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Engineering animal models of dystonia

Janneth Oleas[1], **Fumiaki Yokoi**[1], **Mark P. DeAndrade**[1], **Antonio Pisani**[2], and **Yuqing Li**[1,*]
[1]Department of Neurology, College of Medicine, University of Florida, Gainesville, FL 32610, USA

[2]Department of Neuroscience, University Tor Vergata/Laboratory of Neurophysiology and Synaptic Plasticity, Fondazione Santa Lucia Istituto di Ricovero e Cura a Carattere Scientifico, 00143 Rome, Italy

Abstract

Dystonia is a neurological disorder characterized by abnormal involuntary movements that are prolonged and often cause twisting and turning. Several genetically modified worms, fruit flies, and rodents have been generated as models of genetic dystonias, and in particular DYT1, DYT11, and DYT12 dystonias. Although these models do not show overt dystonic symptoms, the rodent models exhibit pronounced motor deficits in specialized behavioral tasks, such as the rotarod and beam-walking tests. For example, in a rodent model of DYT12 dystonia, which is generally stress triggered, motor deficits are observed only after the animal is stressed. Moreover, in a rodent model of DYT1 dystonia, the motor and electrophysiological deficits can be rescued by trihexyphenidyl, a common anticholinergic medication used to treat dystonic symptoms in human patients. Biochemically, the DYT1 and DYT11 animal models also share some similarities to patients, such as a reduction in striatal D2 dopamine receptor and binding activities. Additionally, conditional knockout mouse models for DYT1 and DYT11 dystonia show that the loss of the causal dystonia related proteins in the striatum lead to motor deficits. Interestingly, loss of the DYT1 dystonia causal protein in Purkinje cells shows an improvement in motor performance, suggesting that gene therapy targeting of the cerebellum or intervention in its downstream pathways may be useful. Finally, recent studies using DYT1 dystonia worm and mouse models led to a potential novel therapeutic agent, which is currently undergoing clinical trials. These results indicate that genetic animal models are an extremely powerful tool to elucidate the pathophysiology and to further develop new therapeutics for dystonia.

1. Introduction

Dystonia is a neurological disorder characterized by sustained contractions of muscles, which cause abnormal movement, twisting, and postures.[1] Several methods of classifying dystonia exist. The earliest method of classifying dystonia was to segregate it as either primary or secondary.[2] In primary dystonia, the dystonic symptoms are not caused by another condition or environmental factor, and can be hereditary or nonhereditary. In secondary dystonia, the dystonic symptoms are usually the result of another condition (e.g. stroke, brain injury, or metabolic disease) or certain medications.[3] A more recent classification system has been developed that segregates dystonia based on clinical characteristics and etiology.[1] Current hypotheses suggest that dystonia is a neurodevelopmental disorder[4] that is caused by an abnormal motor circuitry in the cerebral cortex, basal ganglia, thalamus, and cerebellum, which in turn leads to abnormal synaptic

*Corresponding author – Telephone: +1-352-273-6546; Fax: +1-352-273-5989; yuqing.li@neurology.ufl.edu.
Author Roles
J.O., F.Y., M.P.D., A.P., and Y.L. wrote and edited this review article.

plasticity and altered neurotransmission[5-10], and thereby likely causes the dystonic symptoms.

The animal models of dystonia can be classified into two fundamental groups: phenotypic and genotypic. The primary goal of the phenotypic animal models is to mimic the dystonic phenotype seen in patients. One common method has been to inject pharmacological compounds into specific brain regions, such as the cerebellum or striatum, or to systemically attempt to reproduce an overt dystonic phenotype.[7,11, 12] The advantage of this approach is that it has the possibility of the potential identification of a brain region, cell receptor, or signaling pathway that may contribute to dystonia onset. These models are also particularly useful in analyzing secondary dystonia caused by brain injury or from the side effects of drugs.[13] However, one must consider that the primary goal of genotypic dystonia animal models is to mimic the genetic mutations found in patients, which can be accomplished by targeted mutagenesis or through insertional transgenic techniques. This type of model is particularly useful to analyze the pathophysiology of genetic dystonias by examining the normal function of the causal proteins and to analyze the mutated effects. The primary aim of this review is to highlight the findings of genotypic models of DYT1, DYT11 and DYT12 dystonias using worms (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*), and rodents, and to illustrate their utility in elucidating dystonia pathophysiology, and to uncover novel therapeutics.

2. DYT1 Dystonia

2.1 Background

DYT1 dystonia (OMIM 128100) is a primary, generalized, early-onset torsion dystonia.[14] Patients typically present with symptoms in childhood, but can present as late as 26 years of age.[15, 16] Typically, symptoms first present in the limbs and then over several years in many patients these symptoms become generalized.[17] DYT1 dystonia is an autosomal-dominant disorder with a reduced and incomplete penetrance of 30-40%. It is caused by a trinucleotide (GAG) deletion in the *DYT1/TOR1A* gene, which encodes the ubiquitously expressed torsinA protein.[18] This mutation removes one of a pair of glutamic acid residues from the C-terminal region of the torsinA protein. This mutation is commonly referred to as either *DYT1* ΔGAG or torsin$A^{\Delta E}$. TorsinA is a member of the AAA^{+} superfamily (ATPases associated with diverse cellular activities), and has been implicated in a variety of cellular processes, including chaperone-mediated protein folding and vesiclular trafficking.[19, 20]

2.2 Invertebrate models of DYT1 dystonia

Worms (*C. elegans*) and fruit flies (*D. melanogaster*) have a short generation time and a well-defined nervous system and these properties make them very good model organisms to study neurological disorders.[21-23] *C. elegans* have three torsin related genes: *tor-1, tor-2*, and *ooc-5*. All three genes encode ATPases that are putative proteins for the Clp/Hsp100 and AAA^{+} superfamilies.[24, 25] Caldwell and colleagues conducted *in vivo* assays in *C. elegans* to investigate the effect of these torsin proteins on polyglutamine-induced protein aggregation.[26] First, they observed that human torsinA expression, wild-type TOR-2 overexpression, or TOR-2 and OOC-5 co-expression these approaches were able to reduce protein aggregation in *C. elegans*.[26] However, expression of a mutated TOR-2 had lost its ability to reduce the protein aggregation (Table 1).[26] In another study, overexpression of human torsinA or TOR-2 in *C. elegans,* specifically in dopaminergic neurons, was able to protect dopaminergic neurons from 6-hydroxydopamine (6-OHDA) induced neuronal loss, possibly through down regulation of the dopamine transporter.[27] However, this protection was decreased when either mutant torsinA or mutant TOR-2 proteins were expressed.[27] Lastly, human torsinA was able to suppress endoplasmic reticulum (ER) stress response in

C. elegans, both at baseline and in response to the protein glycosylation inhibitor tunicamycin, which induces ER stress.[28] However, in the presence of mutant human torsinA, baseline ER stress was found to be increased.[28]

When human torsinA$^{\Delta E}$ was expressed either in the muscle or neurons of fruit flies, temperature dependent locomotion deficits were observed.[29] Additionally, protein aggregation of mutant torsinA was observed at normal temperature.[29] Fruit flies expressing an 18 bp deletion in human *DYT1/TOR1A*, a mutation reported in a family with early onset dystonia,[30, 31] showed similar motor deficits after exposure to 38°C. Additionally, abnormalities in synapses and larva neuromuscular junction were observed in these fruit flies.[31] Fruit flies only have a single torsin gene, dtorsin.[32] Down-regulation of *dtorsin* results in increased neuronal degeneration.[33] Furthermore, a null mutation of *dtorsin* will result in semi-lethality, sterility, locomotion deficits, and decreased dopamine levels in the brains of the larva and adult heterozygotes.[34]

These invertebrate studies have been integral in identifying the function of torsinA, such as a role chaperone-mediated protein folding, protection of dopaminergic neurons from neurotoxicity, and ER stress, and the effects of modulating expression or introducing mutations on behavior. These findings taken together support the continued use of invertebrate animal models to study DYT1 dystonia, and in particular the role of cellular functions.

2.3 Rodent Models of DYT1 dystonia

In mammals, there are four genes in the torsin family: torsinA, torsinB, torsin2A, and torsin3A. The human *DYT1/TOR1A* gene and rodent *Dyt1/Tor1a* genes, in particular mice and rats, are highly homologous, and therefore they are excellent model organisms.

2.3.1 Dyt1 ΔGAG knock-in, knockout, and knockdown mouse models of DYT1 dystonia

Multiple DYT1 genotypic dystonia models have been generated using rodents (Table 2). The most significant model is the *Dyt1 ΔGAG* heterozygous knock-in (KI) line of mice, as this line of mice recapitulates the trinucleotide deletion in *Dyt1/Tor1a that is* most often seen in DYT1 dystonia patients. Similar to humans, this deletion results in a loss of one of a pair of glutamic acid residues in torsinA.[35, 36]

The *Dyt1* KI mouse exhibited motor deficits and abnormal gait, which while not overt dystonia, is thought to represent a dystonia-like phenotype.[35] *Dyt1* KI mice also displayed a subtle anxiety-like behavior and enhancement of cued fear memory.[37] These results were similar to *DYT1* dystonia mutation carriers, who exhibited increased anxiety, verbal memory retroactive interference, and higher semantic fluency performance.[38]

Additionally, *Dyt1* KI mice showed reduced release of striatal dopamine at baseline and after amphetamine stimulation.[39] Furthermore, a reduction in striatal D2 dopamine receptor (D2R) binding was observed in the *Dyt1* KI mice,[40] which was consistent with post-mortem and *in vivo* PET imaging studies that revealed a reduction in D2R binding in human DYT1 dystonia mutation carriers.[41]

Lastly, *Dyt1* KI mice exhibited cerebellothalamocortical (CbTC) pathway abnormalities using PET and DTI imaging techniques.[42] This was similar to the white-matter alterations in the sensorimotor cortex[43] and the superior cerebellar peduncle[44] in DYT1 dystonia and primary dystonia patients, which suggest alterations in CbTC tract integrity.[44, 45] Furthermore, the KI mice have shown alterations in corticostriatal long-term depression (LTD).[40] Neurotransmission deficits were also reported in cell culture of hippocampal neurons from *Dyt1* KI mice.[46, 47]

Dyt1 KI mice had reduced torsinA protein levels in the brain,[36, 48] which was consistent with the accelerated degradation of mutant torsinA found in cultured cells.[49, 50] Therefore, to determine whether a loss-of-function mutation of torsinA leads to DYT1 dystonia, *Dyt1* knockdown (KD) mice were developed. These mice had approximately 36% reduction of torsinA protein, and also *Dyt1* knockout mice were similarly developed. Similar to *Dyt1* KI mice, Dyt1 KD mice displayed motor deficits, increased locomotor activity, and alterations of striatal dopamine metabolism[51]. In contrast, *Dyt1* homozygous knockout (KO),[36, 52] *Dyt1* homozygous KI,[35, 36] and KO/KI double mutant mice[53] resulted in neonatal lethality, suggesting that a gross loss of torsinA function impaired normal development in mice. These findings are to date, consistent with no report of human carriers with mutations in both *DYT1* alleles.

2.3.2 Brain-region specific Dyt1 conditional knockout mice

Brain-region specific *Dyt1* conditional knockout mice have been used to understand how specific brain region or cell types contribute to the pathophysiology of the disease (Table 2). When the phenotypes of conditional knockout mice match with those of *Dyt1* KI mice, it is proposed that the corresponding regions or cells have relevance to the pathophysiology. The cerebral cortex-specific *Dyt1* conditional knockout (*Dyt1* cKO) mice, were generated by crossing *Emx1-cre*[54] and *Dyt1 loxP* mice, and exhibited motor deficits and hyperactivity.[52] The striatum-specific *Dyt1* conditional knockout (*Dyt1* sKO) mice, generated by crossing *Rgs9L-cre*[55] and *Dyt1 loxP* mice, exhibited motor deficits and reduced striatal D2R binding activity.[56] However, both cKO[52] and sKO[56] mice showed no significant alteration in striatal monoamine levels. Next, cholinergic neuron-specific *Dyt1* conditional knockout mice, generated by crossing *ChAT-cre*[57] and *Dyt1 loxP* mice, showed motor deficits. Furthermore, striatal cholinergic interneurons in these mice showed alterations in response to muscarinic receptor activation and D2R receptor activation, but no change in response to either $GABA_A$ or metabotropic glutamate receptor activation.[58] The cerebral cortex and striatum, along with cholinergic innervation seemed to be important components of the basal ganglia circuitry. These studies suggested that loss of torsinA function in these areas resulted in motor deficits and these findings may prove important to better understanding the pathogenesis of dystonia.

Another aspect of this puzzle that has yet to be investigated is using conditional knockout techniques to show the contribution of the direct and indirect pathways of the basal ganglia. Striatal medium spiny neurons (MSNs) expressing D1 dopamine receptor mediate the direct pathway, which is thought to be involved in the initiation of movement, while striatal MSNs expressing D2 dopamine receptor mediate the indirect pathway, and this may possibly prevent unwanted movements. Therefore, the motor deficits in DYT1 dystonia may be mediated, at least in part, through changes in D2R function in the basal ganglia circuit. The relative contributions of presynaptic D2R on cholinergic interneurons and postsynaptic D2R on medium spiny neurons to the pathophysiology of DYT1 dystonia remain to be determined.

Several studies have suggested the role of the cerebellum in the pathophysiology of dystonia. For instance, cerebellectomies of either dystonic (*dt*) rats or tottering mutant mice were able to improve their dystonic-like symptoms.[7, 59] Moreover, crossing the tottering mutant mice with *pcd* mutant mice, which have Purkinje cell-specific degeneration, were similarly able to improve in their dystonic-like symptoms.[60] Therefore, Purkinje cell-specific *Dyt1* conditional knockout (*Dyt1* pKO) mice were generated by crossing *Pcp2-cre*[61] with *Dyt1 loxP* mice. These mice had alterations in Purkinje cell dendritic morphology and showed an improvement in motor performance compared to wild-type mice.[6253] Since Purkinje cells integrate cerebellar signals and the cerebellum modulates the basal ganglia

circuits,[63] loss of torsinA function in Purkinje cells may balance the abnormal basal ganglia circuits and attenuate the dystonic symptoms.

2.3.3 Transgenic rodent models of DYT1 dystonia

A line of transgenic mice overexpressing human torsinA$^{\Delta E}$ driven by the neuron-specific enolase promoter displayed self-clasping of hind limbs, hyperkinesia, altered circling behavior, abnormal gait, brain stem pathology, and disrupted striatal dopamine levels.[64, 65] A second line of transgenic mice were generated using the human cytomegalovirus (hCMV) immediate early promoter in order to drive the overexpression of human torsinA$^{\Delta E}$, and these have been referred to as hMT mice. The hMT mice exhibited motor learning deficits,[66] motor deficits,[67] and abnormal gait.[67] Furthermore, there was an increase in dopamine turnover,[67] altered dopamine release, decreased basal locomotion induced by amphetamine,[68] and decreased dopamine transporter function.[69]

The hMT mice have been extensively studied using electrophysiological techniques. The hMT mice exhibited defected LTD, an enhanced long-term potentiation (LTP), and a deficit of synaptic depotentiation (SD).[70] A shortened pause response by thalamic stimulation in cholinergic interneurons was also found, due to an altered D2R function affecting the synaptic convergence between thalamostriatal and corticostriatal responses.[71] Furthermore, the hMT mice exhibited an excitatory, instead of inhibitory, striatal cholinergic interneuron response after dopamine D2R activation,[72, 73] and also had a decreased D2R receptor level.[74] Additionally, electrophysiological studies revealed that there was a critical alteration of striatal dopamine D2R-mediated function both in cholinergic interneurons as well as in the control of GABAergic synaptic transmission in MSNs of the hMT mice.[72, 75]These observations are compatible with current theories on the pathogenesis of dystonia, suggesting that both an increased propensity to “potentiation” (LTP-like) and a failure of “depression” mechanisms (LTD- and SD-like) lead to a “loss of inhibition” in the motor system which might, at least in part, explain the pathogenesis of the excess of abnormal movements observed in dystonic patients, though this remains speculative.[9]

A third line of transgenic mice overexpressing human torsinA$^{\Delta E}$ in dopaminergic neurons of the midbrain resulted in motor deficits and altered dopamine release in response to cocaine. These findings suggested that disruption of torsinA activity by overexpressing the mutant torsinA in dopaminergic neurons affected dopamine transmission. [76]

A fourth line of transgenic mice was generated using a murine prion protein promoter, and this line of mice revealed that overexpressing wild-type or torsinA$^{\Delta E}$ caused inclusion bodies predominantly in the brainstem, nuclear envelope abnormalities, altered monoamine levels, and motor deficits.[77] However, both the wild-type and torsinA$^{\Delta E}$ were expressed as fusion proteins with a carboxyl terminal attachment of a V5-His tag. The functional consequences of these fusion proteins have not been well characterized. Realizing the deficiency of the approach, the same research group generated transgenic rats expressing human torsinA$^{\Delta E}$ from the human torsinA promoter. The mutant rats showed altered synaptic plasticity, motor deficits, and nuclear membrane alterations.[78]

Various transgenic rodent models overexpressing wild-type torsinA or mutant torsinA$^{\Delta E}$ using different promoters have been produced (Table 3). Most of the transgenic rodent models showed an impairment of motor behavior. However, investigators should be cautious when interpreting these results because the observed behavioral and cellular abnormalities could have been a result of non-physiological and ectopic protein expression. Furthermore, it was difficult to generate proper control animals for these transgenic mice due to differences in the transgene insertion site, copy number, expression level, and pattern of expression.

3. DYT11 dystonia

3.1 Background

DYT11 dystonia (OMIM 159900) is the major subtype of myoclonus-dystonia (M-D), and is characterized by myoclonic jerks with dystonic symptoms.[79, 80] Additionally, it is often accompanied by psychiatric symptoms, such as depression and anxiety disorders. DYT11 dystonia generally presents in childhood, but in some individuals can present in late adulthood.[81] It is caused by mutations in *SGCE*, which encode the transmembrane glycoprotein ε-sarcoglycan.[79] *SGCE* is maternally imprinted and paternally expressed.[82] Furthermore, it has been demonstrated that there is exclusive paternal expression of ε-sarcoglycan in the brains of mice[83] and humans.[84]

3.2 Rodent models of DYT11 dystonia

Epsilon-sarcoglycan was first identified in mice as a homolog of α-sarcoglycan.[85, 86] Two lines of *Sgce* knockout mice have been reported. The first lacks exon 4, which results in a frame-shift mutation and has exhibited significant relevant phenotypes.[83, 87] Another mouse line was generated and it lackedexons 6 through 9, which did not result in a frame-shift mutation, and did not exhibit an overt phenotype.[88] We will focus on the findings from the first line of mice.

3.2.1 Sgce heterozygous KO mice

Sgce heterozygous knockout (KO) mice lacking exon 4 were generated by crossing *Sgce loxP*[83] with *CMV-cre*[89] mice (Table 2). Paternally-inherited *Sgce* KO mice showed myoclonus and deficits in fine motor coordination and balance, motor learning in the beam-walking test, and anxiety and depression-like behaviors.[87] The motor learning deficit was recently demonstrated in DYT11 dystonia patients with impaired saccadic adaptation,[90] and this was thought to be a form of cerebellar motor learning.[91] *Sgce* KO mice also exhibited abnormal nuclear envelopes in striatal neurons and cerebellar Purkinje cells, suggesting that DYT11 dystonia belongs to a growing family of nuclear envelopathies.[92-94] Additionally, loss of ε-sarcoglycan did not cause a reduction of other sarcoglycan isoforms (α, δ, and ζ), suggesting that ε-sarcoglycan did not make a sarcoglycan complex similar to other sarcoglycans.[93] Furthermore, the levels of striatal dopamine and its metabolites in *Sgce* KO mice were significantly increased.[87] The hyperdopaminergic state seemed to be potentially further strengthened by evidence that DYT11 dystonia patients had a reduction in striatal D2R binding.[95] Similarly, *Sgce* KO mice exhibited reduced striatal D2R protein levels and an increased dopamine release after amphetamine administration.[96] In conclusion, these studies suggested that DYT11 dystonia had functional alterations of the monoamine system in the striatum, and that the *Sgce* KO mice reasonably modeled DYT11 dystonia.

3.2.2 Brain-region specific Dyt1 conditional knockout mice

Brain region-specific *Sgce* knockout models have been generated to dissect brain circuits involved in the pathogenesis of DYT11 dystonia. Paternally-inherited striatum-specific *Sgce* conditional knockout (*Sgce* sKO) mice exhibited motor deficits, but no myoclonus and also had normal nuclear envelopes in the striatum.[93] Paternally-inherited cerebellar Purkinje cell-specific *Sgce* conditional knockout (*Sgce* pKO) mice showed motor learning deficits, but no myoclonus and a normal nuclear envelope in the cerebellar Purkinje cells.[94] The results suggested that loss of ε-sarcoglycan function in the striatum and in the cerebellar Purkinje cells contributed to motor deficits and motor learning deficits observed in the complete *Sgce* KO mice.[87] In contrast, loss of ε-sarcoglycan function in these regions alone did not contribute to myoclonus or nuclear envelope abnormalities, suggesting that other brain regions or a combination of these brain regions may contribute to these phenotypes.

4. DYT12 dystonia

4.1 Background

DYT12 dystonia (OMIM 128235), or rapid-onset dystonia-parkinsonism (RDP), is characterized by symptoms of both dystonia and parkinsonism, which include resting tremor, akinesia, bradykinesia, and postural instability.[97] In DYT12 dystonia, the human patients' symptoms can appear within minutes to a few days, and do not remit.[97-101] The symptoms can be triggered by a physiological stressor, such as a high fever or pregnancy.[98, 102] DYT12 dystonia is caused by missense mutations in the *ATP1A3* gene, which encodes the Na^+, K^+-ATPase α3 isoform. The α3 isoform is only expressed in neurons and cardiac cells.[97, 103] It is hypothesized that the loss of the α3 isoform during stress may interfere with the normal response to physiological stressors. These responses have been referred to as stress induced channelopathies, and this type of finding has been observed in other diseases, such as myasthenia gravis and chronic fatigue syndrome.[8, 104]

4.2 Rodent model of DYT12 dystonia

Na^+, K^+-ATPase α3 isoform-deficient mice were generated as a DYT12 dystonia model from gene targeting that resulted in aberrant splicing of the gene.[105] The heterozygous *Atp1a3* KO mice exhibited an increase in locomotor activity at baseline and in response to methamphetamine in the open-field test. Additionally, the heterozygous *Atp1a3* KO mice showed impaired learning in the Morris water maze and decreased hippocampal NR1 subunit of NMDA receptor. More importantly, the heterozygous *Atp1a3* KO mice had no motor deficits prior to stress induction, but following an immobilized stress protocol, female heterozygous *Atp1a3* KO mice exhibited motor deficits in the rotarod and beam-walking tests.[106] The stressed *Atp1a3* KO mice also exhibited alterations in their sensory response to warm stimuli, circling behavior, and the monoamine neurotransmitter system. Moreover, in a separate study stress increased the susceptibility to depression-like phenotypes in *Atp1a3* KO mice.[107]

5. Dystonia animal models and preclinical drug discovery

Currently there is no known cure for dystonia. However, physical therapy, medications, and surgery aim to lessen symptoms. Positive symptomatic outcomes have resulted from botulinum toxin treatment for focal dystonia,[108] medications such as the anticholinergic trihexyphenidyl, and from the use of gamma-aminobutyric acid (GABA) derivatives for primary dystonia.[109] Deep brain stimulation for generalized and cervical dystonia have also been shown effective in select cases.[109] However, not all patients respond favorably to these treatments likely as a result of the heterogeneity of dystonia, and the differences in underlying pathophysiology. Therefore, there is tremendous need for new and more effective treatments for dystonia.

Although none of the genetic dystonia rodent models exhibit overt dystonic symptoms, the beam-walking and rotarod tests have successfully detected motor deficits in a majority of genotypic rodent models.35, 39, 48, 51, 56, 58, 66, 67, 76-78, 87, 93, 94, 106 Two particular cases worth noting are the DYT12 and DYT1 dystonia mouse models. In DYT12 dystonia patients, symptoms are often triggered by a stressor, resulting in a rapid progression of dystonic symptoms and parkinsonism. In the mouse model of DYT12 dystonia, there are no beam-walking deficits but when a stress protocol is used, the deficits emerge.[106] Furthermore, the beam-walking deficits in *Dyt1* KI mice, could be rescued using the anticholinergic trihexyphenidyl, a common human dystonia medication.[40] We believe these two studies provide some preliminary evidence that the beam-walking test may be an indirect test for dystonia in genotypic rodent models though this will need further validation.

We posit that potential therapeutics aimed at treating genetic dystonias could possibly rescue beam-walking deficits observed in respective genotypic models. For example, an *in vivo* screen using transgenic *C. elegans* resulted in the identification of the antibiotic ampicillin as being able to enhance wild-type torsinA activity and rescuing beam-walking deficits and torsinA protein levels in the *Dyt1* KI mice.[110] The effect of ampicillin on DYT1 dystonia patients is now currently being investigated in a clinical trial (NCT01433757). In another case, loss of the torsinA protein in the Purkinje cells of the cerebellum (*Dyt1* pKO) in combination with the global *Dyt1* ΔGAG mutation (*Dyt1* KI) in mice exhibited lower numbers of slips compared to *Dyt1* KI mice on the beam-walking test.[53] This finding indicated the possibility that the molecular lesions of torsinA in cerebellar Purkinje cells by gene therapy or intervention in the signaling pathway downstream of the cerebellar Purkinje cells may rescue motor symptoms in DYT1 dystonia patients. Additionally, further refinement of drugs targeting the corticostriatal pathway and its modulatory pathways will hopefully provide new treatments for DYT1 dystonia and other related dystonias. It is likely that other candidate tests like beam-walking will emerge as we better understand the animal models of dystonia.

6. Improving existing models for dystonia and future directions

One approach for the development of a better animal model would be gene targeting mediated by zinc finger nucleases (ZFNs), which are engineered proteins that bind to DNA at specific sites and produce double strand breaks in DNA.[111] The advantage of ZFNs lies in targeting efficiency and the possibility of creating gene modifications from different organisms,[111] omitting the need for producing embryonic stem cell based chimeric animal models.[112] Since ZFN technology can be applicable to various organisms,[113, 114] it can be useful to generate novel *Dyt1* KI or *Dyt1* knockout rats. Rats have larger bodies and brain sizes compared to mice, and this may facilitate easier *in vivo* electrophysiological, behavioral, and imaging studies.

Additionally, the engineered rodent dystonia models developed to date suffer from the lack of overt motor deficits. Future efforts should focus on developing models with early onset and pronounced symptoms. The process of generating such models will also provide insights into novel factors that contribute to onset and severity of symptoms. Recent studies have shown that *Dyt1* homozygous KO mice lethality varied from 12 hours to 3 weeks depending on the genetic background.[115] Therefore, it is possible that motor symptoms might also be influenced by genetic background. Additionally, it has been shown that additional gene mutations can affect the temporal onset of motor deficits, such as is the case of mutant mice harboring both the *Dyt1* KI and *Sgce* KO mutations.[48] Finally, stress induced motor deficits in the DYT12 dystonia mouse model suggest that environmental factors could also influence the onset and severity of the phenotype. Combinations of these approaches will hopefully lead to better mammalian models and facilitate the development of novel therapeutics.

7. Conclusion

Although all engineered rodent models have not revealed overt dystonic symptoms, motor deficits in these models may correspond to dystonic symptoms in humans. In addition to behavioral similarities between genotypic models of dystonia and patients, biochemical similarities also exist. For example, reductions in striatal D2R and/or its binding activities are common findings in both DYT1 and DYT11 dystonia mouse models. Analysis of these rodent models also demonstrates the pathophysiological changes in the corticostriatal, thalamostriatal, and cerebellothalamocortical pathways and understanding these changes will be critical to therapy development. It is our hope that further investigation of these pathways will lead to novel therapeutics to treat dystonia. Using a combination of

invertebrate and rodent models, ampicillin, a common antibiotic, was shown to rescue motor deficits in animals and is a candidate for human trials. Moreover, *Dyt1* pKO mice indicated that molecular lesions of torsinA in cerebellar Purkinje cells placed by gene therapy or alternatively intervening in the signaling pathways downstream of cerebellar Purkinje cells may both be able to rescue motor symptoms. These results indicate that the genetic animal models developed can be useful to further study the pathophysiology of dystonia and to attempt to develop novel therapeutics.

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Table 1

Invertebrate models of DYT1 dystonia

Model	Important findings
C. elegans containing fluorescent polyglutamine-induced protein aggregates.	↓ Polyglutamine-induced protein aggregation when overexpressing human torsinA, TOR-2, and co-expressing of TOR-2 and OOC-5. TOR-2 (Δ368) mutant (DYT1 associated mutation) was not able to reduce protein aggregation.[26]
C. elegans (dopamine-specific neuronal promoter)	↑ Resistance of DA neurons to 6-OHDA by overexpression of human torsinA or worm TOR-2. ↓ Resistance of DA neurons to 6-OHDA by expression of torsinA$^{\Delta E}$, mutant TOR-2 proteins, or by co-expression of wild-type torsinA and torsinA$^{\Delta E}$[27]
C. elegans (ges-1 intestinal promoter)	↓ ER stress response at basal and when stress was induced by tunicamycin with wild type torsinA expression. ↑ ER stress response at basal conditions with torsinA$^{\Delta E}$ expression, and by co-expression of wild-type torsinA and torsinA$^{\Delta E}$, but no significant change in response to tunicamycin.[28]
D. melanogaster Gal4/UAS system for overexpression of torsinA$^{\Delta E}$ in the nervous system and muscle	Protein aggregation by torsinA$^{\Delta E}$. Impaired locomotion after exposure to 38°C. Ultrastructure alterations at the neuromuscular junction in larvae.[29]
D. melanogaster Gal4/UAS system for overexpression or down-regulation of dtorsin	Downregulation or overexpression of dtorsin caused subtle rough eye phenotypes (neurotoxicity) and abnormal eye pigment granule configuration in young flies. Downregulation of *dtorsin* caused retinal degeneration in aged flies. Overexpression of *dtorsin* caused protection from neural degeneration. *dtorsin* localizes to nuclear envelope and ER.[33]
D. melanogaster Gal4/UAS system for overexpression of torsinA$^{\Delta E}$, and ΔFY HtorA in neurons or muscles	Protein aggregation in neurons and muscles by torsinA$^{\Delta E}$, but absent in ΔFY HtorA mutants. Impaired locomotion after exposure to 38°C in flies expressing torsinA$^{\Delta E}$, and ΔFY HtorA. Ultrastructure alterations at the neuromuscular junction in larvae, and synapses.[31]
D. melanogaster null mutant for *dtorsin*	Prepulpal semi-lethality, sterility and altered locomotion behavior ↓ Dopamine levels in brain. ↓GTP cyclohydrolase protein and activity[34]

Abbreviations: 6-OHDA (6-hydroxydopamine), ER (endoplasmic reticulum), GTP (Guanosine-5′-triphosphate), TOR-2(Δ368) (lacking a codon for amino acid 368), torsinA$^{\Delta E}$ (human mutant torsinA with a GAG deletion), ΔFY HtorA (human mutant torsinA with an 18 bp deletion).

TABLE 2

ES cell based dystonia models

	Mouse	Microdialysis	Monoamines	Locomotion	Rotarod	Beam Walking	Gait Analysis	Neuronal Phenotype	Additional findings
dYT1 Rodent Models	***Dyt1* AGAG KI**	↓ DA at baseline and by AMPH in striatum(~2.3 months male/ female)[39]	↓ HVA in striatum (11 months-male)[35] ↑ HVA in striatum (10 months)[39] ↓ DA in midbrain (6 and 10 months male/female)[39]	↑ Activity (6 months-male)[35] ↑ Daytime motility (3 and 6 months male/female)[39]	Not affected[35] (6months male/female) ↓ Motor skills (3,6,10 months male/female)[39]	↑ Slips (6months- male)[35] ↑ Transversal time and slips (3 months) (male/female)[39]	↑ Paw overlap distance (6months-male)[35] Affected gait (male/female)[39]	Abnormal nuclear envelope in CNS (*Dyt1* ΔGAG homozygous KI and KO embryonic day 15,18)[36] Abnormal nuclear envelope of cerebral cortical neurons (*Dyt1* ΔGAG homozygous KI-newborn)[56] Inclusions for ubiquitin and torsinA in brain stem (6months-male)[35] Enlarged and reduced TH positive cells in substantia nigra (3-6 months male/ female)[39] Shorter primary dendrites (8 months)and reduced spine numbers in Purkinje cells (3-8months)[62]	Microstructural abnormalities in cerebellothalamocortical pathway (4 months)42 ↓ Corticostriatal LTD (3-4 weeks-male)[40] ↓ D2R binding[40] ↓ D2R in striatum (6 months-male)[40] ↑ Synaptic vesicle recycling[46] ↑ Glutamate mEPSC frequency[47] (hippocampus cultured neurons from heterozygous and homozygous *Dyt1* ΔGAG KI mice postnatal Days 0–1) ↓ torsinA in brain[53] ↓ torsinA in striatum[48]
	***Dyt1* KD mice**	NA	↓ DOPAC in striatum (8–9 months-male)[51]	↑ Horizontal activity (6 months-male)[51]	Not affected (6 months-male/female)[51]	↑ Slips (6 months-male)[51]	↓ Hind base (male)52	NA	
	Purkinje cell-specific *Dyt1* conditional KO	NA	NA	NA	Not affected (~5.2-7.7months-male/female)[53]	↓ Slips (~5.6-8 months-male/female)[53] No difference in slip numbers between CT and *Dyt1* ΔGAG KI/Dyt1 pKO double mutant mice (~5.3-5.7months-male/female)[53]	Normal gait (~5.4-7.8 months-male/female)[53]	Shorter primary dendrites (8 months)and reduced spine numbers (3-8 months) in Purkinje cells[62] Normal nuclear envelope in cerebellar cells ~2-4 months male/female)[53]	

	Mouse	Microdialysis	Monoamines	Locomotion	Rotarod	Beam Walking	Gait Analysis	Neuronal Phenotype	Additional findings
DYT1 dystonia models	**Cortex-specific *Dyt1* conditional KO**	NA	Not affected in striatum (~8.7-11.3 months-male/ female)52	↑ Activity (~3.8-6.5 months male/ female)52	Not affected (~3.8- 6.5 months male/female)52	↑ Slips (~3.8-6.3 months male/female)52	↓ Hind base (~5.4- 8 months male)52	Normal nuclear envelope in cerebral cortical neurons (8 weeks)[56]	
	Striatum-specific *Dyt1* conditional KO	NA	Not affected in striatum (~10-11.3 months- male/ female)[56]	Not affected (~4-5.5 months-male/female)[56]	Not affected (~ 3.4- 4.8 months-male/female)[56]	↑ Slips (~4.6-6 months-male/female)[56]	NA	Normal nuclear envelope in striatal medium spiny neurons (6 weeks)[56]	↓ D2R binding in striatum (~8.8-10.5 months)[56]
	Cholinergic-specific *Dyt1* conditional KO mice	Normal DA at baseline and by AMPH in striatum (7 months)[58]	NA	Not affected (3-8 months-male/female)[58]	↓ Latency to fall (8-11 months-male/female)[58]	Not affected (8-11 months-male/female)[58]	NA	NA	Normal striatal acetylcholine content, uptake and release abnormal D2R response and altered muscarinic receptor function (8-10 weeks)[58]
	Sgce KO mice	Normal DA at baseline ↑ DA release by AMPH in striatum (5 months)[96]	↑ DA, DOPAC, HVA in striatum (~11-11.7 months-male/ female)[87]	↑ Horizontal activity (female) ↑ Vertical movement number, movement time, activity (male/ female)[87]	Not affected[87]	↑ Slips, impaired motor learning (~6.5-7.5 months male/female)[87]	Not affected[87]	Abnormal nuclear envelope in striatum (2-5.5 months)[93] Abnormal nuclear envelopes in cerebellar Purkinje cells (2-4 months)[94]	Myoclonus (~7.2-8 months male/female)[87] Anxiety-like behavior (~7.3-8.2 months male)[87] Depression-like behaviors (~7.8-8.6 months female)[87] ↓ D2R,normal D1R and DAT in striatum (5 months)[96]
	Striatum-specific Sgce conditional KO mice	NA	NA	Not affected (~5.2-8 months male/female)[93]	↓ Latency to fall in trials 3 and 5 ↓ Total latency to fall (~5.7-8.3 months male/female)[93]	↑ Slips (~5.5-8 months-male/female)[93]	NA	Normal nuclear envelope in striatum[93]	No myoclonus(~6.3-9 months-male/female).[93]
	Purkinje cell-specific Sgce conditional KO mice	NA	NA	↓Stereotypic activity, movement numbers, movement time (~6.2-7.5 months-male/female)[94]	↓ Latency to fall on 4th trial (~7-8 months male/female)[94]	Impaired motor learning (~6.6-8 months-male/female)[94]	NA	Normal nuclear envelope in cerebellar Purkinje cells[94]	No significant myoclonus (~8.7-10 months-male/female)[94]
DYT1 and DYT11 Dystonia model	***Dyt1* and Sgce double mutant mice**	NA	NA	↓ Horizontal movement numbers compared to	NA	↑ Slips (5.5 months-male/female)[48]	NA	Normal nuclear envelope in cortical neurons(5.5-	↓ torsinA in striatum (5.5 months male/female)[48]

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	Mouse	Microdialysis	Monoamines	Locomotion	Rotarod	Beam Walking	Gait Analysis	Neuronal Phenotype	Additional findings
				*Dyt1*ΔGAG KI (~4.4-5.3 months-male/ female)[48]				months-male/ female)[48]	
DYT12 Dystonia model	**Heterozygous *Atp1a3* KO mice**	NA	Not affected, but individual neurotransmitter levels correlated to open field activity levels showed that stressed mice had changes in the dopaminergic and serotonergic systems[106]	↓ Clockwise (CW) circling (stressed 6months male-female) Voluntary activity levels by wheel running: one group hyperactive, the other group hypoactive (6months-stressed female)[106]	↓ Latency to fall (stressed 6 months-female)[106]	↑ Slips (stressed 6 months-female)[106]	NA	NA	↓ Sensitivity to warm stimuli (stressed female)[106]

Abbreviations: AMPH (amphetamine), CNS (central nervous system), CT(control littermate), DA (dopamine), DAT (DA transporter), DOPAC (3, 4-dihydroxyphenylacetic acid), *Dyt1*ΔGAG KI (heterozygous mutant *Dyt1* gene with a GAG deletion), D1R D2R (dopamine receptor type 1 and 2), HVA (homovanillic acid), KD (knockdown), KI (knock-in), KO (knockout), LTD (long term depression), NA (Not analyzed),TH (tyrosine hydroxylase).

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Table 3

Transgenic DYT1 dystonia rodent models

Promoter	Microdialysis	Monoamines	Locomotion	Rotarod	Beam Walking	Gait Analysis	Neuronal phenotype	Additional findings
Rat neuron-specific enolase	NA	↓ DA in striatum (mice with affected phenotype) 64 ↓ DOPAC/DA in striatum (mice with unaffected and affected phenotype) (1.5months)64	Self-clasping, hyperkinesia, circling (mice with affected phenotype (1.5-months)64 ↓ Rearing (3 months)65	Not affected (3,6,9,12 months)65	NA	↓ Forelimb and hind limb stride length (9 months)65	Perinuclear inclusions for ubiquitin and TorsinA in PPN and PAG64	Transgene expression lost after repeated breeding65
Human cytomegalovirus (CMV)	Not affected DA at baseline in striatum68 ↓ DA by AMPH in striatum (6months-male)68 ↓ DAT activity in striatum (6-months-male)69	Not affectedin striatum (6 months-male)68 ↑ DOPAC/DA and HVA/DA in striatum (3-4months-male)67	Not affected (9months-male)66 ↓ Basal locomotion activity and by AMPH (6months-male)69	↓ Motor learning (9 months-male)66 Not affected (3-4 months male)67	↑ Transversal time and slips (3-4months-male)67	↑ Hind base width (3-4 months-male)67	No torsinA or ubiquitin inclusions in striatum (male)66 No torsinA or ubiquitin inclusions No bleb formation in nuclear envelope (male)67	Abnormal response to D2 dopamine receptor stimulation of Chls (9 months)72 Normal DAT, VMAT2, striatal DA receptors density (6 months-female)68 Impaired LTD and SD in medium spiny neurons70 ↑ Striatal acetylcholinesterase activity (~2months)70 Altered GABAergic function in striatum (~2 months)75 ↓ D2R, ↓ RGS9 in striatum (~2 months)74 ↓Pause response by thalamic stimulation in Chls (male)71
Murine prion	NA	htorsinA$^{\Delta E}$ mice: ↑ DOPAC, 5-HT, and 5-HIAA in brain stem htorsinA mice: ↓DA, 5-HT and 5-HIAA in striatum (5 months- male/female)77	htorsinA$^{\Delta E}$ mice: ↑ Activity (6months-male/female)77 htorsinA mice: ↓ Activity	htorsinA$^{\Delta E}$: ↓ Latency to fall, ↓ Learning (6months-ale/female)77 htorsinA mice: Not affected	htorsinA$^{\Delta E}$: Not affected (6months-male/female)77 htorsinA mice: ↑ Transversal time	htorsinA$^{\Delta E}$: Not affected (6months-male/female)77 htorsinA mice: ↓ Mean stride length for forelimbs and hind limbs	htorsinA$^{\Delta E}$ and htorsinA: torsinA or ubiquitin inclusions mainly in brainstem, nuclear envelope disruption in brainstem and striatum (1.5months male)77	Altered motor circuits integrity by diffusion tensor imaging in cortex, striatum, cerebellum (5months -male)77

Promoter	Microdialysis	Monoamines	Locomotion	Rotarod	Beam Walking	Gait Analysis	Neuronal phenotype	Additional findings
Tor1A promoter (rat model)	NA	NA	NA	↓ Latency to fall at training sessions (2 months - male)78	↑ Transversal time 3,5 cm round beam (2months-male)78	↑ Variation of step length (hind limb) (11months male-)78	Abnormal protein localization of human torsinA$^{\Delta E}$ to nuclear envelope (cortex, hippocampus, striatum, olfactory bulb, substantia nigra)(2 months-male)78 Altered nuclear envelope structure in cortex and striatum (3 months- male)78	Limb grasping and clasping (11 months-male) Impaired LTD and SD in medium spiny neurons (8-12 weeks old-male)78
Human TH promoter fragment (transgene expression in dopaminergic neurons of midbrain)	TH-htorsinA$^{\Delta E}$: Not affected DA at baseline and ↓ DA by cocaine in striatum (male) TH-htorsinA mice: ↑ DA release by cocaine in striatum76	TH-htorsinA$^{\Delta E}$mice: Normal DA and DOPAC in striatum TH-hTorsinA mice: ↓ DA and ↓ DOPAC in striatum (2.5 months-female)76	TH- htorsinA$^{\Delta E}$ mice: Normal baseline locomotion TH-htorsinA mice: ↓ Baseline locomotion (4-10months male)76	Not affected (2,4,6 months male/female)76	TH- htorsinA$^{\Delta E}$ mice: ↑ Transversal time and slips male/female)76	NA	NA	

Abbreviations: 5-HIAA (5-hydroxyindoleacetic acid), 5-HT (5-hydroxytryptophan), AMPH (amphetamine), Chls (cholinergic interneurons), DA (dopamine), DAT (DA transporter), DOPAC (3, 4-dihydroxyphenylacetic acid), D2R (dopamine receptor type 2), HVA (homovanillic acid), htorsinA$^{\Delta E}$ (human wild-type torsinA), htorsinAAE (human mutant torsinA with a GAG deletion), LTD (long-term depression, NA (Not analyzed), PPN (pedunculopontine nucleus), PAG (periaqueductal gray), RGS9 (Regulator of G-protein signaling 9), SD (synaptic depotentiation),TH (tyrosine hydroxylase), VMAT2 (vesicular monoamine transporter).