1* is nested within intron 8 of the extended form of the *SUMF1* (*sulphatase modifying factor 1*) gene. *LRRN1* regulates boundary formation within the brain. *LRRN3* is nested within intron 3 of the *IMMP2L* (*inner mitochondrial membrane peptidase-like*) gene. *Lrrn3* exhibits regulated expression in the developing ganglia and motor neurons of the neural system, and is upregulated during neuronal cortical injury.<sup>64</sup>

The *LRTM* (*leucine-rich repeats 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 *CTNNA3*<sup>55,62–63</sup> and for both of their nested genes, *LRRN3* and *LRRTM3*, respectively,<sup>55,56</sup> 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)<sup>64–68</sup> 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.<sup>39,56,69</sup> *LRRN3* is localised within the genomic region most commonly duplicated in ASD<sup>62,70</sup> and it also appears duplicated in TS<sup>35</sup> and *LRRN1* is also duplicated in ASD,<sup>71</sup> 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.<sup>39–40,45</sup> 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.<sup>39</sup> 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.<sup>59</sup> 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 *Lrrm3* transcription.<sup>72</sup> 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.<sup>73</sup> Studies in the chick demonstrate that the MHB is established by the downregulation of *Lrrn1* by *Fgf8* on the posterior side of the future boundary<sup>73</sup> by creating a differential cellular affinity between the two compartments. The molecular basis of this



**Figure 1** Schematic of domain architecture for a selection of neuronal leucine-rich repeat transmembrane protein families.

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.<sup>73</sup>

The LRRTMs have also been implicated in brain development and disease: LRRTM1 expression levels have been implicated in schizophrenia, left right brain asymmetry and handedness.<sup>74</sup> 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 NRXNs.<sup>64–67,75,76</sup> *LRRTM1*, *LRRTM2* and *LRRTM3* are all nested within other genes. However, in contrast to the discordant expression of *LRRN3* and *IMMP2L*, the high-level expression of *LRRTM*'s in the brain is concordant with the high-level expression of their host genes.<sup>77</sup> Here it is interesting to note that the *LRRTM1* and *LRRTM2* genes have bidirectional promoters that they share with their host genes.<sup>77</sup> 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 ( $\alpha$ - and  $\beta$ -) 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

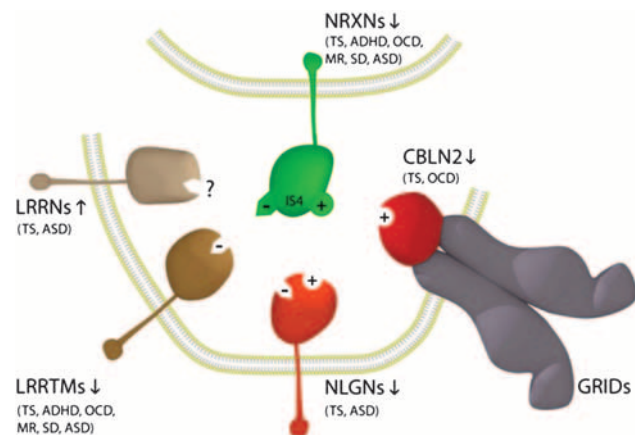
to organise synapses by mediating cellular adhesion. The extracellular domain of presynaptic NRXNs binds to post-synaptic ligands (neuroligins (NLGNs), LRRTMs or cerebellin precursors (CBLNs)) to form trans-synaptic cell-adhesion complexes.<sup>8,78</sup> The three alpha-NRXNs<sup>1–3</sup> are essential for survival and have a pivotal role in neurodevelopment where their roles partially overlap.<sup>78</sup> *NRXN1* and *NRXN4/CNTNAP2*, two of the genes recurrently disrupted in TS, both encode members of the NRXN superfamily.<sup>53</sup> *NLGN4X*, 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.<sup>8,36,39–40,45–46</sup> 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.<sup>73</sup> 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.<sup>10,25,33,44</sup> In 2003, Mathew State and colleagues<sup>44</sup> 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.<sup>44</sup> 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 (<1 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<sup>44</sup> but we can now clearly locate a second TS translocation breakpoint<sup>25,44</sup> within the same gene desert, albeit, much closer to *CBLN2*.<sup>44</sup> The *CBLN2* gene has also been deleted in TS.<sup>10,25,33,44</sup>

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 similar manner to its most closely related family member CBLN1.<sup>79</sup> 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).<sup>79,80</sup> 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<sup>79</sup> (Figure 2). Thus, CBLN2 demonstrates an integrated competitive relationship with other members of the genetic model for TS (Figure 2) to provide the strongest of support for its role in the pathogenesis of TS. These findings expand the breadth of known NRXN trans-synaptic ligands/complexes now associated with TS and further strengthen the model (Figure 2) as a useful framework for understanding the broader pathogenesis of TS.

We searched other TS intergenic breakpoint loci<sup>24,26-29,31-33,35,37,41-43,45</sup> for genes that are structurally or functionally related to those within the model (Figure 2). Other *LRR*-coding genes *LRRTM1*, *LRTM1* and *SLITRK1* are located near breakpoints at 2p12, 3p21 and 13q31, respectively (Table 2).<sup>24,26,29,37</sup> Likewise, we searched critical TS linkage regions<sup>11,13-22</sup> and found that the strongest linkage markers identified (D2S139 and D3S1289) from two separate parametric linkage analyses of single multi-generation TS



**Figure 2** Neuropathogenetic model for Tourette syndrome (TS) implicates the full complement of known neurexin (NRXN) trans-synaptic cell-adhesion ligand gene families through multiple means of enquiry: neuroligins (NLGNs); leucine-rich repeat transmembrane proteins (LRRTMs); and the cerebellin precursors (CBLNs). The presynaptic NRXNs form trans-synaptic complexes with postsynaptic ligands NLGNs, LRRTMs and CBLNs in the formation and/or maintenance of neuronal circuitry within the brain. Vertical arrows indicate putative pathogenic dose effects. Neurexin isoforms with (+) and without (-) the 30 amino-acid insert at splice site 4 (IS4) dictate the different/competitive binding of NRXNs between the ligands. Comorbidities listed are those associated with the TS translocations and copy number variations (CNVs) affecting the respective genes.

pedigrees were positioned within the genes *CTNNA2* and *CACNA2D3*, respectively (Table 3).<sup>17,20</sup> Surprisingly, both of these genes harbour nested neuronal *LRR* transmembrane coding genes, namely *LRRTM1* and *LRTM1*, respectively. *LRRTM1* and *LRTM1* are the same two *LRR* candidates identified near TS translocation breakpoints (Table 2).<sup>24,26,29,37</sup> *LRRTM1* and *LRTM1* are structurally related to each other (Box 1) and to the two neuronal *LRR* transmembrane coding genes (*LRRTM3* and *LRRN3*) nested within genes that are disrupted in TS (*CTNNA3* and *IMMP2L*, respectively) (Table 1).<sup>64-67</sup> *LRRTM1* is actually a member of the same gene family as *LRRTM3*. *LRRTM1* has an important role in brain development (discussed earlier).<sup>74</sup> Further independent support for *LRRTM1* as a neurological disease gene comes from a separate non-parametric linkage analysis for schizophrenia where the strongest linkage marker was also located within the *CTNNA2* gene.<sup>74</sup> However, it was not the *CTNNA2* gene that was demonstrated to be pathogenic for schizophrenia but rather the expression of its nested gene, *LRRTM1*.<sup>74</sup> Could the differential regulation of *LRRTM1* result in schizophrenia or TS?<sup>74,81,82</sup> Viewed together, this stunning molecular correspondence between TS disrupted genes, intergenic breakpoints and parametric linkage regions, assorted ASD association studies and linkage analyses of schizophrenia provides impressive support for the pathogenic role for these nested *LRR* transmembrane coding genes in TS and for the broader application of our neuropathogenetic model (Figure 2). The association of *LRRTM1* with TS further increases the number of NRXN postsynaptic cell-adhesion ligands now associated with TS (Figure 2).<sup>64,67</sup>

Also of interest are the three main suggestive linkage peaks reported by the TSAICG parametric study of affected sib pairs on 3p, 3q and 14q.<sup>11</sup> These three peaks span additional neuronal transmembrane protein genes *LRRN1*, *NLGN1* and *NRXN3*, respectively (Table 3). The gene desert 3' of *NRXN3* has also been linked to TS by parametric analysis within a large Italian kindred (Table 3).<sup>13</sup> Impressively, all three of these genes are members of the same small specialised gene families now implicated in the TS model (Figure 2; Box 1).<sup>56,67,71</sup> Furthermore, *NLGN1* is a trans-synaptic ligand for *NRXN3* and *NRXN1*,<sup>67,83</sup> *NRXN3*, *NRXN1* and *NRXN2* are mutated in ASD;<sup>54,84</sup> and *LRRN1* like its close relation *LRRN3* has been duplicated in ASD.<sup>71</sup>

### 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).<sup>11</sup> 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

**Table 3** Candidate genes within linkage regions for Tourette syndrome

Study	Pedigree	Linkage marker/locus	Candidate genes
Breedveld <sup>13</sup>	Italian pedigree	D14S1000	Linkage spans gene desert adjacent to NRXN3
TSAICG <sup>11</sup>	NPL pairs	14q 31	Suggested linkage region spans NRXN3
TSAICG <sup>11</sup>	NPL pairs	3p 26	Suggested linkage region spans SUMF1/LRRN1
TSAICG <sup>11</sup>	NPL pairs	3q 26	Suggested linkage region spans NLGN1
Knight <sup>17</sup>	Utah pedigree	D3S1289	Marker located within CACNA2D3/LRRTM1
Simonik <sup>20</sup>	Africana families	D2S139	Marker located within CTNNA2/LRRTM1
		GATA28F12	Marker near SLC26A7-Cl/HCO3 exchange
		D11S1377	GRIK 4-glutamate transport channel
Merette <sup>18</sup>	Single Canadian pedigree	D11S1377	GRIK 4-glutamate transport channel
Laurin <sup>22</sup>	Single pedigree	D5S430	SLC1A3-glutamate transport channel
Knight <sup>17</sup>	Utah pedigree	D1S207	Linkage spans ZnT7-zinc transporter
TSAICG <sup>11</sup>	Non-parametric	D2S165	Linkage spans ZnT3-synaptic zinc transporter
TSAICG <sup>11</sup>	Multigeneration families	5p	Linkage spans MSNP1AS-moesin antisense
Verkerk <sup>21</sup>	Single Dutch pedigree	D3S1311	Marker located within SAP97-synaptogenesis
Paschou <sup>19</sup>	Two large pedigrees	D17S928/784	Critical linkage region spans NPTX1
Zhang <sup>14</sup>	Sib pair families	D17S784	Marker located near NPTX1
Curtis <sup>15</sup>	Single pedigree	D5S400	Marker within SLIT3-LRR axonal guidance
		D14S288	Marker adjacent to LRFN5
Ercan-Sencicek <sup>16</sup>	Single pedigree	D15S126	Non-sense mutation located within HDC

Underline highlights linkage marker located with the candidate gene.

have been mutated in ASD.<sup>85</sup> 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.<sup>79,80</sup> 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).<sup>46</sup> 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.<sup>82</sup> 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<sup>86</sup> 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.<sup>87</sup> Overexpression of *SAP97* enhances glutamatergic synaptic transmission.<sup>88</sup>

### 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 1* 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<sub>A</sub>-receptors and the secreted neurexophilins 1 and 3.<sup>89-91</sup> In hindsight, this finding should not have been altogether unexpected, given the recurrent disruptions of *NRXN1* in TS (Table 1).<sup>45</sup> 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).<sup>67</sup> 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 (*NRXNs1<sup>IS4-</sup>*).<sup>67</sup> In contrast, *CBLN1* and *CBLN2* only bind NRXN isoforms that include this same insert at splice site 4 (*NRXNs1<sup>IS4+</sup>*)<sup>79</sup> compared with *NLGNs*, which appear to bind both of these isoform types but with different affinity (Figure 2).<sup>67</sup> These common affinities in turn allow for competition for synaptogenesis, for example, competition

exists between the NRXNs1<sup>IS4+</sup>-NLGN1 trans-synaptic complex and the tripartite NRXNs1<sup>IS4+</sup>-CBLN1-GRID2 complex<sup>79</sup> and likely the NRXNs1<sup>IS4+</sup>-CBLN2-GRID1 complex (Figure 2). There is also clear potential for competition for synaptogenesis between the NRXNs1<sup>IS4+</sup>-NLGN and NRXNs1<sup>IS4+</sup>-LRRTM complexes (Figure 2).<sup>26,65,67,75,76</sup> 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:<sup>92</sup> 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.<sup>92</sup> 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.*<sup>92</sup> support this interpretation by their demonstration that NLGN1 and NLGN3 act redundantly with the LRRTMs in the maintenance of excitatory synapses.<sup>67,79,92</sup> 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.<sup>92</sup>

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.<sup>79,93,94</sup> In contrast to the enrichment of CBLN1 and its receptor GRID2 within the cerebellum,<sup>95–97</sup> CBLN2 expression patterns in the brain more closely overlap those of its receptor GRID1 including enrichment within the cortex.<sup>97,98</sup> 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,<sup>98</sup> which contrasts markedly with the ataxic presentation of the GRID2 knockout mouse.<sup>99</sup> The behavioural phenotype of the CBLN2 knockout mouse<sup>100</sup> is eagerly anticipated. At the molecular level GRID1's preferential role with CBLN2 in the induction of inhibitory presynaptic differentiation<sup>101</sup> 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.<sup>80,97</sup> GRID1 is also widely expressed in the brain and cortex, while CBLN1, CBLN3 and GRID2 are more selectively enriched

within the cerebellum.<sup>79,97</sup> All LRRTMs are expressed within the dentate gyrus while their expression is distinct and complimentary within the different laminae of the cortex.<sup>56,65,67</sup> 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.<sup>97</sup> 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.<sup>73</sup> 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.<sup>102</sup> 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.<sup>73</sup> FGF8 appears to have a similar role in the regionalisation and growth of the frontal cortex.<sup>102</sup> 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.<sup>102</sup> 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.<sup>103</sup> 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.<sup>104</sup> These investigators also observed an increased number and proportion of the GPi 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,<sup>48,105</sup> 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.<sup>106</sup> 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* knock-out 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.<sup>107</sup>

**LRRTMs compete for neural circuitry.** The imbalance in striatal- and GPi-inhibitory neuron distribution described above suggests that the functional dynamics of cortico-striato-thalamic 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 LRRNs, 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.<sup>1</sup> 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<sup>108,109</sup> 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).<sup>56,67,79,83</sup> 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.<sup>74,110</sup> 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.<sup>111</sup> Furthermore, as evident from animal models, phenotypic characterisation can show large modifying effects of genetic background and complex and unpredictable epistatic interactions. Zhang and Meaney,<sup>112</sup> 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.<sup>108</sup> 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.<sup>113</sup> In this regard, the role for testosterone in the extreme male brain hypothesis has been suggested in ASD.<sup>114</sup> 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.<sup>4,115</sup> 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<sup>101</sup> that appears in opposition with reduced number of excitatory synapses associated with the downregulation of the LRRTMs and NLGN4X (Figure 2),<sup>67,75</sup> albeit the downregulation/disruption of NRXN1 infers loss of both excitatory and inhibitory synaptic connections. Neural 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.<sup>116,117</sup> 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.<sup>118</sup> 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;<sup>75–76,78,119</sup> 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  $\alpha$ NRXN1 is deleted from the mouse genome; however, this compromises synaptic function.<sup>78</sup> Release of neurotransmitters like glutamate requires the presynaptic co-assembly of  $\text{Ca}^{2+}$  channels with the secretory apparatus and this  $\text{Ca}^{2+}$  channel function is impaired in  $\alpha$ NRXN knockout mice with consequent reductions in neurotransmitter release.<sup>78</sup> NRXN-NLGN and NRXN-LRRTM connections, which are sensitive to extracellular  $\text{Ca}^{2+}$  concentrations, appear to trigger postsynaptic differentiation and control the balance of inhibitory GABA-ergic and excitatory glutamatergic inputs.<sup>79</sup> By comparison, LRRTM1 null mice have altered distribution of the excitatory presynaptic vesicular glutamate transporter VGLUT1.<sup>75,76</sup> 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,<sup>101</sup> 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 LRRNs 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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## Glossary

**Motor and vocal tics:** A simple motor tic is a sudden, brief, involuntary, repetitive, nonpurposeful movement of a single muscle group, such as an eye blink, face twitch, shoulder shrug, arm or leg jerk. Complex tics include forced touching, pulling clothes, a whole body jump or an abnormal walk. Vocal tics are involuntary sounds produced by moving air through the nose, mouth or throat, or vocalizations. These are also called phonic tics and examples include throat clearing, grunting and

coughing. Tourette syndrome affects ~1% of the school-aged population and ~10% of these require lifelong therapy.

**Echolalia:** The automatic repetition of vocalizations made by another person.

**Stereotypies:** These are repetitive and ritualistic movements or posture, such as body rocking, swaying movements, or crossing and uncrossing of legs.

**Obsessive-compulsive disorder (OCD):** A disorder characterized by intrusive, persistent thoughts (obsessions) and/or repetitive, intentional behaviours (compulsions) that result in

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 inter-cellular 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.

*Cortex*: 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.<sup>57–61</sup> 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<sup>57</sup> 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*)<sup>120</sup> 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.