

## Emerging Common Molecular Pathways for Primary Dystonia

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**ABSTRACT:** The dystonias are a group of hyperkinetic movement disorders whose principal cause is neuron dysfunction at 1 or more interconnected nodes of the motor system. The study of genes and proteins that cause familial dystonia provides critical information about the cellular pathways involved in this dysfunction, which disrupts the motor pathways at the systems level. In recent years study of the increasing number of *DYT* genes has implicated a number of cell functions that appear to be involved in the pathogenesis of dystonia. A review of the literature published in

English-language publications available on PubMed relating to the genetics and cellular pathology of dystonia was performed. Numerous potential pathogenetic mechanisms have been identified. We describe those that fall into 3 emerging thematic groups: cell-cycle and transcriptional regulation in the nucleus, endoplasmic reticulum and nuclear envelope function, and control of synaptic function. © 2013 *Movement Disorder Society*

**Key Words:** *DYT* genes; cell cycle; endoplasmic reticulum; nuclear envelope; synaptic function

The dystonias are a heterogeneous group of hyperkinetic movement disorders characterized by sustained involuntary muscle spasms and postures.<sup>1</sup> The most common forms are primary, in which dystonic movements are the only clinical feature and there is no evidence of neurodegeneration. In the majority of these cases, the cellular processes that lead to functional abnormalities of neurons, sufficient to disrupt the finely tuned control of movement, are unknown. However, in recent years the identification of genes that cause rare monogenic familial dystonia has given insight into the neurobiology of dystonia and shed light on the molecular mechanisms involved. Further evidence has come from the study of more complex forms of secondary dystonia, in which there is evidence of neurodegeneration or central nervous system

(CNS) damage and the dystonic movements are part of a more complex neurological phenotype.

The purpose of this review is to summarize the evidence, predominantly from the study of genetic forms of dystonia, and highlight cellular pathways that are important to the genesis of dystonia. In particular, it focuses on areas where there are common themes to the cellular pathogenesis. Most of this information comes from study of monogenic primary dystonias and the proteins that the various *DYT* genes encode. The genetic classification of dystonia has subtypes *DYT* 1–25 and includes pure primary dystonias, dystonia-plus syndromes, in which other features such as myoclonus or parkinsonism are present, and paroxysmal dyskinesias, in which dystonia is often a prominent feature. Table 1 describes the *DYT* loci, showing key clinical features and information about the protein encoded by the *DYT* gene. The following sections describe the specific forms of primary and dystonia-plus syndromes for which the gene and protein have been identified and studied and that are discussed in this review.

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### DYT1 Dystonia: TorsinA

*DYT*1 dystonia is caused by a heterozygous 3-bp GAG deletion in the *TOR1A* gene.<sup>2</sup> TorsinA is a 332-amino acid protein typical of the AAA+ (ATPases associated with a variety of cellular activities) protein

**TABLE 1.** Summary of genes and proteins involved in genetic forms of primary dystonia and dystonia-plus syndromes

Locus	Designation	Clinical features	Gene/inheritance	Protein	Putative functions
<b>Pure dystonia</b>					
<i>DYT1</i> Chr9q34.11	Early-onset primary dystonia	Childhood onset dystonia in limb with generalization	<i>TOR1A</i> Autosomal dominant	TorsinA	AAA <sup>+</sup> protein, nuclear envelope, ER secretory and stress response, regulation of synaptic function
<i>DYT2</i>	Early-onset dystonia	Adolescent-onset segmental or generalized	Autosomal recessive	Unknown	
<i>DYT4</i> Chr19p13.3	Whispering dysphonia	Childhood-onset laryngeal abductor spasm with cervical dystonia	<i>TUBB4a</i> Autosomal dominant	Beta-tubulin 4a	Structural cytoskeleton protein
<i>DYT6</i> Chr 8p11.21	AD early-onset focal dystonia	Early-onset dystonia with prominent cervical and laryngeal involvement	<i>THAP1</i> Autosomal dominant	Thanatos-associated domain-containing apoptosis associated protein 1	Atypical zinc-finger protein; THAP domain is chromatin-binding factor and regulates transcription
<i>DYT7</i> Chr 8p	Familial focal dystonia	Adult-onset focal dystonia	Unknown Autosomal dominant	Unknown	
<i>DYT13</i> Chr1p36.32-p36.13	Familial craniocervical dystonia	Focal or segmental dystonia of craniocervical region and upper limbs	Unknown Autosomal dominant	Unknown	
<i>DYT17</i> Chr 20p11.2-2q13.12	Early-onset AR dystonia	Early-onset focal dystonia progressing to generalized with dysphonia and dysarthria	Unknown Autosomal recessive	Unknown	
<i>DYT21</i> Chr 2q14.3-q21.3	Late-onset dystonia	Late-onset multifocal and generalized dystonia	Unknown Autosomal dominant	Unknown	
<i>DYT23</i> Chr9q34.11	Cervical dystonia	Late-onset primary cervical dystonia	<i>CIZ1</i> Autosomal dominant	Cip1-interacting zinc finger protein 1	Regulation of G1-S cell cycle and DNA replication
<i>DYT24</i> Chr11p14.2	Late-onset dystonia	Cranial and cervical dystonia	<i>ANO3</i> Autosomal dominant	Anoctamin 3	Calcium-gated chloride channel
<i>DYT25</i> Chr18p	Cervical dystonia with local spread	Predominantly late-onset primary cervical dystonia with spread to face	<i>GNAL</i> Autosomal dominant	Alpha subunit of G protein	Probable interaction with D1 and adenosine 2A receptors.
<b>Dystonia plus syndromes</b>					
<i>DYT3</i> Xq13.1	X-linked dystonia (Lubag)	Segmental or generalized dystonia with parkinsonism	<i>TAF1</i> X-linked	TATA box-binding protein associated factor 1	Regulation of transcription initiation and cell cycle
<i>DYT5/14</i> Chr2q13.2	Dopa-responsive dystonia	Dystonia with parkinsonism, diurnal variation, very good response to L-dopa	<i>GCH1</i> Autosomal dominant	GTP cyclohydrolase 1	Rate-limiting enzyme in synthesis of tetrahydrobiopterin, key cofactor in monoamine synthesis; results in deficient dopamine synthesis
<i>DYT11</i> Chr7q21.3	Myoclonic dystonia syndrome	Upper body myoclonic jerks with dystonia; responsive to alcohol	<i>SGCE</i> Autosomal dominant	Epsilon-sarcoglycan	Cell membrane protein that may act as structural platform for other protein interactions
<i>DYT12</i> Chr19q13.2	Rapid onset dystonia parkinsonism	Acute-onset generalized dystonia with parkinsonism, rostrocaudal gradient of symptoms	<i>ATP1A3</i> Autosomal dominant	Alpha 3 subunit of Na/K ATPase	Subunit of Na/K ATPase on neuronal membrane

**TABLE 1. Continued**

Locus	Designation	Clinical features	Gene/inheritance	Protein	Putative functions
<i>DYT15</i> Chr 18p11	Myoclonus dystonia	Childhood-onset myoclonus with variable dystonia	Unknown Autosomal dominant	Unknown	
<i>DYT16</i> Chr2q31.2	DRD and parkinsonism	Dystonia-parkinsonism with bulbar involvement and bradykinesia	<i>PRKRA</i> Autosomal recessive	Protein kinase, interferon-inducible double-stranded RNA dependent activator	<i>PRKRA</i> is activator of proteins involved in response to cell stress
<b>Paroxysmal dystonia</b>					
<i>DYT8</i> Chr2q35	Paroxysmal nonkinesigenic dyskinesia (PKND)	Attacks of dystonia or choreoathetosis. Precipitants: stress, alcohol, fatigue, coffee	<i>MR-1</i> Autosomal dominant	Myofibrillo-genesis regulator 1	Affect neuronal stress response pathways
<i>DYT9</i> and <i>DYT18</i> Chr1p34.2	Paroxysmal exercise induced dystonia Episodic ataxia	Dystonia (usually limb) brought on by exercise; ataxia/spasticity between attacks	<i>GLUT1</i> Autosomal dominant	Glucose transporter type 1	Reduced glucose transport to neurons with unstable metabolic state
<i>DYT10</i> and <i>DYT19</i> Chr16p11.2	Paroxysmal kinesigenic choreoathetosis (PKC)	Attacks of dystonia or choreoathetosis precipitated by sudden movement; associated with migraine and epilepsy	<i>PRRT2</i> Autosomal dominant	Proline-rich trans-membrane protein 2	Interacts with SNAP25 and may affect synaptic functions/neuronal excitability
<i>DYT20</i> Chr 2q31	Canadian PKND	PKND phenotype	Unknown Autosomal dominant	Unknown	

family; these proteins typically function as oligomers and use the energy of ATP hydrolysis for many functions including protein trafficking, membrane fusion, protein refolding, and degradation.<sup>3</sup> The pathogenic codon deletion ( $\Delta E$ ) in torsinA removes a glutamic acid residue from a C-terminal alpha helix believed to be critical for oligomerization and/or tertiary structure.<sup>4</sup> TorsinA is ubiquitously expressed in numerous cell types including neurons and glia within the CNS.<sup>5-8</sup> TorsinA mRNA is expressed in sensorimotor regions of the brain including the cerebral cortex, striatum, substantia nigra pars compacta, thalamus, hippocampus, midbrain, pons, cerebellum, and spinal cord.<sup>5-8</sup> It has been suggested that the neuron-specific effect of mutant torsinA occurs, as there is low expression of other forms of torsin, notably torsinB, and cannot therefore correct for the presence of dysfunctional torsinA.<sup>9</sup>

**DYT4 Dystonia: Beta-Tubulin 4a**

Study of a large family with autosomal dominant whispering dysphonia and generalized dystonia revealed a single cosegregating mutation in the beta-tubulin 4a gene.<sup>10</sup> The mutation in the beta-tubulin autoregulatory domain was highly expressed in the nervous system and implicates the cytoskeleton in dystonia pathogenesis.

**DYT6 Dystonia: THAP1**

Dystonia-causing mutations in *THAP1* (Thanatos-associated protein domain containing apoptosis

associated protein 1) have been widely reported.<sup>11,12</sup> *THAP1* is a transcription factor and a member of THAP protein family that contains an evolutionarily conserved zinc-dependent DNA-binding domain. Mutations in *THAP1* include several reported deletions and nonsense mutations, including one that removes the start codon. Many of the mutations occur in the DNA-binding domain and several appear to disrupt the nuclear localization signal.<sup>13-15</sup> In addition, at least 1 mutation appears to cause disease through recessive inheritance.<sup>16</sup> Considered together, these observations suggest that *THAP1* may cause dystonia through a loss-of-function mechanism.

Before its identification as a dystonia-causing gene, *THAP1* was reported to regulate cell proliferation via regulation of pRB/E2F cell-cycle target genes, and another report pointed to a potential proapoptotic role related to localization in promyelocytic leukemia nuclear bodies.<sup>17,18</sup>

**DYT11 Myoclonus Dystonia: Epsilon-Sarcoglycan**

Epsilon-sarcoglycan (SGCE) is a member of the sarcoglycan protein family that comprises plasma trans-membrane proteins. Most *SGCE* mutations are deletions or nonsense mutations that eliminate gene function.<sup>19</sup> The *SGCE* gene is maternally imprinted, meaning that individuals are dependent on expression from the paternal allele. Nearly all myoclonus-

dystonia patients inherit a loss-of-function allele from their father and therefore lack functional SGCE protein.

Sarcoglycans are components of the dystrophin-glycoprotein complex (DGC), a membrane-spanning complex that makes connections with the extracellular matrix and the intracellular actin cytoskeleton.<sup>20,21</sup> Although DGC function is typically described in striated muscle, where its dysfunction is linked to several muscular dystrophies, DGC proteins are also expressed in the CNS. SGCE is found in many brain regions and is associated with dopaminergic neurons in the substantia nigra and ventral tegmental areas.<sup>22</sup> In neurons, DGC proteins (including SGCE) concentrate at postsynaptic sites and have been localized to GABAergic inhibitory synapses.<sup>23,24</sup> This association with GABAergic synapses may contribute to the deficient inhibition that is observed in many forms of primary dystonia.

### DYT16 Dystonia-Parkinsonism: PRKRA

DYT16 is less prevalent than DYT1 and DYT6, having been described in only a small number of families.<sup>25</sup> The gene encodes the protein PRKRA (protein kinase, interferon-inducible double-stranded RNA-dependent activator), also known as PACT, which regulates activity of protein kinase R.<sup>26</sup>

### DYT23 Dystonia: Cip1-Interacting Zinc Finger Protein 1

A recent study has identified a Cip1-interacting zinc finger protein 1 (*CIZ1*; c.790A>G, p.S264G) mutation in a large white pedigree with late-onset primary cervical dystonia.<sup>27</sup> Screening in subjects with adult-onset cervical dystonia identified 2 additional missense mutations in *CIZ1* (p.P47S and p.R672M).

*CIZ1* encodes Cip1-interacting zinc finger protein 1, a DNA replication factor. *CIZ1* was first identified through its interaction with p21<sup>Cip1/Waf1</sup> (CDKN1A), a cyclin-dependent kinase inhibitor involved in G<sub>1</sub>-S cell-cycle regulation and cellular differentiation.<sup>27</sup> *CIZ1* is expressed in the cerebellum, cerebral cortex, substantia nigra, and putamen of the adult human brain.<sup>28</sup>

### DYT24 Dystonia: Anoctamin 3

Exome sequencing in an autosomal dominant family with craniocervical dystonia identified a putative mutation in the anoctamin 3 (*ANO3*) gene, and additional mutations were found in other familial and sporadic cases of cervical dystonia.<sup>29</sup> *ANO3* is expressed highly in the striatum, but also the neocortex, hippocampus, and amygdala. Anoctamin 3 is believed to act as a calcium-gated chloride channel, and functional studies using Ca<sup>2+</sup> imaging in case and control

fibroblasts demonstrated abnormalities in endoplasmic reticulum-dependent Ca<sup>2+</sup> signaling.

### DYT25 Dystonia: GNAL

Recently, mutations in *GNAL* have been identified in 11 multiplex families (autosomal dominant) predominantly with cervical and segmental dystonia.<sup>30,31</sup> *GNAL* encodes the stimulatory alpha subunit G $\alpha_{olf}$ , first identified as a G-protein that mediates odorant signaling in the olfactory epithelium, coupling D1 and A2a receptors to adenylyl cyclase and histone H3 phosphorylation.

The following sections focus on mechanisms that have been derived from the study of a number of the studied genes described above but will also consider pathogenesis of selected forms of secondary dystonia. Three key areas can be identified in which there appear to be shared molecular pathways: (1) cell cycle and transcription, (2) nuclear envelope/endoplasmic reticulum (ER) interface and ER secretory pathways, and (3) synaptic function.

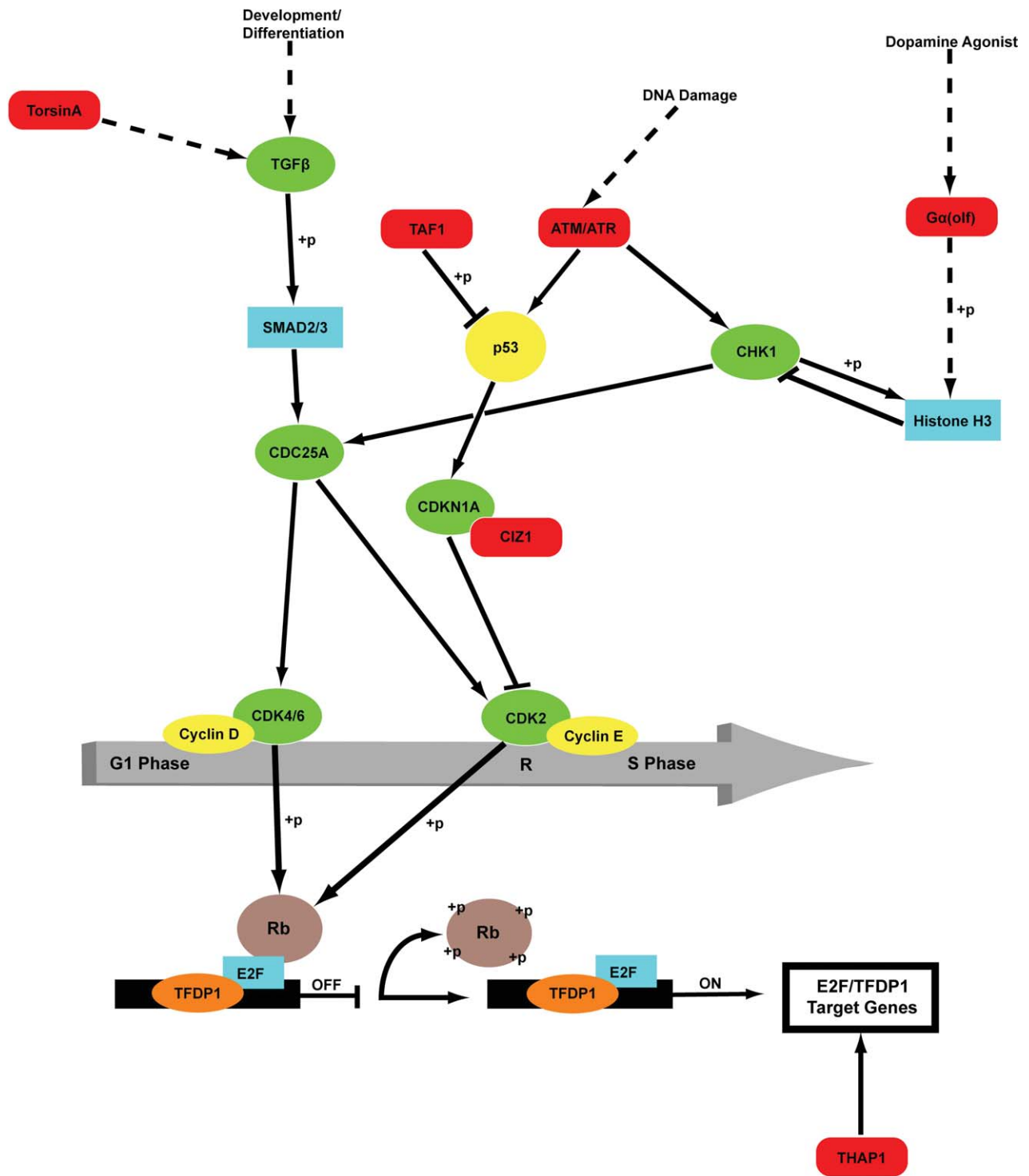
### Defects at the G<sub>1</sub>-S Cell-Cycle Checkpoint

The eukaryotic cell cycle consists of 4 distinct phases: gap 1 (G<sub>1</sub>), synthesis (S), gap 2 (G<sub>2</sub>), and mitosis (M). In addition, terminally differentiated cells like neurons enter a quiescent state call gap 0, or G<sub>0</sub>. The G<sub>1</sub> phase is commonly known as the growth phase. During G<sub>1</sub>, cells prepare for the DNA synthesis that will occur in the S phase. DNA is synthesized and chromosomes are replicated during the S phase. The G<sub>1</sub>-S cell-cycle checkpoint pathway controls the transition from the end of G<sub>1</sub> to S. The G<sub>1</sub>-S cell-cycle checkpoint ensures that the cell is fully prepared for DNA synthesis. Numerous factors such as DNA damage from irradiation, contact inhibition, TGF $\beta$ , and oxidative stress can inhibit transition from G<sub>1</sub> to S, and the G<sub>1</sub>-S checkpoint has also been implicated in the molecular pathology of dystonia.

### *CIZ1*

The cellular role and neural localization of *CIZ1* are compatible with current themes in dystonia research. *CIZ1*, *TOR1A*, *THAP1*, and genes associated with neurodegenerative dystonia seen in DYT3 dystonia (*TAF1*) and ataxia telangiectasia (*ATM*) are involved in G<sub>1</sub>-S cell-cycle regulation (Fig. 1).

In cell-free systems, *CIZ1* is able to promote DNA replication after replication complex formation.<sup>32</sup> The C-terminal domain anchors *CIZ1* to the nonchromatin nuclear matrix, whereas DNA replication activity resides in the N-terminal half of the protein. The C-terminal domain of *CIZ1* also recognizes the consensus DNA sequence ARYSR(0-2)YYAC.<sup>33</sup> Studies of GFP-tagged *CIZ1* have shown that formation of



**FIG. 1.** G1/S cell-cycle checkpoint and dystonia. Dystonia-associated proteins are shaded red. Indirect, multistep, and putative pathways are denoted with hashed lines. In general, arrows indicate excitatory interactions, and stops mark inhibitory interactions. However, some relationships are nonlinear and the result of combinatorial actions of heteromeric complexes and posttranslational modifications. +p, Phosphorylation; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; CDC25A, cell division cycle 25 homolog A; CDK2, cyclin-dependent kinase 2; CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; CDKN1A, p21/WAF1 or cyclin-dependent kinase inhibitor 1; CHK1, checkpoint kinase 1; CIZ1, Cip1-interacting zinc finger protein; E2F, transcription factor E2F; G1 phase, gap 1 phase of the cell cycle; p53, protein 53; R, restriction point; Rb, retinoblastoma protein; SMAD2, mothers against decapentaplegic homolog 2; SMAD3, mothers against decapentaplegic homolog 3; S phase, synthesis phase; TAF1, transcription initiation factor TFIID subunit 1; TFDP1, transcription factor Dp1; TGFβ, transforming growth factor β.

subnuclear particles or foci requires both the N- and C-terminal domains.<sup>34</sup>

The N-terminal region of CIZ1, including the first zinc finger motif, binds to the N-terminal CDK2-interacting domain of CDKN1A, and this interaction is disrupted by overexpression of CDK2.<sup>35</sup> When CIZ1 and CDKN1A are individually overexpressed, they localize primarily to the nucleus. However, overexpression of CIZ1 can induce cytoplasmic distribution of CDKN1A, suggesting that CIZ1 regulates the cellular localization of CDKN1A.

In cell-free experiments, CIZ1 increases the number of nuclei that initiate DNA replication, and in intact wild-type and CDKN1A-null cells, CIZ1 stimulates DNA synthesis. Consistent with a role in DNA replication, endogenous CIZ1 was found to colocalize with proliferating cell nuclear antigen (PCNA) during S phase, and targeted depletion of CIZ1 retracts cell proliferation by inhibiting entry into S phase.<sup>31</sup> CIZ1-depleted cells accumulate chromatin-bound minichromosome maintenance complex component 3 MCM3 and PCNA but fail to synthesize DNA efficiently.<sup>31</sup>

CIZ1 is an estrogen-responsive gene.<sup>36</sup> CIZ1 coregulates estrogen receptor alpha (ER $\alpha$ ) by enhancing ER $\alpha$  transactivation activity and promoting the recruitment of the ER $\alpha$  complex to target genes. CIZ1 overexpression confers estrogen hypersensitivity and promotes growth rate, anchorage independence, and tumorigenic properties in breast cancer cells. It is tempting to speculate that aberrant interactions between ER $\alpha$  and CIZ1 or other, as yet unidentified dystonia-related proteins contribute to the relative increased prevalence of cervical dystonia in females.

### THAP1

THAP1 plays an important role in transcriptional regulation in the context of cell proliferation and pRB/E2F cell-cycle pathways.<sup>18,37,38</sup> Both RNAi silencing and overexpression of THAP1 inhibit G<sub>1</sub>-S progression. Overexpression of THAP1 in primary human endothelial cells inhibited proliferation, and gene expression profiling showed that this effect was a result of repression of pRB/E2F cell-cycle target genes. The antiproliferative effects of THAP1 on endothelial cells were not dependent on apoptosis. THAP1 appears to inhibit cell-cycle progression at the G<sub>1</sub>-S transition, localizes to promyelocytic leukemia nuclear bodies with the proapoptotic leucine-zipper protein Par-4, and potentiates TNF $\alpha$ -induced apoptosis.<sup>17</sup>

Clouaire and colleagues (2005) identified a consensus DNA-binding sequence (AGTACGGGCAA) recognized by the THAP domain of THAP1.<sup>39</sup> Nucleotide positions upstream of the core motif appear to modulate the strength and affinity of the GGCA/THAP interaction. Other THAP zinc fingers, including human THAP2 and THAP3, share structural

homology but do not recognize the same DNA target sequence. Protein-protein interactions, including multimerization, mediated by the coiled-coil domain of THAP1 may increase binding of the THAP zinc finger. Human THAP1, human THAP9, and *Drosophila* THAP bind DNA through a bipartite interaction using both the major and minor grooves.<sup>40</sup>

Overexpression of THAP1 in endothelial cells has been used as an indirect means of identifying THAP1 targets.<sup>18</sup> A total of 16 genes were upregulated >1.5-fold, and 80 downregulated. Of the latter group, the genes were concentrated in classes related to cell-cycle/cell proliferation, and the majority were also regulated by the pRB/E2F pathway. The cellular effects of THAP1 knock-down with RNAi were similar to the effects of overexpression. In addition, THAP1 knock-down was associated with decreased expression of 8 pRB/E2F cell-cycle target genes: *RRM1*, *Mad2*, *survivin*, *HMMR*, *RRM2*, *CDC2*, *cyclin B1*, and *DLG7*. *RRM1* was shown to be a direct transcriptional target of THAP1. Potential THAP1 binding sites were also identified in the promoters of other genes: *RRM2*, *BIRC2*, *survivin*, and *cyclin B1*.

Studies have also focused on a potential functional interaction with torsinA. THAP1 was reported to bind to and activate the promoter of the *Tor1a* gene that encodes torsinA, although these studies did not demonstrate changes in torsinA mRNA following manipulation of THAP1.<sup>41,42</sup> Moreover, there do not appear to be sequence alterations in the genes encoding THAP1 or torsinA that explain disease penetrance in DYT1 dystonia.<sup>43,44</sup>

Two hybrid studies have identified several protein interactions for THAP1.<sup>17,45</sup> Analysis of the set of interacting proteins suggests that THAP1 may contribute to transcription, splicing, and, possibly, RNA transport. Interestingly, several interacting proteins indirectly implicate THAP1 in cerebellar development. For example, NKAP is a transcriptional repressor acting on Notch target genes and is required for T-cell development. Notch 1 is required for neuronal and glial differentiation in the cerebellum.<sup>46</sup> FXR2, a homologue of the fragile X mental retardation protein (FMRP), is involved in mRNA transport. Deletion of FMRP in mice is associated with cerebellar ultrastructural abnormalities and motor learning deficits.<sup>47</sup> Finally, DVL2 appears to be involved in the Wnt signaling pathway, which is critical for cerebellar morphogenesis.<sup>48</sup> There is an increasing body of evidence that implicates the cerebellum in the genesis of dystonic movements,<sup>49</sup> and these data suggest that THAP1 could lead to subtle functional abnormalities of cerebellar functions.

Although often considered a neurodegenerative disorder, some patients with Lubag (DYT3) manifest isolated focal (including cervical) or segmental dystonia years before the development of parkinsonism.<sup>50</sup>

DYT3 is associated with deficiency of a neuronal isoform of TAF1 (N-TAF1) that is expressed preferentially in medium spiny neurons of the striosome compartment.<sup>51,52</sup> TAF1 (TATA box-binding protein [TBP] associated factor 1) forms part of the TFIID transcriptional complex that is composed of TBP and up to 13 additional TAFs. TFIID binds to TATA boxes and participates in transcriptional initiation. Deficiency of defective TAF1 could exert deleterious effects on cell-cycle control via multiple mechanisms. For instance, TAF1 induces G<sub>1</sub>-S progression by phosphorylating p53 at threonine-55.<sup>53</sup> In TAF1-defective cell lines, ATR localizes to subnuclear foci and contributes to phosphorylation of downstream targets such as p53 and CHK1, which induces cell-cycle arrest.<sup>54</sup> Finally, TAF1 has been shown to undergo alternative splicing in response to developmental or DNA damage signals.<sup>55</sup>

### TorsinA

TorsinA binds the KASH domain of nesprin-3, which spans the nuclear envelope (NE) outer membrane, and TA is concentrated at the nuclear envelope in its mutant form. TorsinA appears to play a role in interactions between the nucleus and cytoskeleton.<sup>26</sup> These interactions may be important for cell polarity and/or transcription.<sup>56,57</sup> Alternatively, torsinA may participate in transcriptional regulation and/or G<sub>1</sub>-S cell-cycle regulation via the TGF $\beta$  pathway.<sup>58,59</sup> *Drosophila* larvae that overexpress mutant  $\Delta$ E torsinA exhibit overt ultrastructural defects at the neuromuscular junction similar to defects reported for mutants with defective TGF $\beta$  signaling.<sup>60,61</sup> Overexpression of SMAD2, a downstream effector of the TGF $\beta$  pathway, corrects morphological and behavioral defects associated with expression of mutant torsinA.<sup>58</sup>

Variant ataxia-telangiectasia from recessive mutations in *ATM* may present with dystonia.<sup>62</sup> This is similar to THAP1 dystonia, with frequent craniocervical and upper limb involvement, and can occur in the absence of cerebellar atrophy on MRI or ataxia on clinical examination. *ATM* (ataxia telangiectasia mutated) is a serine/threonine kinase activated by DNA double-strand breaks and can phosphorylate p53, effector kinase CHK2, and an inhibitor of p53, MDM2.<sup>63</sup> CHK2 also phosphorylates p53. Activation of p53 leads to increased expression of the Cdk inhibitor p21 and contributes to maintenance of cell-cycle arrest.

### GNAL

G $\alpha$ (olf) is found widely in brain with particularly robust expression in the striatum and cerebellar Purkinje cells.<sup>31</sup> In the striatum, G $\alpha$ (olf) may contribute to cell-cycle control via its role in phosphorylation of histone H3.<sup>64</sup> At the transcriptional level, mutations in *GNAL*

have been associated with upregulation of genes involved in cell-cycle control and development.<sup>31</sup>

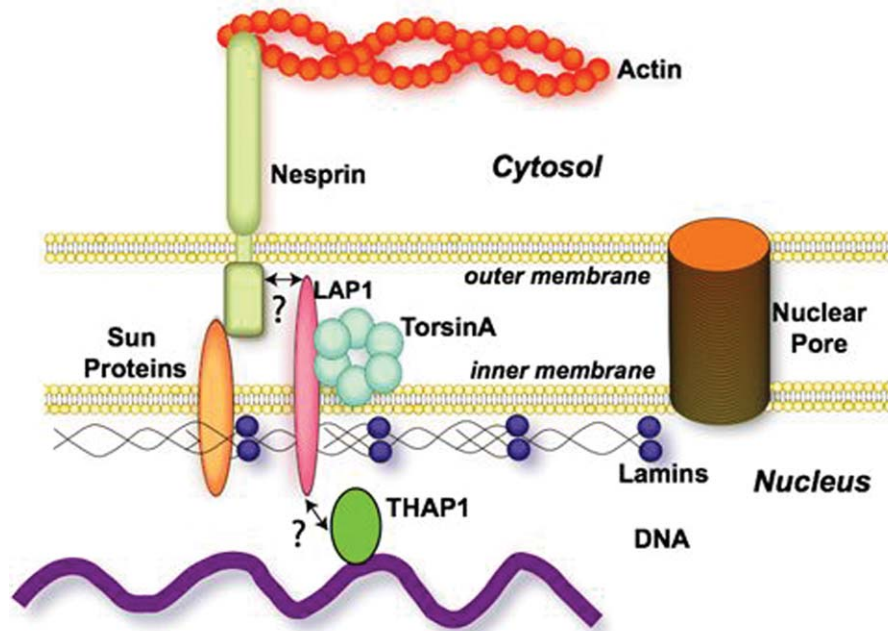
Neuronal death is an important phenomenon in the development of the nervous system, and the above suggests 1 or more dystonia-associated proteins (CIZ1, THAP1, torsinA, N-TAF1, G $\alpha$ [olf], and ATM) may contribute to this process.<sup>7,12,65</sup> For example, cell-cycle control and the G<sub>1</sub>-S checkpoint are known to be critical to olivocerebellar development.<sup>66</sup> In the cerebellar cortex, for instance, cell death may maintain numerical matching between granule and Purkinje cells (175:1). The downstream consequences of mutant or deficient dystonia-associated proteins could be aberrant cell-cycle reentry in terminally differentiated neurons, leading to numerical mismatch between specific neuronal populations or ultrastructural defects of specific neuronal types such as Purkinje cells or medium spiny neurons.<sup>67-70</sup>

### Endoplasmic Reticulum/Nuclear Envelope-Associated Dystonia Proteins

Another area in which there appears to be convergence in pathogenic mechanisms in dystonia is the endoplasmic reticular/nuclear envelope (ER/NE) endomembrane system. Several genes that cause dystonia when mutated encode proteins associated with the ER/NE system. Included in this group are torsinA, epsilon-sarcoglycan, and THAP1. The ER/NE membrane system is in the cell interior, and critically, all proteins must pass through this organelle to become membrane-embedded. Membrane-embedded proteins such as ion channels and neurotransmitter receptors and transporters are precisely the types of molecules that would be expected to cause the neural dysfunction seen in primary dystonia.

### TorsinA

TorsinA resides in the ER lumen, and several observations support a role for torsinA in membrane trafficking. It can inhibit trafficking of polytopic membranebound proteins, including the dopamine transporter, an effect suppressed by the  $\Delta$ E mutation.<sup>71</sup> Similarly, protein processing through the secretory pathway is defective in fibroblasts from patients with DYT1 dystonia, an abnormality that is rescued by downregulating the mutant protein.<sup>72,73</sup> TorsinA function in the ER also appears to affect the ability of a neuron to withstand cellular stress. Overexpression of torsinA protects cells from ER stress.<sup>74</sup> Conversely torsinA knockdown or the  $\Delta$ E mutation sensitizes cells to ER stress.<sup>73</sup> These effects may relate to a role for torsinA in endoplasmic reticular-associated degradation, a process whereby misfolded proteins are removed from the ER lumen and degraded by the proteasome.<sup>75,76</sup> Interestingly, the DYT16 protein PRKRA (PACT) may also play a role in regulating ER stress via induction of protein kinase R.<sup>77</sup>



**FIG. 2.** Potential interactions of torsinA and THAP1 at the nuclear envelope.

TorsinA also functions at the nuclear envelope.<sup>78,79</sup> A “substrate trap” version of torsinA that prevents it from uncoupling from protein partners causes it to accumulate abnormally in the NE.<sup>80–82</sup> Similarly, disease mutant  $\Delta E$ -torsinA concentrates abnormally at the nuclear membrane, indicating that the DYT1 mutation may lead to an abnormal interaction with an NE partner,<sup>80,83</sup> possibly lamina-associated polypeptide 1 (LAP1).<sup>84</sup> The neuronal nuclear membranes from torsinA null of  $\Delta E$ -homozygous knock-in mice exhibit abnormal nuclear envelope membranes, providing *in vivo* evidence for an NE role for torsinA.<sup>80</sup> Considered together, these observations suggest that the DYT1 mutation impairs normal torsinA function.

The role of torsinA within the NE is not well understood but may involve regulating connections between proteins that tether the nucleus to the cytoskeletal networker transcriptional regulation at the inner NE membrane.<sup>78,85</sup> These functions may intersect with that of epsilon-sarcoglycan, a protein that participates in nucleo-cytoskeletal connections, and the transcription factor THAP1 (Fig. 2).

### **Epsilon-Sarcoglycan**

The connection of epsilon-sarcoglycan (SCGE) function to the actin cytoskeleton, which itself has links to the nuclear membrane, points to a possible connection between torsinA and SCGE. TorsinA has been implicated in regulating connections between the nucleus and the cytoskeleton, in particular through its reported interactions with the nesprins, a family of outer NE proteins that themselves can interact with the actin cytoskeleton. TorsinA has been reported to interact with

nesprin-3, which mislocalizes in cells lacking torsinA or that harbor DYT1 mutant torsinA.<sup>86</sup> Several reports have demonstrated that nesprin-3 can connect to the actin cytoskeleton, consistent with a potential functional connection between torsinA and SCGE. This connection between torsinA and SCGE may also be in part direct, as torsinA has been reported to interact with and promote the degradation of SCGE mutants in the ER.<sup>87</sup> Abnormalities of the actin cytoskeleton could account for observations of neural process abnormalities in cell lines overexpressing torsinA.<sup>88,89</sup>

### **THAP1**

Although THAP1 does not appear to have a direct effect on torsinA expression, there are other ways that nuclear-localized THAP1 could potentially interact with torsinA-related pathways. The nuclear membrane is increasingly recognized as a site of transcriptional regulation through several mechanisms.<sup>90,91</sup> It is possible that THAP1 targets are dysregulated in DYT1 dystonia, in which mutant torsinA mislocalizes to the nuclear membrane. It is also possible that THAP1 and torsinA pathways connect via pRB, as this is a THAP1-binding partner that also indirectly connects to torsinA via laminA and LAP1. Direct testing of these possibilities awaits the identification of THAP1 target genes in neurons, which can then be assessed in DYT1 and forms of primary dystonia.

### **Synaptic Function**

Primary dystonia and the dystonia-plus syndromes are characterized by the absence of neurodegeneration, implying a functional neuronal defect that leads to the



abnormal movement command. This may be a neurochemical defect, possibly for multiple neurotransmitter pathways. The hypothesis that primary dystonia is a result of disturbance of neurotransmitters or synaptic transmission is an attractive one, and there is evidence to support this, particularly involving the dopaminergic pathway (Fig. 3). The clearest example is from a study of dopa-responsive dystonia in which mutations encoding proteins critical for dopamine (DA) biosynthesis, including GTP-cyclohydrolase 1 and tyrosine hydroxylase, cause dystonia.<sup>92,93</sup> Most cases of DRD are due to GTPCH1 mutations; GTPCH1 is the rate-limiting step in production of tetrahydrobiopterin, which is a key cofactor in the synthesis of monoamines, particularly dopamine. Reduced levels of functional GTPCH1 lead to reduced DA levels and dystonia. DRD, therefore, responds very well to L-dopa therapy, which corrects the presynaptic deficit of DA.<sup>94</sup>

D2 receptor antagonists can cause tardive dystonia,<sup>95</sup> and dystonia is also seen in Parkinson's disease, particularly young onset,<sup>96</sup> and Lesch-Nyhan syndrome, in which there is preferential loss of nigral dopaminergic neurons.<sup>97</sup> Furthermore, other DA-related abnormalities have been found in various forms of focal primary dystonia, including reduced basal ganglia D2 receptor binding in imaging studies and possible association of dystonia with polymorphisms in the D5 receptor gene.<sup>98,99</sup>

### TorsinA

The finding of torsinA mRNA in neurons of the substantia nigra led to neurochemical analysis of dopamine and its metabolites in postmortem brain tissue.<sup>5,100</sup> However, these studies were inconclusive.<sup>101,102</sup> Similarly, studies in various transgenic mouse models provided inconsistent results regarding metabolite levels.<sup>103–106</sup> However, more recent work in a model using the TH promoter to drive expression of wild-type or mutant torsinA has suggested a defect in DA reuptake implicating the DA transporter (DAT).<sup>107,108</sup> This has been supported by findings of reduced DA reuptake in hMT-CMV mice<sup>109</sup> and a recent study in DYT knock-in mice showing that TH-positive substantia nigra neurons were slightly reduced in number and increased in size,<sup>110</sup> and evidence of a direct interaction between torsinA and DAT<sup>71</sup> as well as vesicle monoamine transporter 2 (VMAT2).<sup>111</sup> Recent work using more sensitive voltammetry to measure extracellular DA, however, found evidence to suggest that it was release not reuptake that was impaired, arguing against DAT dysfunction.<sup>107,112</sup>

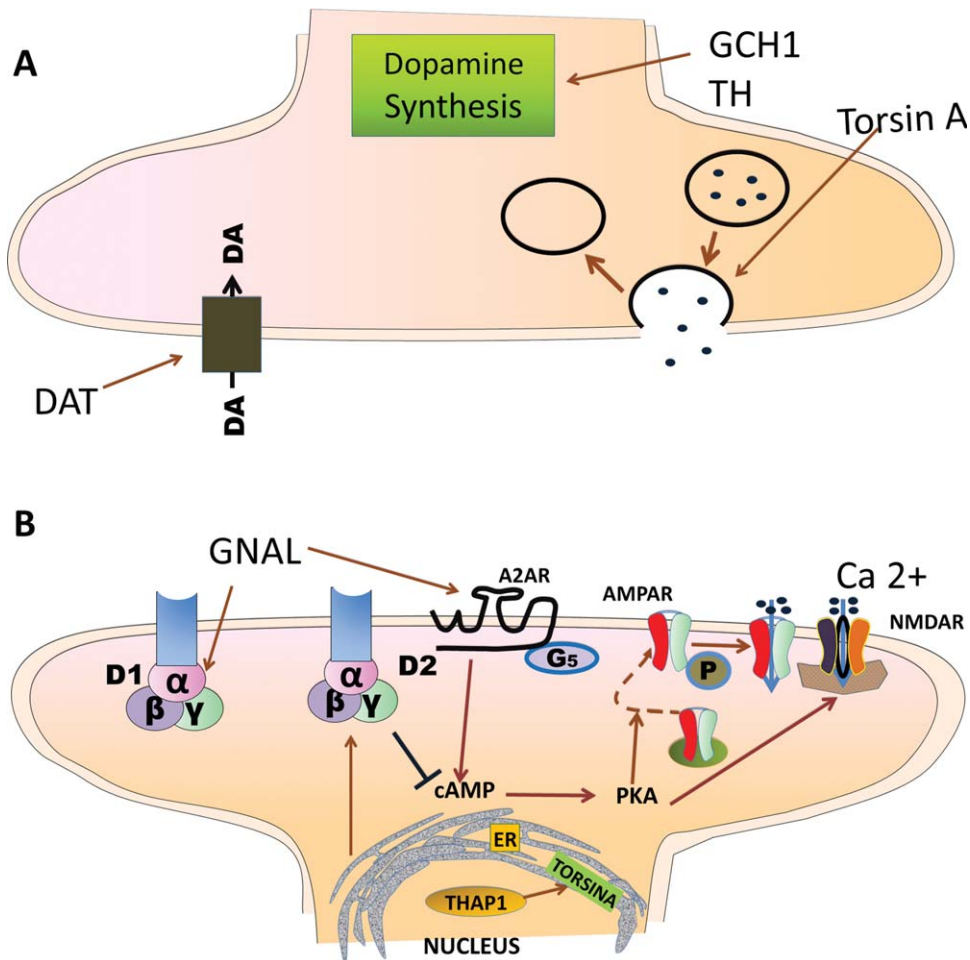
Further evidence for involvement of presynaptic vesicles comes from cellular studies. It has been detected that torsinA is associated with vesicles in axons and presynaptic terminals, and biochemical

fractionation analysis showed enrichment of torsinA in the fraction containing synaptosomal membranes.<sup>113</sup> TorsinA has also been found to colocalize with snapin (SNARE-associated protein) on dense-core granules at the tips of differentiated PC12 cells.<sup>114</sup> Functional analysis of SH-SY5Y cells expressing wild-type or mutant torsinA has shown that it regulates the degradation of snapin and stonin-2 (synaptotagmin specific endocytic adapter) by the proteasome, with mutant torsinA leading to reduced levels and compromised synaptic vesicle recycling.<sup>115</sup>

Abnormalities in synaptic vesicle recycling has been supported by recent work in cultured hippocampal neurons from a knock-in mouse model of DYT1 dystonia that suggested that torsinA regulates recycling at a level at or upstream of the rise in calcium concentration in nerve terminals and that the regulation is influenced by neuronal activity.<sup>116</sup> Furthermore, using patch-clamp electrophysiology, it was found that neurons with mutant torsinA had more frequent miniature glutamate release, which may underlie the excitability of the CNS in DYT1 dystonia.<sup>117</sup> The authors have not looked at other neurons, potentially more relevant to basal ganglia dysfunction.

Postsynaptic defects in hMT-CMV-derived striatal slices showed that activation of D2 receptors (D2Rs) led to abnormal activation and inappropriate firing of cholinergic interneurons<sup>118</sup> and GABAergic medium spiny neurons.<sup>119</sup> In addition, medium spiny neurons from hMT-CMV mice had decreased expression of surface D2Rs with impaired G-protein coupling, despite normal levels of D2R mRNA.<sup>120</sup> It was suggested that there was a posttranslational defect in receptor processing. This may occur in the ER for TA, where it acts as a molecular chaperone, leading to abnormal folding or oligomerization of the D2R, and a direct interaction between TA and the D2R has previously been demonstrated.<sup>71</sup> In support of this hypothesis are data from [<sup>11</sup>C]-raclopride PET studies that showed reduced levels of D2R binding in patients with DYT1 and DYT6 dystonia, suggesting a pathogenetic link between these 2 forms and a D2R defect.<sup>121,122</sup> For DYT1, reductions in radioligand binding were found in the caudate, putamen, and ventrolateral thalamus.<sup>123</sup> Further work needs to be performed to clarify this area, not least is whether the potential D2R defect in DYT6 dystonia is also at a posttranslational level or relates to disrupted transcription.

The abnormal synaptic plasticity in transgenic models of primary dystonia has been reviewed recently and highlighted disruption of synaptic scaling, with facilitation of synaptic potentiation, together with loss of synaptic inhibitory processes.<sup>124</sup> In the hMT-CMV model described above, impaired D2R postsynaptic function was suggested by the inability of the D2R agonist to reestablish normal corticostriatal synaptic plasticity. Interestingly, blockade of A2A receptors



**FIG. 3.** Schematic diagram of synapse indicating potential impact of proteins whose mutant forms cause dystonia. **A:** Presynaptic terminal showing interaction of torsinA, DAT, and mutations in *GCH1* and *TH* on synaptic vesicle recycling, dopamine uptake, and synthesis, respectively. **B:** Postsynaptic terminal. TorsinA and THAP1 may affect dopamine receptor expression or function. D2R and A2AR function may be influenced by these proteins or directly by GNAL influencing PKA phosphorylation and promoting delivery of GluA1-containing AMPARs to extrasynaptic pools, increasing their availability for subsequent synaptic recruitment on LTP induction and NMDAR activation. Calcium influx through synaptic NMDARs is endogenously facilitated by A<sub>2</sub>AR, but it can also be inhibited by activation of D<sub>2</sub>R through PKA-dependent mechanisms.  $\alpha, \beta, \gamma$ , subunits of G protein; DA, dopamine; D2, dopamine type 2 receptor; D1, dopamine type 1 receptor; GCH1, GTP-cyclohydrolase 1; GNAL, stimulatory  $\alpha$  subunit of G protein; TH, tyrosine hydroxylase, ER, endoplasmic reticulum; A2AR, adenosine 2 receptor; AMPAR, AMPA receptor; NMDAR, NMDA receptor.

fully restored the impairment of synaptic plasticity. A2A receptors and D2 receptors oppose each other in the induction of bidirectional synaptic plasticity, with D2Rs promoting long-term depression and A2A receptors favoring induction of long-term potentiation. The effect of the A2AR antagonist suggests the deficit in D2R function in the model can be reversed by eliminating the negative tone exerted by A2ARs on this. The possible role of A2ARs in genetic dystonia is also implicated from preliminary work on GNAL.

### GNAL

G-proteins link seven-transmembrane-domain receptors to downstream effectors and function as heterotrimers composed of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits. Evidence suggests that  $G\alpha_{olf}$  acts in medium spiny neurons to couple dopamine type 1 receptors (D1Rs) of the direct pathway and adenosine A2A receptors (A2ARs) of the

indirect pathway to the activation of adenylate cyclase type 5.<sup>125</sup> A2ARs and  $G\alpha_{olf}$  are also expressed in striatal cholinergic interneurons. The mutations identified in GNAL appear to lead to loss of function and implicate abnormalities in D1R and/or A2AR transmission in the pathogenesis of dystonia.

### DYT12: Alpha-3 Subunit of Na<sup>+</sup>/K<sup>+</sup> ATPase

Neuronal dysfunction leading to abnormal neurophysiology has been implicated for the DYT12 protein, alpha-3 subunit of the Na<sup>+</sup>/K<sup>+</sup> ATPase, which belongs to the group of P-type ATPases, which utilize energy liberated during the hydrolysis of ATP for active transport of cations across cell membranes. Biochemical enzyme assays have revealed that mutations in  $\alpha 3$  cause a reduction in both Na<sup>+</sup> affinity and extrusion of intracellular Na<sup>+</sup>, leading to disrupted electrochemical ionic gradients across the neuronal

cell membrane.<sup>126</sup> It is possible that this may lead to downstream abnormalities of synaptic function. A phenotypic model of RDP was recently generated by chemically inhibiting the  $\alpha 3$  isoform of the ATPase function in selected brain regions using the targeted infusion of ouabain, which selectively reduces  $\alpha 3$ -ATPase function in a dose-dependent manner in genetically wild-type mice.<sup>127</sup> Ouabain infusions in the basal ganglia and cerebellum induced a parkinsonism-like or dystonic-like phenotype, respectively, but only concomitant infusions in both structures yielded a stress-inducible phenotype resembling features of RDP. In the mouse model, dystonic postures were reduced following transient inhibition of cerebellar input by GABA injection, again underlining the emerging importance of cerebellar dysfunction in dystonia and RDP.

Further evidence to support a dopaminergic etiology for dystonia comes from the study of dopamine transporter deficiency syndrome.

### Dopamine Transporter Deficiency Syndrome

Dopamine transporter deficiency syndrome is an autosomal recessive condition caused by loss-of-function mutations in the *DAT* gene.<sup>128,129</sup> *DAT* is a transmembrane protein exclusively expressed in dopaminergic neurons, in which it mediates the reuptake of dopamine into presynaptic terminals after synaptic transmission. This rapid recycling of neurotransmitter is crucial to synaptic function, as it replenishes dopamine stores in the presynaptic terminal and prevents desensitization of the postsynaptic terminal. Children presented in infancy with either hyperkinesia, parkinsonism, or a mixed hyperkinetic and hypokinetic movement disorder. Some individuals had previously been misdiagnosed with cerebral palsy. During childhood they developed severe dystonia-parkinsonism associated with an eye movement disorder and pyramidal tract features. Investigations revealed raised ratios of homovanillic acid to 5-hydroxyindoleacetic acid in cerebrospinal fluid. *DAT* SCAN imaging in 1 patient showed complete loss of dopamine transporter activity in the basal nuclei, although a trial of L-dopa had no effect on either the patient's symptoms or CSF parameters.

### Common Themes in Dystonia Molecular Pathways

The increasing knowledge of proteins whose mutant forms cause dystonia has implicated a large number of neurobiological pathways that lead to dystonic movements. A number of themes have emerged that have been identified in this review—from abnormal transcription and cell cycle because of the nuclear effects of dystonia genes to ER dysfunction and synaptic abnormalities. It is right to seek common pathways that may represent targets for therapeutic strategies

for this group of incurable movement disorders. However, it may also lead to oversimplification in the search for unifying mechanisms. Most cases of dystonia are primary and not associated with neuronal death. Thus, the pathogenic mechanisms may be subtle and only cause relatively mild defects in the relevant pathways, leading to abnormal processing of the motor command within the CNS.<sup>70</sup>

There is increasing awareness of the role of abnormal inhibition and plasticity affecting sensorimotor pathways in dystonia.<sup>1</sup> This may be a template laid down in early life, supporting the view that primary dystonia is a neurodevelopmental circuit disorder. In support of this are the developmental patterns of expression of the best-studied primary dystonia gene products: *torsinA* and *THAP1*. *TorsinA* expression in the mouse was highest during prenatal and early postnatal development, particularly in the cortex, striatum, thalamus, and cerebellum.<sup>130</sup> In the human brain, *torsinA* protein is detectable at 4–8 weeks postnatally in the cerebellum (Purkinje cells), substantia nigra hippocampus, and basal ganglia.<sup>131</sup> Similarly, *THAP1* is expressed in the rat in early development, particularly in the cerebellum (Purkinje cells), cortical pyramidal neurons, relay neurons in the thalamus, medium and cholinergic striatal neurons, dopaminergic substantia nigra neurons, and hippocampal neurons.<sup>132</sup> This developmentally regulated expression of 2 dystonia-associated proteins suggests a role in terminal regulation and establishment of key circuits involved in motor control. The themes identified in this review would have a significant effect on neurodevelopment in terms of altered transcription of key genes at the nuclear level, protein processing and trafficking through the ER, or altered ER stress response in important periods of neural cell differentiation, through to abnormal synaptic function affecting neurotransmission or synaptic plasticity. Any or all of the above mechanisms could lead to abnormal patterns or responsiveness of sensorimotor circuits, leading to a susceptibility to developing an abnormal “dystonic state.”

It may be that with the increasing number of *DYT* loci and better understanding of recently identified genes encoding *CIZ1*, *GNAL*, and *ANO3*, the key cellular pathogenic mechanisms involved in the genesis of dystonic movement will become clearer. ■

## References

1. Phukan J, Albanese A, Gasser T, Warner T. Primary dystonia and dystonia-plus syndromes: clinical characteristics, diagnosis, and pathogenesis. *Lancet Neurol.* 2011;10:1074–1085.
2. Ozelius L, Kramer P, Page CE et al. The early-onset torsion dystonia gene (*DYT1*) encodes an ATP-binding protein. *Nat Genet.* 1997;17:40–48.
3. Hanson PI, Whiteheart SW. AAA<sup>+</sup> proteins: have engine will work. *Nat Rev Mol Cell Biol.* 2005;6:519–529.

4. Kock N, Naismith TV, Boston HE, et al. Effects of genetic variations in the dystonia protein torsinA: identification of polymorphism at residue 216 as protein modifier. *Hum Mol Genet.* 2006;15:1355–1364.
5. Augood SJ, Penney JB, Friberg IK, et al. Expression of the early onset torsion dystonia gene (DYT1) in human brain. *Ann Neurol.* 1998;43:669–673.
6. Rostasy K, Augood SJ, Hewett JW, et al. TorsinA protein and neuropathology in early onset generalized dystonia with GAG deletion. *Neurobiol Dis.* 2003;12:11–24.
7. Xiao J, Gong S, Zhao Y, LeDoux MS. Developmental expression of rat torsinA transcript and protein. *Brain Res Dev Brain Res.* 2004;152:47–60.
8. Zhao Y, Xiao J, Ueda M, et al. Glial elements contribute to stress-induced torsinA expression in the central and peripheral nervous systems. *Neuroscience.* 2008;155:439–453.
9. Jungwirth M, Dear ML, Brown P, Holbrook K, Goodchild R. Relative expression of homologous torsinB correlates with the neuronal specific importance of DYT1 dystonia-associated torsinA. *Hum Mol Genet.* 2010;19:888–900.
10. Hersheson J, Menacci NE, Davis M, et al. Mutations in the auto-regulatory domain of  $\beta$ -tubulin 4a cause hereditary dystonia. *Ann Neurol.* 2012 [Epub ahead of print].
11. Blanchard A, Ea V, Roubertie A, et al. DYT6 dystonia: review of the literature and creation of the UMD Locus-Specific Database (LSDB) for mutations in the THAP1 gene. *Hum Mutat.* 2011;32:1213–1224.
12. LeDoux MS, Xiao J, Rudzińska M, et al. Genotype-phenotype correlations in THAP1 dystonia: molecular foundations and description of new cases. *Parkinsonism Relat Disord.* 2012;18:414–425.
13. Fuchs T, Gavarini S, Saunders-Pullman R, et al. Mutations in the THAP1 gene are responsible for DYT6 primary torsion dystonia. *Nat Genet.* 2009;41:286–288.
14. Djarmati A, Schneider SA, Lohmann K, et al. Mutations in THAP1 (DYT6) and generalised dystonia with prominent spasmodic dysphonia: a genetic screening study. *Lancet Neurol.* 2009;8:447–452.
15. Bressman SB, Raymond D, Fuchs T, Heiman GA, Ozelius LJ, Saunders-Pullman R. Mutations in THAP1 (DYT6) in early-onset dystonia: a genetic screening study. *Lancet Neurol.* 2009;8:441–446.
16. Schneider SA, Ramirez A, Shafiee K, et al. Homozygous THAP1 mutations as cause of early-onset generalized dystonia. *Mov Disord.* 2011;26:858–861.
17. Roussigne M, Cayrol C, Clouaire T, Amalric F, Girard JP. THAP1 is a nuclear proapoptotic factor that links prostate-apoptosis-response-4 (Par-4) to PML nuclear bodies. *Oncogene.* 2003;22:2432–2442.
18. Cayrol C, Lacroix C, Mathe C, et al. The THAP-zinc finger protein THAP1 regulates endothelial cell proliferation through modulation of pRB/E2F cell-cycle target genes. *Blood.* 2007;109:584–594.
19. Zimprich A, Grabowski M, Asmus F, et al. T. Mutations in the gene encoding epsilon-sarcoglycan cause myoclonus-dystonia syndrome. *Nat Genet.* 2001;29:66–69.
20. Sunada Y, Campbell KP. Dystrophin-glycoprotein complex: molecular organization and critical roles in skeletal muscle. *Curr Opin Neurol.* 1995;8:379–384.
21. Straub V, Campbell KP. Muscular dystrophies and the dystrophin-glycoprotein complex. *Curr Opin Neurol.* 1997;10:168–175.
22. Chan P, Gonzalez-Maeso J, Ruf F, et al. Epsilon-sarcoglycan immunoreactivity and mRNA expression in mouse brain. *J Comp Neurol.* 2005;482:50–75.
23. Brunig I, Suter A, Knuesel I, Luscher B, Fritschy JM. GABAergic terminals are required for postsynaptic clustering of dystrophin but not of GABA(A) receptors and gephyrin. *J Neurosci.* 2002;22:4805–4813.
24. Levi S, Grady RM, Henry MD, Campbell KP, Sanes JR, Craig AM. Dystroglycan is selectively associated with inhibitory GABAergic synapses but is dispensable for their differentiation. *J Neurosci.* 2002;22:4274–4285.
25. Camargos S, Scholz S, Simon-Sanchez J, et al. DYT16, a novel young-onset dystonia-parkinsonism disorder: identification of a segregating mutation in the stress response protein PRKRA. *Lancet Neurol.* 2008;7:207–215.
26. Bragg DC, Armata IA, Nery FC, et al. Molecular pathways in dystonia. *Neurobiol Dis.* 2011;42:136–147.
27. Xiao J, Uitti RJ, Zhao Y, et al. Mutations in CIZ1 cause adult-onset primary cervical dystonia. *Ann Neurol.* 2012;71:458–469.
28. Mitsui K, Matsumoto A, Ohtsuka S, et al. Cloning and characterization of a novel p21(Cip1/Waf1)-interacting zinc finger protein, ciz1. *Biochem Biophys Res Commun.* 1999;264:457–464.
29. Charlesworth G, Plagnol V, Holmstrom KM, et al. Mutations in ANO3 cause dominant craniocervical dystonia: ion channel implicated in pathogenesis. *Am J Hum Genet.* 2012;91:1041–1050.
30. Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in GNAL cause primary torsion dystonia. *Nat Genet.* 2013;45:88–92.
31. Vemula SR, Puschmann A, Xiao J, et al. Role of G $\alpha$ (Olf) in familial and sporadic adult onset primary dystonia. *Hum Mol Genet.* 2013 [Epub ahead of print].
32. Coverley D, Marr J, Ainscough J. Ciz1 promotes mammalian DNA replication. *J Cell Sci.* 2005;118:101–112.
33. Warder DE, Keherly MJ. Ciz1, Cip1 interacting zinc finger protein 1 binds the consensus DNA sequence ARYSR(0 2)YYAC. *J Biomed Sci.* 2003;10:406–417.
34. Ainscough JF, Rahman FA, Sercombe H, et al. C-terminal domains deliver the DNA replication factor Ciz1 to the nuclear matrix. *J Cell Sci.* 2007;120:115–124.
35. Copeland NA, Sercombe HE, Ainscough JF, Coverley D. Ciz1 cooperates with cyclin-A-CDK2 to activate mammalian DNA replication in vitro. *J Cell Sci.* 2010;123:1108–1115.
36. den Hollander P, Rayala SK, Coverley D, Kumar R. Ciz1, a novel DNA-binding coactivator of the estrogen receptor alpha, confers hypersensitivity to estrogen action. *Cancer Res.* 2006;66:11021–11029.
37. Bessiere D, Lacroix C, Campagne S, et al. Structure-function analysis of the THAP zinc finger of THAP1, a large C2CH DNA-binding module linked to Rb/E2F pathways. *J Biol Chem.* 2008;283:4352–4363.
38. Campagne S, Saurel O, Gervais V, Milon A. Structural determinants of specific DNA-recognition by the THAP zinc finger. *Nucleic Acids Res.* 2010;38:3466–3476.
39. Clouaire T, Roussigne M, Ecochard V, Mathe C, Amalric F, Girard JP. The THAP domain of THAP1 is a large C2CH module with zinc-dependent sequence-specific DNA-binding activity. *Proc Natl Acad Sci U S A.* 2005;102:6907–6912.
40. Sabogal A, Lyubimov AY, Corn JE, Berger JM, Rio DC. THAP proteins target specific DNA sites through bipartite recognition of adjacent major and minor grooves. *Nat Struct Mol Biol.* 2010;17:117–123.
41. Gavarini S, Cayrol C, Fuchs T, et al. Direct interaction between causative genes of DYT1 and DYT6 primary dystonia. *Ann Neurol.* 2010;68:549–553.
42. Kaiser FJ, Osmanovic A, Rakovic et al. The dystonia gene DYT1 is repressed by the transcription factor THAP1 (DYT6). *Ann Neurol.* 2010;68:554–559.
43. Palada V, Stiern S, Glockle et al. K. Lack of sequence variations in THAP1 gene and THAP1-binding sites in TOR1A promoter of DYT1 patients. *Mov Disord.* 2012;27:917.
44. Kamm C, Uflacker N, Asmus F, et al. No evidence for THAP1/DYT6 variants as disease modifiers in DYT1 dystonia. *Mov Disord.* 2011;26:2136–2137.
45. Rual JF, Venkatesan K, Hao T, et al. Towards a proteome-scale map of the human protein-protein interaction network. *Nature.* 2005;437:1173–1178.
46. Lütolf S, Radtke F, Aguet M, Suter U, Taylor V. Notch1 is required for neuronal and glial differentiation in the cerebellum. *Development.* 2002;129:373–385.
47. Koekkoek SK, Yamaguchi K, Milojkovic BA, et al. Deletion of FMR1 in Purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates cerebellar eyelid conditioning in Fragile X syndrome. *Neuron.* 2005;47:339–352.
48. Andersson T, Södersten E, Duckworth JK, et al. CXXC5 is a novel BMP4-regulated modulator of Wnt signaling in neural stem cells. *J Biol Chem.* 2009;284:3672–3681.

49. Sadnicka A, Hoffland BS, Bhatia KP, van de Warrenburg BP, Edwards MJ. The cerebellum in dystonia—help or hindrance. *Clin Neurophysiol.* 2012;123:65–70.
50. Evidente VG, Advincola J, Esteban R, et al. Phenomenology of “Lubag” or X-linked dystonia-parkinsonism. *Mov Disord.* 2002;17:1271–1277.
51. Makino S, Kaji R, Ando S, et al. Reduced neuron-specific expression of the TAF1 gene is associated with X-linked dystonia-parkinsonism. *Am J Hum Genet.* 2007;80:393–406.
52. Sako W, Moridaki R, Kaji R, et al. Identification and localization of a neuron-specific isoform of TAF1 in rat brain; implications for neuropathology of DYT3 dystonia. *Neuroscience.* 2011;189:100–107.
53. Li HH, Li AG, Sheppard HM, Liu X. Phosphorylation on Thr-55 by TAF1 mediates degradation of p53: a role for TAF1 in cell G1 progression. *Mol Cell.* 2004;13:867–878.
54. Buchmann AM, Skaar JR, DeCaprio JA. Activation of a DNA damage checkpoint response in a TAF1-defective cell line. *Mol Cell Biol.* 2004;24:5332–5339.
55. Katzenberger RJ, Marengo MS, Wassarman DA. ATM and ATR pathways signal alternative splicing of *Drosophila* TAF1 pre-mRNA in response to DNA damage. *Mol Cell Biol.* 2006;26:9256–9267.
56. Basham SE, Rose LS. The *Caenorhabditis elegans* polarity gene *ooc-5* encodes a Torsin-related protein of the AAA ATPase superfamily. *Development.* 2001;128:4645–4656.
57. Baptista MJ, O’Farrell C, Hardy J, Cookson MR. Microarray analysis reveals induction of heat shock proteins mRNAs by the torsion dystonia protein, TorsinA. *Neurosci Lett.* 2003;343:5–8.
58. Koh YH, Rehfeld K, Ganetzky B. A *Drosophila* model of early onset torsion dystonia suggests impairment in TGF-beta signaling. *Hum Mol Genet.* 2004;13:2019–2030.
59. Erol A. Genotoxic stress-mediated cell cycle activities for the decision of cellular fate. *Cell Cycle.* 2011;10:3239–3248.
60. Aberle H, Haghighi AP, Fetter RD, McCabe BD, Magalhães TR, Goodman CS. wishful thinking encodes a BMP type II receptor that regulates synaptic growth in *Drosophila*. *Neuron.* 2002;33:545–558.
61. McCabe BD, Marqués G, Haghighi AP, et al. The BMP homolog *Gbb* provides a retrograde signal that regulates synaptic growth at the *Drosophila* neuromuscular junction. *Neuron.* 2003;39:241–254.
62. Saunders-Pullman R, Raymond D, Stoessl AJ, et al. Variant ataxia-telangiectasia presenting as primary-appearing dystonia in Canadian Mennonites. *Neurology.* 2012;78:649–657.
63. Pommier Y, Weinstein JN, Aladjem MI, Kohn KW. Chk2 molecular interaction map and rationale for Chk2 inhibitors. *Clin Cancer Res.* 2006;12:2657–2661.
64. Bertran-Gonzalez J, Håkansson K, Borgkvist A, et al. Histone H3 phosphorylation is under the opposite tonic control of dopamine D2 and adenosine A2A receptors in striatopallidal neurons. *Neuropsychopharmacology.* 2009;34:1710–1720.
65. Herrup K, Sunter K. Numerical matching during cerebellar development: quantitative analysis of granule cell death in staggerer mouse chimeras. *J Neurosci.* 1987;7:829–836.
66. Subkhankulova T, Zhang X, Leung C, Marino S. *Bmi1* directly represses *p21Waf1/Cip1* in Shh-induced proliferation of cerebellar granule cell progenitors. *Mol Cell Neurosci.* 2010;45:151–162.
67. Wang L, Wang R, Herrup K. E2F1 works as a cell cycle suppressor in mature neurons. *J Neurosci.* 2007;27:12555–12564.
68. Li J, Chen J, Vinters HV, Gatti RA, Herrup K. Stable brain ATM message and residual kinase-active ATM protein in ataxia-telangiectasia. *J Neurosci.* 2011;31:7568–7577.
69. Prudente CN, Pardo CA, Xiao J, et al. Neuropathology of cervical dystonia. *Exp Neurol.* 2013;241:95–104.
70. Song CH, Bernhard D, Bolarinwa C, Hess EJ, Smith Y, Jinnah HA. Subtle microstructural changes of the striatum in a DYT1 knock-in mouse model of dystonia. *Neurobiol Dis.* 2013;54:362–371.
71. Torres GE, Sweeney AL, Beaulieu JM, Shashidharan P, Caron MG. Effect of torsinA on membrane proteins reveals a loss of function and a dominant-negative phenotype of the dystonia-associated DeltaE-torsinA mutant. *Proc Natl Acad Sci U S A.* 2004;101:15650–15655.
72. Hewett JW, Tannous B, Niland BP, et al. Mutant torsinA interferes with protein processing through the secretory pathway in DYT1 dystonia cells. *Proc Natl Acad Sci U S A.* 2007;104:7271–7276.
73. Hewett JW, Nery FC, Niland B, et al. siRNA knock-down of mutant torsinA restores processing through secretory pathway in DYT1 dystonia cells. *Hum Mol Genet.* 2008;17:1436–1445.
74. Chen P, Burdette AJ, Porter JC, et al. The early-onset torsion dystonia-associated protein, torsinA, is a homeostatic regulator of endoplasmic reticulum stress response. *Hum Mol Genet.* 2010;19:3502–3515.
75. Nery FC, Armata IA, Farley JE, et al. TorsinA participates in endoplasmic reticulum-associated degradation. *Nat Commun.* 2011;2:393.
76. Bernasconi R, Molinari M. ERAD and ERAD tuning: disposal of cargo and of ERAD regulators from the mammalian ER. *Curr Opin Cell Biol.* 2011;23:176–183.
77. Lee ES, Tang N, Thompson S, Miller S, Katze MG. The double-stranded RNA-activated protein kinase (PKR) plays a significant role in a sustained ER stress-induced apoptosis. *FEBS J.* 2007;281:4325–4332.
78. Gerace L. TorsinA and torsion dystonia: unraveling the architecture of the nuclear envelope. *Proc Natl Acad Sci U S A.* 2004;101:8839–8840.
79. Goodchild RE, Dauer WT. Mislocalization to the nuclear envelope: an effect of the dystonia-causing torsinA mutation. *Proc Natl Acad Sci U S A.* 2004;101:847–852.
80. Goodchild RE, Kim, CE, Dauer WT. Loss of the dystonia-associated protein torsinA selectively disrupts the neuronal nuclear envelope. *Neuron.* 2005;48:923–932.
81. Naismith TV, Heuser JE, Breakefield XO, Hanson PI. TorsinA in the nuclear envelope. *Proc Natl Acad Sci U S A.* 2004;101:7612–7617.
82. Gonzalez-Alegre P, Paulson HL. Aberrant cellular behavior of mutant torsinA implicates nuclear envelope dysfunction in DYT1 dystonia. *J Neurosci.* 2004;24:2593–2601.
83. Giles LM, Chen J, Li L, Chin LS. Dystonia-associated mutations cause premature degradation of torsinA protein and cell-type-specific mislocalization to the nuclear envelope. *Hum Mol Genet.* 2008;17:2712–2722.
84. Goodchild RE, Dauer WT. The AAA+ protein torsinA interacts with a conserved domain present in LAP1 and a novel ER protein. *J Cell Biol.* 2005;168:855–862.
85. Worman HJ, Gundersen GG. Here come the SUNs: a nucleocytoskeletal missing link. *Trends Cell Biol.* 2006;16:67–69.
86. Nery FC, Zeng J, Niland et al. TorsinA binds the KASH domain of nesprins and participates in linkage between nuclear envelope and cytoskeleton. *J Cell Sci.* 2008;121:3476–3486.
87. Esapa CT, Waite A, Locke M, et al. SGCE missense mutations that cause myoclonus-dystonia syndrome impair epsilon-sarcoglycan trafficking to the plasma membrane: modulation by ubiquitination and torsinA. *Hum Mol Genet.* 2007;16:327–342.
88. Ferrari-Toninelli G, Paccioretti S, Francisconi S, Uberti D, Memo M. TorsinA negatively controls neurite outgrowth of SH-SY5Y human neuronal cell line. *Brain Res.* 2004;1012:75–81.
89. Hewett JW, Zeng J, Niland BP, Bragg DC, Breakefield XO. Dystonia-causing mutant torsinA inhibits cell adhesion and neurite extension through interference with cytoskeletal dynamics. *Neurobiol Dis.* 2006;22:98–111.
90. Srsen V, Korfali N, Schirmer EC. Nuclear envelope influences on cell-cycle progression. *Biochem Soc Trans.* 2011;39:1742–1746.
91. Egecioglu D, Brickner JH. Gene positioning and expression. *Curr Opin Cell Biol.* 2011;23:338–345.
92. Ichinose H, Ohye T, Takahashi E, et al. Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene. *Nat Genet.* 1994;8:236–242.
93. Knappskog PM, Flatmark T, Mallet J, Ludecke B, Bartholome K. Recessively inherited L-DOPA-responsive dystonia caused by a point mutation (Q381K) in the tyrosine hydroxylase gene. *Hum Mol Genet.* 1995;4:1209–1212.

94. Trender-Gerhard I, Sweeney MG, Schwingenschuh P, et al. Autosomal-dominant GTPCH1-deficient DRD: clinical characteristics and long-term outcome of 34 patients. *J Neurol Neurosurg Psychiatry*. 2009;80:839–845.
95. Casey DE. Pathophysiology of antipsychotic drug-induced movement disorders. *J Clin Psychiatry*. 2004;65:25–28.
96. Tolosa E, Compta Y. Dystonia in Parkinson's disease. *J Neurol*. 2006;(Suppl 7):VII7–VII13.
97. Egami K, Yitta S, Lewers JC, et al. Basal ganglia dopamine loss due to defect in purine cycling. *Neurobiol Dis*. 2007;26:391–407.
98. Perlmutter JS, Stambuk MK, Markham J, et al. Decreased [18F]spiperone binding in putamen in idiopathic focal dystonia. *J Neurosci*. 1997;17:843–850.
99. Misbahuddin A, Placzek MR, Chaudhuri KR, Wood NW, Bhatia KP, Warner TT. A polymorphism in the dopamine receptor DRD5 is associated with blepharospasm. *Neurology*. 2002;58:124–126.
100. Augood SJ, Martin DM, Ozelius LJ, Breakefield XO, Penney JB Jr, Standaert DG. Distribution of the mRNAs encoding torsinA and torsinB in the normal adult human brain. *Ann Neurol*. 1999;46:761–769.
101. Augood SJ, Hollingsworth Z, Albers DS, et al. Dopamine transmission in DYT1 dystonia: a biochemical and autoradiographical study. *Neurology*. 2002;59:445–458.
102. Furukawa Y, Hornykiewicz O, Fahn S, Kish SJ. Striatal dopamine in early-onset primary torsion dystonia with the DYT1 mutation. *Neurology*. 2000;54:1193–1195.
103. Shashidharan P, Sandu D, Potla U, et al. Transgenic mouse model of early-onset DYT1 dystonia. *Hum Mol Genet*. 2005;14:125–133.
104. Dang MT, Yokoi F, McNaught KS, et al. Generation and characterization of Dyt1 DeltaGAG knock-in mouse as a model for early-onset dystonia. *Exp Neurol*. 2005;196:452–463.
105. Grundmann K, Hubener J, Habig K, et al. Gene expression changes in a transgenic mouse model overexpressing human wild-type and mutant torsinA. *Proteomics Clin Appl*. 2008;2:720–736.
106. Zhao Y, Decuyper M, Ledoux MS. Abnormal motor function and dopamine neurotransmission in DYT1 DeltaGAG transgenic mice. *Exp Neurol*. 2008;210:719–730.
107. Page ME, Bao L, Andre P, et al. Cell-autonomous alteration of dopaminergic transmission by wild type and mutant (DeltaE) TorsinA in transgenic mice. *Neurobiol Dis*. 2010;39:318–326.
108. Balcioglu A, Kim M-O, Sharma N, Cha J-H, Breakefield XO, Standaert DG. Dopamine release is impaired in a mouse model of DYT1 dystonia. *J Neurochem*. 2007;102:783–788.
109. Hewett J, Johanson P, Sharma N, Standaert DG, Balcioglu A. Function of dopamine transporter is compromised in DYT1 transgenic animal model in vivo. *J Neurochem*. 2010;113:228–235.
110. Song CH, Fan X, Exeter CJ, Hess EJ, Jinnah HA. Functional analysis of Dopaminergic system in a DYT1 knock-in mouse model of dystonia. *Neurobiol Dis*. 2012;48:66–78.
111. Misbahuddin A, Placzek MR, Taanman JW, et al. Mutant torsinA, which causes early-onset primary torsion dystonia, is redistributed to membranous structures enriched in vesicular monoamine transporter in cultured human SH-SY5Y cells. *Mov Disord*. 2005;20:432–440.
112. Bao L, Patel JC, Walker RH, Shashidharan P, Rice ME. Dysregulation of striatal dopamine release in a mouse model of dystonia. *J Neurochem*. 2010;114:1781–1791.
113. Augood SJ, Hollingsworth Z, Albers DS, et al. Dopamine transmission in DYT1 dystonia: a biochemical and autoradiographical study. *Neurology*. 2002;59:445–458.
114. Granata A, Watson R, Collinson L, Schiavo G, Warner TT. The dystonia-associated protein torsinA modulates synaptic vesicle recycling. *J Biol Chem*. 2008;283:7568–7579.
115. Granata A, Koo SJ, Haucke V, et al. CSN complex controls the stability of selected synaptic proteins via a torsinA-dependent process. *EMBO J*. 2011;30:181–193.
116. Kakazu Y, Koh J-Y, Ho D, Gonzalez-Alegre P, Harata C. Synaptic vesicle recycling is enhanced by torsinA that harbours the DYT1 dystonia mutation. *Synapse*. 2012;66:453–464.
117. Kakazu Y, Koh J-Y, Iwabuchi S, Gonzalez-Alegre P, Harata C. Miniature release events of glutamate from hippocampal neurons are influenced by the dystonia-associated protein TorsinA. *Synapse*. 2012;66:807–822.
118. Pisani A, Martella G, Tschertner A, et al. Altered responses to dopaminergic D2 receptor activation and N-type calcium currents in striatal cholinergic interneurons in a mouse model of DYT1 dystonia. *Neurobiol Dis*. 2006;24:318–325.
119. Sciamanna G, Bonsi P, Tassone A, et al. Impaired striatal D2 receptor function leads to enhanced GABA transmission in a mouse model of DYT1 dystonia. *Neurobiol Dis*. 2009;34:133–145.
120. Napolitano F, Pasqualetti M, Uziel A, et al. Dopamine D2 receptor dysfunction is rescued by adenosine A2A receptor antagonism in a model of DYT1 dystonia. *Neurobiol Dis*. 2010;38:434–445.
121. Asanuma K, Carbon-Correll M, Eidelberg D. Neuroimaging in human dystonia. *J Med Invest*. 2005;52:272–279.
122. Carbon M, Niethammer M, Peng S, et al. Abnormal striatal and thalamic dopamine neurotransmission: genotype-related features of dystonia. *Neurology*. 2009;72:2097–2103.
123. Carbon M, Argyelan M, Eidelberg D. Functional imaging in hereditary dystonia. *Eur J Neurol*. 2010;(Suppl 1):58–64.
124. Quatarone A, Pisani A. Abnormal synaptic plasticity in dystonia: disruption of synaptic homeostasis. *Neurobiol Dis*. 2011;42:162–170.
125. Herve D, Le Moine C, Corvol JC, et al.  $G_{\alpha_{off}}$  levels are regulated by receptor usage and control dopamine and adenosine action in the striatum. *J Neurosci*. 2001;21:4390–4399.
126. Ashmore LJ, Hrizo SL, Paul SM, Van Voorhies WA, Beitel GJ, Palladino MJ. Novel mutations affecting the Na, K ATPase alpha model complex neurological diseases and implicate the sodium pump in increased longevity. *Hum Genet*. 2009;126:431–447.
127. Calderon DP, Fremont R, Kraenzlin F, Khodakhah K. The neural substrates of rapid-onset dystonia-parkinsonism. *Nat Neurosci*. 2011;14:357–365.
128. Kurian MA, Zhen J, Cheng S Y, et al. Homozygous loss-of-function mutations in the gene encoding the dopamine transporter are associated with infantile parkinsonism-dystonia. *J Clin Invest*. 2009;119:1595–1603.
129. Kurian MA, Li Y, Zhen J, et al. Clinical and molecular characterisation of hereditary dopamine transporter deficiency syndrome: an observational cohort and experimental study. *Lancet Neurol*. 2011;10:54–62.
130. Vasudevan A, Breakefield XO, Bhide PG. Developmental patterns of torsinA and torsinB expression. *Brain Res*. 2006;1073–1074:139–145.
131. Siegert S, Bahn C, Kramer ML, et al. TorsinA expression is detectable in human infants as young as 4 weeks old. *Brain Res Dev Brain Res*. 2005;757:19–26.
132. Zhao Y, Xiao J, Gong S, Clara JA, Ledoux MD. Neural expression of transcription factor THAP1 during development in rat. *Neuroscience*. 2013;231:282–295.