REVIEW

Emerging Molecular-Genetic Families in Dystonia: Endosome-Autophagosome-Lysosome and Integrated Stress Response Pathways

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ABSTRACT: Advances in genetic technologies and disease modeling have greatly accelerated the pace of introducing and validating molecular-genetic contributors to disease. In dystonia, there is a growing convergence across multiple distinct forms of the disease onto core biological processes. Here, we discuss two of these, the endosome-autophagosome-lysosome pathway and the integrated stress response, to highlight recent advances in the field. Using these two pathomechanisms as examples, we further discuss the opportunities that molecular-genetic grouping of dystonias present to transform dystonia care.

Key Words: autophagy; endosomal trafficking; dystonia; eIF2 α; Eif2s1; HOPS complex; integrated stress response; ISR; lysosome.

What does it mean to be part of a family? In dystonia, relationships have been proposed across a number of features. There are dystonia groupings based on clinical features, neuroimaging findings, and increasingly, on molecular genetic mechanisms. Just as the understanding of Parkinson's disease has followed a trajectory from clinical phenomena to neuropathology to isolated monogenic causes to biological groupings,[1,2] the field of dystonia is also rapidly approaching this possibility and the benefits of moving toward biological classifications are increasingly recognized.[3]

In this review, we present accumulated evidence from human genetic and functional studies that support the consideration of molecular-genetic families in dystonia for two processes, the endosome-autophagosome-lysosome pathway (EALP) and the integrated stress response (ISR). We further submit that the time is ripe for biological classifications of dystonias. Molecular mechanistic groupings matter because they help move toward more accurate and personalized treatments for people with dystonia. Mechanistic groupings provide a path for diagnostic assays and patient stratification. They may also shed light on a common conundrum in dystonia by helping to predict genetic penetrance. Ultimately, they provide a focused lens to improve therapeutic approaches in a

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field that has many unmet treatment needs and currently largely relies on a "one size fits all" approach.

Here, we present two examples of multiple dystonias converging around common cellular mechanisms and discuss the issues and opportunities that arise within this mechanistic framework. We selected endolysosomal trafficking and protein synthesis regulation by the ISR because of the substantial recent advancements in these areas. We recognize that emerging evidence can support even more "dystonia families" than we are able to cover in this format and the reader is encouraged to consider additional emerging findings regarding the significance of synaptic signaling,[3] nuclear pore complexes,[4,5] and myelination.[6,7] This abundance also raises the point that some of these mechanistic families are likely to be inter-related, intersecting at either upstream or downstream nodes. Later, we highlight an example of this possibility between EALP and ISR.

Last, associating particular cellular pathways with dystonia helps to alert clinicians working with individuals with rare syndromic (complex) disorders to consider this clinical component in instances where underlying genes intersect the dystonia-implicated pathway. Dystonia can be a subtle clinical presentation and is often overlooked or mistaken by non-subspecialty clinicians. The overarching goal of this conceptual approach is to shorten the time to diagnosis and optimize the therapeutic approach for the movement disorder patient while contributing to a fuller appreciation of the underlying causes of dystonia.

Dystonia and the EALP

The EALP is the central cellular mechanism for degrading macromolecules and defective organelles, mediating clearance of extracellular particles by endocytosis and intracellular contents by autophagy[8] (Fig. 1). Lysosome-related degradation is a dynamic process, regulated by cellular signaling and environmental influences, and plays an essential role in the function and control of the abundance of many proteins. Ensuring adequate protein levels is critical for homeostasis of highly dynamic structures in the nervous system including synapses and myelin.[9]

Defects in vesicular trafficking, fusion, export, import, and degradation within the EALP often result in lysosomal dysfunction syndromes. Lysosomal disorders are classically characterized by progressive multi-system clinical manifestations and abnormal accumulation of undigested macromolecules or catabolic materials inside cytoplasmic vesicles and/or enlarged dysfunctional lysosomes.[10] These conditions, many of which are collectively referred to as "lysosomal storage disorders" (LSDs), are mostly caused by genetic defects in lysosomal hydrolases, but may also be a consequence of mutational lesions in genes encoding non-enzymatic components of the EALP.[10-12] For instance, a growing number of Mendelian disorders linked to specific impairments in endosome/autophagosome trafficking to the lysosome are being recognized.[13,14] The majority of causally implicated genes in EALP-related disorders are associated with autosomal recessive inheritance, whereas X-linked transmission has also been documented.[15] This is consistent with a strong effect of bi-allelic or hemizygous variants on disease with expressions of classic severe LSDs.[10] Many LSDs and other EALP-related conditions are classically associated with variable presentations of dysmorphia, musculoskeletal abnormalities, organomegaly, and heterogeneous neurological disturbances. Neurological problems are present in up to two-thirds of cases and most frequently include developmental delay, hypotonia, seizures, cognitive impairment, and/or ataxia.[10]

Moreover, a growing body of evidence suggests that hyperkinetic movement disorders including dystonia can also be the predominant or even the sole expression of monogenic syndromes caused by EALP defects[15,16] (Table 1). Three early examples introducing this were prominent dystonic phenotypes in X-linked and autosomal recessive diseases with dysfunctional autophagy. These clinical presentations were mixed neurodevelopmental-neurodegenerative pathologies, such as a form of neurodegeneration with brain-iron accumulation (NBIA) and predominant dystonia linked to *WDR45* mutations (abnormal autophagosome formation)[17]; a striatonigral-degeneration disorder with progressive dystonia and status dystonicus caused by *VAC14* mutations (defective maturation of the autophagosome)[18]; and a childhood-onset combined movement disorder with ataxia, spasticity, and dystonia related to variants in *VPS13D* (impaired autophagic clearance of mitochondria).[19] Similarly, typical lysosomal enzyme-deficient LSDs including fucosidosis,[20] Gaucher disease type III,[21] GM1 and GM2 gangliosidosis,[22] Krabbe disease,[23] and ceroid lipofuscinosis,[24] all of which result in abnormal accumulation of substrates with formation of neurotoxic deposits and stored material, can present with dystonic phenotypes in some rare cases.[15] Although these observations supported the idea that deficits in autophagic/lysosomal function may be sufficient to produce dystonia, it remained unclear whether EALP defects are a major principal mechanism in the broader group of dystonic diseases including the more common isolated dystonias.

Most recently, the lysosome and the EALP have gained new prominence as cellular structures that may be crucially implicated in dystonia pathogenesis in a wider set of patients. A series of independent publications reported (isolated) dystonia-associated variants in genes encoding components of the homotypic fusion and protein sorting (HOPS) complex (ie, *VPS16*, *VPS11*, *VPS41*), which represents the structural bridge

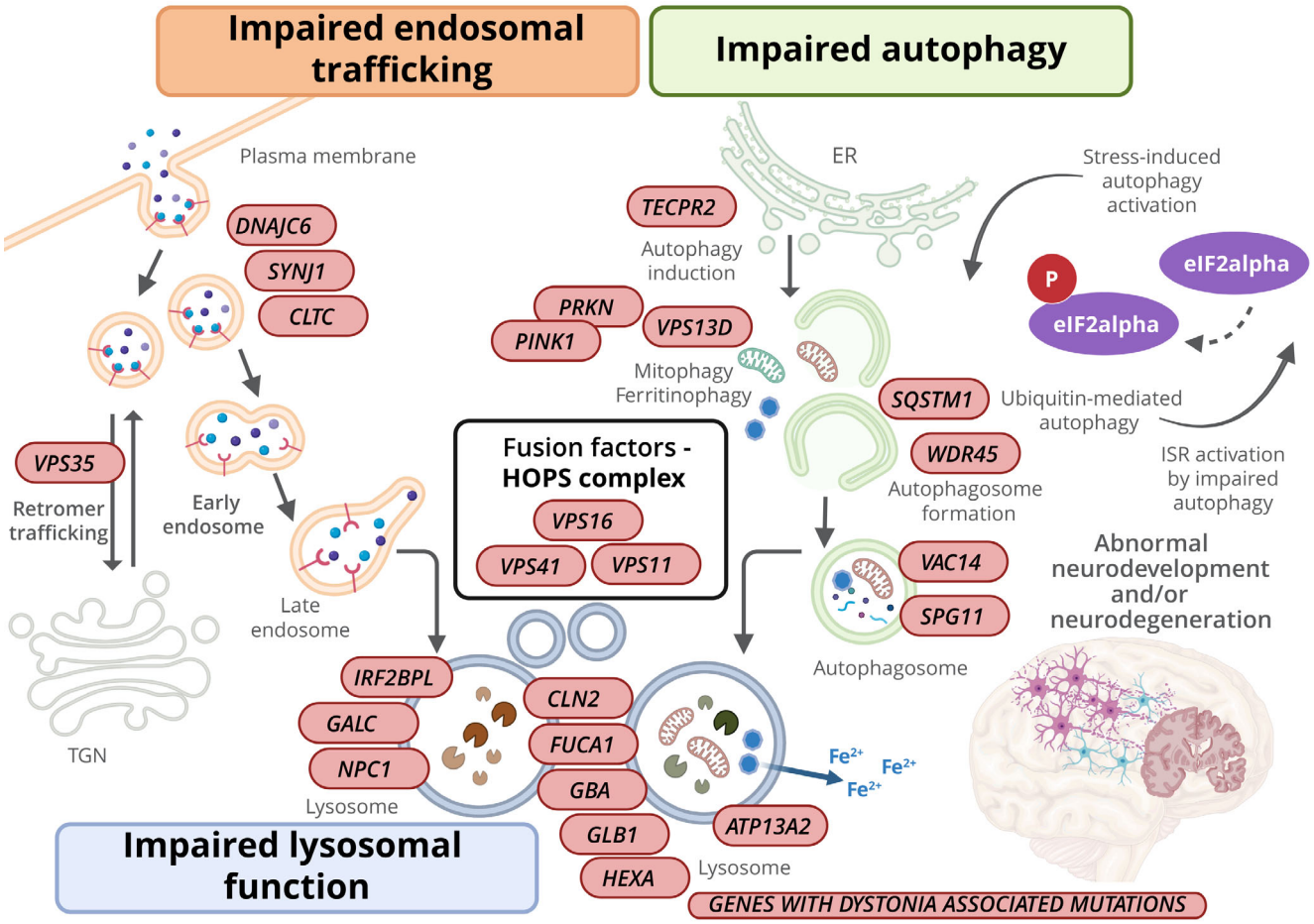

FIG. 1. Schematic of genes associated with dystonia and endosome-autophagosome-lysosome function overlaid onto potential relevant biological processes. Endosomal vesicle trafficking, autophagosome formation, and fusion with the lysosomes are illustrated. Endocytosis is depicted in the upper left corner. The endosomal system selects molecules for recycling via the trans-golgi network (TGN) or lysosomal degradation. The endoplasmic reticulum (ER) is involved in autophagy induction (upper right corner). Mitophagy and ferritinophagy, specific autophagic clearance processes required for degradation of mitochondria and ferritin, respectively, are also shown. Fusion of autophagosomes and late endosomes with lysosomes is essentially mediated by the assistance of the homotypic fusion and vacuole protein sorting (HOPS) complex, a highly conserved functional tether consisting of vacuolar protein sorting (Vps) proteins (black box). Following fusion, target molecules are degraded via lysosomal hydrolases. Dystonia-related genes and products affecting various stages of the endosome-autophagosome-lysosome pathway (EALP) are highlighted, alongside the mechanism(s) to which they are functionally linked. EALP impairments are likely to impact neurodevelopment and neuronal survival. The figure is a modification of previous illustrations of HOPS-complex defects in dystonia[25,27] and was created with Biorender.com. Brain-2 icon by Servier https://smart.servier.com/ is licensed under CC-BY 3.0 Unported https://creativecommons.org/licenses/by/3.0/. [Color figure can be viewed at wileyonlinelibrary.com]

required for fusion of endosomes and autophagosomes with the lysosomes.[25-28] The findings provided evidence for a remarkable degree of allelic heterogeneity (both dominant and recessive variants were found in *VPS16*)[29,30] and phenotypic variability (specific variants were associated with either isolated dystonia or severe classic LSD-like multisystem syndromes, eg, in *VPS16* and *VPS11*).[25,27,31,32] In various families of different geographic origins, heterozygous loss-of-function and bi-allelic missense variants in *VPS16* were identified as a cause of isolated dystonia as well as dystonia with comorbid neurodevelopmental features.[25,27,29,30] Incomplete penetrance in dominant *VPS16*-related dystonia seems to be common, and the condition was associated with NBIA-like structural alterations on magnetic resonance imaging (MRI) (pallidal T2 hypointensities) in a subset of described patients.[25,27] In two published pedigrees, a completely different phenotype resembling LSDs with dysmorphic features, skeletal abnormalities, and progressive psychomotor impairment has been documented in association to bi-allelic *VPS16* mutations.[33] Bi-allelic *VPS11* variants were originally linked to an infantile classic LSD-like disease with developmental delay, epilepsy, tetraplegia, hearing loss, and neuroimaging changes suggestive of cerebral hypomyelination.[32] Recently, Monfrini et al[28] expanded the clinical spectrum of *VPS11*-related diseases to include slowly progressive generalized dystonia with onset in adulthood and no major neurologic comorbidity and bilateral T2 hypointensities in the globus pallidus on MRI. Finally, several back-to-back papers reported bi-allelic *VPS41* variants as a new etiology of

TABLE 1 *List of EALP−/stress response-related genes involved in dystonia.*

Gene	Process/compartment	Inheritance disease/ mutation mode	Functional defect	Disease (OMIM)	Dystonia phenotype
EALP					
ARSA	Lysosomes	AR	Lysosomal enzyme defect	Metachromatic leukodystrophy	Part of complex picture
ATP13A2	Lysosomes	AR	Lysosomal ATPase defect	Kufor-Rakeb syndrome	Part of complex picture; combined dystonia (dystonia-parkinsonism)
CLN2	Lysosomes	AR	Lysosomal enzyme defect	Ceroid lipofuscinosis, neuronal, 2	Part of complex picture
CLTC	Endosomes	AD/het LoF	Endosomal trafficking defect	Intellectual developmental disorder, autosomal dominant, 56	Part of complex picture
DNAJC6	Endosomes	AR	Endocytosis defect	Parkinson's disease, 19	Part of complex picture; combined dystonia (dystonia-parkinsonism)
FUCA1	Lysosomes	AR	Lysosomal enzyme defect	Fucosidosis	Part of complex picture
GALC	Lysosomes	AR	Lysosomal enzyme defect	Krabbe disease	Part of complex picture
GBA	Lysosomes	AR	Lysosomal enzyme defect	Gaucher disease	Part of complex picture
GLB1	Lysosomes	AR	Lysosomal enzyme defect	GM1-gangliosidosis	Part of complex picture
HEXA	Lysosomes	AR	Lysosomal enzyme defect	Tay-Sachs disease	Part of complex picture
IRF2BPL	Lysosomes	AD/het LoF	Lysosomal defect, specific mechanism unknown	Neurodevelopmental disorder with regression, abnormal movements, loss of speech, and seizures	Part of complex picture
NPC1	Lysosomes	AR	Lysosomal transporter defect	Niemann-Pick disease, type C1	Part of complex picture
PINK1	Autophagy	AR	Mitophagy defect	Parkinson's disease, 6	Combined dystonia (dystonia-parkinsonism)
PRKN	Autophagy	AR	Mitophagy defect	Parkinson disease, juvenile, type 2	Combined dystonia (dystonia-parkinsonism)
SPG11	Autophagy/lysosomes	AR	Autophagosome-lysosome fusion defect	Spastic paraplegia, 11, autosomal recessive	Part of complex picture
SQSTM1	Autophagy	AR	Ubiquitin-mediated autophagy defect	Neurodegeneration with ataxia, dystonia, and gaze palsy, childhood-onset	Part of complex picture

(Continues)

TABLE 1 *Continued*

Gene	Process/compartment	Inheritance disease/ mutation mode	Functional defect	Disease (OMIM)	Dystonia phenotype
SYNJ1	Endosomes	AR	Endosomal trafficking defect	Parkinson's disease, 20	Part of complex picture; combined dystonia (dystonia-parkinsonism)
TECPR2	Autophagy	AR	Autophagy induction defect	Neuropathy, hereditary sensory and autonomic, type IX, with developmental delay	Part of complex picture
VAC14	Autophagy	AR	Autophagosome maturation defect	Striatonigral degeneration, childhood-onset	Part of complex picture
VPS11	Autophagosome-endosome-lysosome fusion	AR	Autophagosome-endosome-lysosome fusion defect	Dystonia, 32	Isolated dystonia
VPS13D	Autophagy	AR	Mitophagy defect	Spinocerebellar ataxia, autosomal recessive, 4	Part of complex picture
VPS16	Autophagosome-endosome-lysosome fusion	AD/het LoF and AR	Autophagosome-endosome-lysosome fusion defect	Dystonia, 30	Isolated dystonia
VPS35	Retromer/recycling endosomes	AD/het missense	Endosome recycling defect	Parkinson disease, 17	Combined dystonia (dystonia-parkinsonism)
VPS41	Autophagosome-endosome-lysosome fusion	AR	Autophagosome-endosome-lysosome fusion defect	Spinocerebellar ataxia, autosomal recessive, 29	Part of complex picture
WDR45	Autophagy	XL	Autophagosome formation defect	Neurodegeneration with brain iron accumulation, 5	Part of complex picture
ISR pathway					
AARS1	Translation	AR	Translation defect	Developmental and epileptic encephalopathy, 29	Part of complex picture
AARS2	Mitochondrial translation	AR	Mitochondrial translation defect	Combined oxidative phosphorylation deficiency, 8; leukoencephalopathy, progressive, with ovarian failure	Part of complex picture
AIMP1	Translation	AR	Translation defect	Leukodystrophy, hypomyelinating, 3	Part of complex picture
ATF4	Transcription factor	AD/het LoF	ISR pathway transcriptional defect	NA	Isolated dystonia

(Continues)

TABLE 1 *Continued*

Gene	Process/compartment	Inheritance disease/ mutation mode	Functional defect	Disease (OMIM)	Dystonia phenotype
CARS2	Mitochondrial translation	AR	Mitochondrial translation defect	Combined oxidative phosphorylation deficiency, 27	Part of complex picture
EARS2	Mitochondrial translation	AR	Mitochondrial translation defect	Combined oxidative phosphorylation deficiency 12	Part of complex picture
EIF2AK2	ISR pathway kinase	AD/het missense	ISR pathway defect	Dystonia 33; leukoencephalopathy, developmental delay, and episodic neurologic regression syndrome	Part of complex picture; isolated dystonia
EIF2B5	ISR pathway GEF	AR	ISR pathway GEF defect	Leukoencephalopathy with vanishing white matter, 5	Part of complex picture
EIF4A2	Translational initiation	AD/het LoF, AR	Translation initiation defect	Neurodevelopmental disorder with hypotonia and speech delay, with or without seizures	Isolated dystonia
EPRS1	Translation	AR	Translation defect	Leukodystrophy, hypomyelinating, 15	Part of complex picture
FARS2	Mitochondrial translation	AR	Mitochondrial translation defect	Combined oxidative phosphorylation deficiency, 14; spastic paraplegia, 77	Part of complex picture
KARS1	Translation	AR	Translation defect	Leukoencephalopathy, progressive, infantile-onset, with or without deafness	Part of complex picture
LARS2	Mitochondrial translation	AR	Mitochondrial translation defect	Perrault syndrome, 4	Part of complex picture
PARS2	Mitochondrial translation	AR	Mitochondrial translation defect	Developmental and epileptic encephalopathy, 75	Part of complex picture
PRKRA	ISR pathway kinase activator	AR	ISR pathway defect	Dystonia, 16	Combined dystonia (dystonia-parkinsonism); isolated dystonia
TARS2	Mitochondrial translation	AR	Mitochondrial translation defect	Combined oxidative phosphorylation deficiency, 21	Part of complex picture
THAP1	Transcription factor	AD/het LoF and missense	Transcriptional defect	Dystonia, 6	Isolated dystonia
TOR1A	Endoplasmic reticulum/ nuclear envelope	AD/het inframe	ISR pathway defect, secretion defect, NPC formation defect	Dystonia-1, torsion	Isolated dystonia

(Continues)

TABLE 1 *Continued*

Gene	Process/compartment	Inheritance disease/ mutation mode	Functional defect	Disease (OMIM)	Dystonia phenotype
WARS2	Mitochondrial translation	AR	Mitochondrial translation defect	Neurodevelopmental disorder, mitochondrial, with abnormal movements and lactic acidosis, with or without seizures; parkinsonism-dystonia, 3; childhood-onset	Part of complex picture; combined dystonia (dystonia-parkinsonism)

Note: Isolated dystonia: dystonia, associated or not with tremors that are the only additional movement disorder; combined dystonia: dystonia is combined with other movement disorders; dystonia as part of complex picture: dystonia is accompanied by neurologic or systemic manifestations beyond movement disorders.
Abbreviations: EALP, endosome-autophagosome-lysosome pathway; OMIM, Online Mendelian Inheritance in Man; AR, autosomal recessive; AD, autosomal dominant; het, heterozygous; LoF, loss of function; XL, X-linked; ISR, integrated stress response; NA, not available; GEF, guanine exchange factor; NPC, nuclear pore complex; inframe, inframe deletion.

neurodegenerative-neurodevelopmental syndromes with intellectual disability, optic atrophy, neuropathy, mixed movement disorders with prominent dystonia, and NBIA-like T2 hypointensities in globus pallidus.[25,26,34]

Functional studies using cellular systems and model organisms in the context of dystonia-related VPS gene variants demonstrated abnormalities in the cellular pathways that deliver intracellular cargo to lysosomes for degradation. This result is consistent with impaired function of the HOPS complex, because it is a specialized adaptor connecting the endosomal-autophagosomal-lysosomal compartments.[27] In one series of experiments performed on fibroblasts and lymphoblasts carrying heterozygous *VPS16* mutations, Steel et al[25] showed the presence of microscopic vacuolar changes consistent with defects in fusion of vesicles containing cargo and lysosomal dysfunction. Similar abnormalities were found in cultured cells from patients with bi-allelic *VPS11* and *VPS41* variants.[27] Moreover, suggestive of a transport block in the EALP with accumulation of autophagosomes and upregulation of lysosomal enzymes, *VPS11*-mutant dystonia-patient fibroblasts displayed a significant increase in expression of autophagic markers and lysosomal-hydrolase activity.[28] Zebrafish studies of *VPS41* mutations revealed lysosomal dysregulation throughout the brain, accompanied by microglial abnormalities.[26] Additionally, an in vitro study of *VPS41*-depleted HeLa cells, modeling the situation in some patients with recessive *VPS41*-related dystonia, showed impaired formation of a functional HOPS complex with aberrant lysosomal delivery of endocytic and autophagic cargo.[34] All of these results identify EALP defect-associated lysosomal cytopathology in dystonia models.

Although there is clarity that the EALP process is disrupted, it remains to be understood how these defects lead to dystonia. Three mechanistic considerations with potential wider relevance for dystonia and associated research initiatives deserve mention. First, it is interesting to note that the EALP broadly influences many cellular processes because they rely on lysosomal degradation of components to regulate protein levels.[10] To what extent does the impaired EALP function in VPS gene-related dystonias impact other dystonia-associated pathways, perhaps indicating a convergence of final downstream mechanisms? For example, it is conceivable that lysosomal dysfunction in *VPS16*-related dystonia may result in alterations of mitochondrial integrity (via impaired mitophagy)[35] and/or integrated stress response signaling,[36,37] because both are mechanistically linked to lysosomal homeostasis.[38] This intersection could connect processes implicated by other monogenic dystonias. Second, there has been strong interest in understanding the mechanistic underpinnings of neuroanatomical changes associated with dystonia.[39] The striking observation of NBIA-like signal alterations in basal ganglia as a unifying

feature across multiple VPS-gene-related dystonias[27] provides evidence to support the idea that EALP impairments, potentially also in a wider context of dystonia-manifesting NBIAs, may directly or indirectly contribute to this pathology. Notably, the lysosome is the key organelle for recycling and handling of intracellular iron, indicating that changes in lysosomal function could disrupt iron transit through the lysosomal compartment,[40] potentially leading to abnormal iron deposition and/or NBIA-typical neuroimaging signals in patients with dystonia. Here, again, there is potential for mechanistic connections between dystonia-implicated pathways, as iron homeostasis perturbations may engage the ISR to prevent ferroptosis.[41,42] Third, one key question in dystonia research is whether the disease is of neurodevelopmental or neurodegenerative origin (or results from combined defects).[43] Here, the study of VPS-gene-related dystonias may offer critical clues. Moreover, it is well known that EALP abnormalities, particularly arrested autophagy and/or primary lysosomal dysfunction, act as key pathogenic events in diverse neurodegenerative disorders with protein aggregates such as Parkinson disease.[44] As such, further studies of VPS-gene-related dystonias may provide the potential to better characterize the functional relationships between dystonia, Parkinsonism, and neurodegeneration. On the other hand, mounting evidence indicates that the EALP is also critically implicated in neurodevelopmental cellular processes, including maintenance of neural stem-cell homeostasis, differentiation, and synaptogenesis.[45,46] In this regard, it would be of major interest to identify the lysosomal defect-associated target molecules that contribute to dystonia pathogenesis in a neurodevelopmental context. One hypothesis may be that certain unprocessed substrates or abnormal lysosomal byproducts could directly impair neurodevelopment. It has been shown that mutational defects of an isolated dystonia-related gene product, the transcription factor THAP1, can be associated with abnormal catabolism of glycosaminoglycans (a core class of lysosomal substrates) and neurodevelopmental myelination deficits in the CNS.[47] Intriguingly, abnormal glycosaminoglycan levels and myelination abnormalities were also seen in several cases of VPS gene-related disorders.[27,33] Further work is necessary to define the multiple roles of defective EALP in dystonia and elucidate pathophysiological mechanisms that may range from neurodevelopmental to synaptic/network dysfunction to accelerated neuronal death.

Ultimately, it will be an important objective of future studies to clarify whether therapeutic modulation of autophagy, lysosomal function, and/or other components of the EALP might be a successful strategy to treat one or more types of dystonia.[48] Potential considerations include repurposed drugs and products that are already known to have beneficial potential in the therapy of lysosomal defect-associated disorders, such as autophagy activators (eg, trehalose), substrate-reducing molecules (eg, small molecule inhibitors of glycosphingolipid metabolism), as well as other strategies that may stimulate the pathway to restore normal levels of activity (eg, modulation of Zn2+ levels to control acidity, glycosaminoglycan-clearance therapy). However, the identification of clinical therapeutic windows, definition of optimal target molecules, and evaluation of pharmacodynamic markers for monitoring of EALP activity (among others) would also be necessary to establish these therapies for application in dystonia.

Dystonia and Translational Regulation by the ISR Pathway

The ISR is a highly conserved biochemical pathway that is activated by a diverse range of cellular stressors from classic unfolded proteins, to nutrient deficiencies and viral infection[49] (Fig. 2). The ISR restores cellular homeostasis by regulating protein synthesis and is used body-wide. Although originally named for its identified role in proteostasis, additional uses for this biochemical pathway have been discovered in specific organs/cells. Examples of these roles include circadian rhythms,[50] learning, memory and synaptic plasticity,[51] developmentally regulated axonogenesis,[52] and cholinergic neuromodulation.[53]

Genetic diseases involving ISR pathway genes tend to cause phenotypes involving pancreas, liver, lung, and brain organ systems. Examples of clinical manifestations include diabetes, acute liver failure, pulmonary hypertension, demyelination, and intellectual disability.[54-57] More recently, convergent human genetics and functional studies in model systems supports ISR dysfunction as a cause for dystonia (Table 1).

The earliest direct connection between the ISR and dystonia was made when mutations in the gene encoding the Prkra protein were identified in Brazilian families with an autosomal recessive disorder that featured prominent dystonia and parkinsonism, DYT16/DYT-*PRKRA*.[58] Prkra plays a role in activating the eif2α kinase, PKR (EIF2AK2). Because of those initial findings, causative mutations have been confirmed by multiple groups across several ethnicities.[59,60] The most common clinical phenotype is early onset generalized dystonia with mild parkinsonism that has been described as a non-levodopa response bradykinesia. Substantial variation exists within and across families, and reports of worsening with febrile illnesses highlight the potential for a two-hit process involving genetic and environmental factors. Isolated early and adult-onset dystonia presentations have also been described,[59,61] including finding Prkra mutations in approximately 5% of idiopathic dystonia cases in Brazil.[61] A separate study of idiopathic cervical dystonia cases in the United States implicated another ISR gene, *ATF4/CREB2*, finding pathological variants in approximately 3% of cases.[62] The discovery of *ATF4* variants were reported alongside

functional studies in DYT1/TOR1A that identified a role for the ISR. The mechanistic convergence between Prkra, ATF4, and TOR1A dystonias led to the hypothesis that ISR dysregulation was a pathological driver for dystonia.[62] Subsequently, variants in EIF2AK2/PKR were identified as a cause for autosomal dominant syndromic disorders in which dystonia was either a prominent or sole clinical feature furthering the human genetic evidence for the ISR.[63,64]

Several syndromic genetic disorders that include dystonia are also predicted to dysregulate the ISR. These include leukoencephalopathy, vanishing white matter disease (VWMD) because of *EIF2B* mutations[65]; dystonia with tremor because of frameshift mutations in the *EIF4A2* gene[66]; transfer RNA (tRNA) synthetase genetic disorders such as *WARS2*[67-69] and *CARS*[70]; and even mitochondrial disorders[35] because mitochondrial dysfunction is a potent activator of the ISR.[37,71,72]

A number of functional studies using cellular systems and model organisms has helped define the effects of mutations in the ISR-associated genes, and also introduced evidence of ISR dysregulation in models for isolated dystonia genetic disorders that would not have otherwise been connected to the ISR. Current evidence supports that *PRKRA* mutations enhance and prolong ISR activation[73,74]; Atf4 missense variants reduce Atf4 transcriptional activity[62]; and EIF2AK2/PKR mutations have shown disparate effects across two studies characterizing distinct variants—reduced ISR activity[64] and delayed resolution of ISR activation.[63] Finally, the causative mutations for DYT1/TOR1A and DYT6/THAP1 have been shown to dysregulate the ISR.[62,75-77] Because findings indicating ISR directionality have been done in

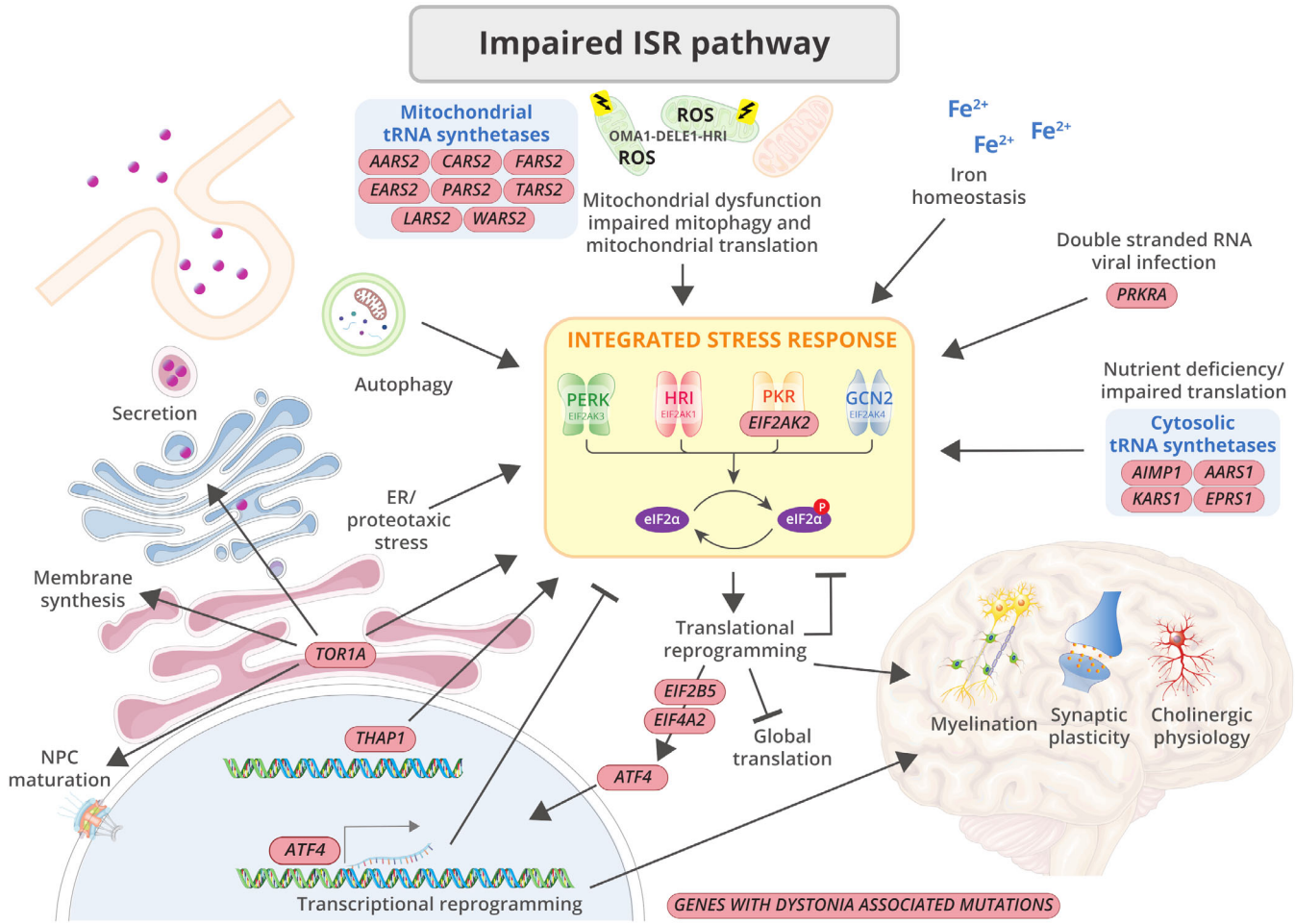

FIG. 2. Schematic of genes associated with dystonia and the integrated stress response (ISR) overlaid onto potential relevant biological processes. Summary diagram illustrating cellular components, biological processes and dystonia-associated genes that intersect the function of the ISR pathway. Several regulators function in the activation of the ISR and the ISR impacts many downstream effector cascades including translational control. Dystonia-associated genes are highlighted in light pink. NPC, nuclear pore complex; ER, endoplasmic reticulum; ROS, reactive oxygen species. Brain-2, mitochondrium-1, DNA-nucleotides, endoplasmatic-reticulum-medium, and golgi-2d-1 icons by Servier https://smart.servier.com/ is licensed under CC-BY 3.0 Unported https://creativecommons.org/licenses/by/3.0/. Interneuron, Nuclear_pore_complex, and Motor_neuron icons by DBCLS https://togotv.dbcls.jp/en/pics.html is licensed under CC-BY 4.0 Unported https://creativecommons.org/licenses/by/4.0/. [Color figure can be viewed at wileyonlinelibrary.com]

disparate assays and research teams, it is as yet uncertain whether signaling differences relate to technical/model choices or biological mechanistic differences. This is an important area for future research.

Experiments involving bi-directional ISR pathway manipulation provide insight into the causal relationship between ISR activity and dystonia-related phenotypes in DYT1/TOR1A and DYT6/THAP1 models. In DYT1/TOR1A knockin mice, boosting ISR activity with selective eIF2α phosphatase inhibition by salubrinal[78] in vivo, delayed neonatal mortality of homozygous mice and by Sal-003[79] ex vivo, rescued synaptic plasticity defects in brain slices.[62] Similar to findings in DYT1/TOR1A knockin mice, some striatal synaptic plasticity deficits in DYT6/THAP1 mouse models were rescued when brain slices were preincubated with Sal-003.[75] Another drug recognized to activate the ISR is ritonavir,[79] which has also shown corrective effects for multiple DYT1/TOR1A model phenotypes—protein mislocalization,[80] microstructural/white matter brain defects,[80] paradoxical cholinergic interneuron responses,[80] and the protein composition of extracellular vesicles.[81] Additionally, in wild-type mice, ISR inhibition with integrated stress response inhibitor (ISRIB)[82] has been shown to reproduce DYT1/TOR1A knockin phenotypes of impaired corticostriatal synaptic plasticity[62] and paradoxical dopamine regulation of cholinergic interneuron activity.[53] These two brain phenotypes have also been found in DYT6/THAP1 models,[75,83] indicating a potential for shared therapeutic benefit if the ISR is targeted.

The use of the ISR in cells body-wide poses a conundrum when considering the relatively selective expression of only one or a few symptoms in human genetic diseases involving ISR genes. What aspect of cellular function might lead to vulnerabilities that generate dystonia? Below, we briefly discuss three hypothesized pathomechanisms for dystonia that intersect biological processes associated with the ISR. These are dysfunction of brain white matter tracts,[84] synaptic plasticity,[85,86] and striatal cholinergic interneuron signaling.[83,87]

In the normal brain, the ISR has been shown to be critical for early developmental establishment of white matter tracts.[52] *EIF2B* mutations that activate the ISR cause a progressive leukoencephalopathy (VWMD).[65] Both DYT1/TOR1A and DYT6/THAP1 show reduced white matter microstructural integrity on diffusion tensor magnetic resonance imaging (DTI) sequences in humans and mouse models.[88-90] Using ritonavir to activate the ISR,[91] a brief neonatal period of drug treatment was sufficient to normalize DTI abnormalities of DYT1 knockin mice when examined in adulthood.[80] This result establishes a mechanistic link between ISR dysregulation during development and the brain white matter abnormalities in DYT1/TOR1A dystonia.

Outside of disease settings, the ISR has been shown in numerous studies to be a potent modifier of synaptic plasticity and learning and memory behavior.[51,92] A number of clinical features in dystonia have led to the hypothesis that corruption of motor skill learning may contribute to dystonia.[85,86] In dystonia mouse models, abnormalities in the brain's main biological process for learning and memory, synaptic plasticity have been described.[86] The corticostriatal circuitry, which is central to motor skill learning has been most extensively studied. ISR signaling is required for a form of long-term synaptic depression (LTD) in corticostriatal circuits.[62] Deficits in corticostriatal LTD have been described in DTY1/TOR1A and DYT6/THAP1 mouse models.[75,93] Pharmacologically boosting the ISR (phosphatase inhibitor, Sal-003) rescues corticostriatal LTD deficits in DYT1/TOR1A knockin mice[62] and a drug-induced form of corticostriatal LTD in DYT6/THAP1 model mice.[75] These results establish causal relationships between ISR pathway signaling and dystonia animal model synaptic plasticity deficits.

Finally, striatal cholinergic interneurons in the normal healthy brain have been found to constitutively activate the ISR rather than to activate it transiently in response to stressors.[53] Dysregulation of the striatal cholinergic system figures prominently in dystonia. Clinically, anti-cholinergic medications remain the most effective (though poorly tolerated) drugs for multiple forms of dystonia. Alongside this clinical observation, deficits in striatal cholinergic signaling have been described in multiple mouse models of monogenic forms of dystonia. Originally reported in DYT1/DYT-*TOR1A*, and later found in two other models, DYT6/DYT-*THAP1* and *GNAL*, the tonic action potential firing of striatal cholinergic interneurons (CIN) shows an abnormal response to dopamine (via type 2 dopamine receptors), in that rather than causing the normal slowing in firing, it paradoxically accelerates CIN firing.[83,94] This paradoxical dopamine response phenotype is also observed when the ISR is inhibited in CINs.[53] Here again, ISR dysregulation is connected to dystonia phenotypes, in this case cholinergic dysfunction.

In summary, multiple brain abnormalities associated with dystonia animal models have also been shown to result from perturbations of the ISR alone. Additionally, in dystonia models, corrective effects of ISR interventions have been shown for several of these phenomena.[62,75,80] Although these phenotypes are ostensibly disparate, it may be that the broad proteomic effects of ISR activity cause multiple consequences. This would also explain the broad corrective effects of ISR interventions.

Exactly which circuits and developmental timing that the ISR's role in myelination, synaptic plasticity, cholinergic signaling, or as yet unrecognized ISR role contribute to dystonia is an important future area for study. Additionally, the aforementioned studies focus on cortical and striatal brain regions. Relatively little has been

studied about the ISR in other brain regions implicated in dystonia, such as cerebellum and spinal cord.

Can the insight of the ISR's role in dystonia be harnessed to develop more effective therapies for dystonias? There are several ISR-modifying compounds with drug-like properties that may be suitable for human clinical trials in the near future. These include compounds with activating (sephin1, raphin1) and inhibiting (ISRIB, 2Bact) properties.[82,95-97] For clinical translation, directionality of ISR modulation and timing of treatment should be addressed for each type of dystonia. For ISR treatment directionality, so far, only the DYT1/DYT-*TOR1A* knockin mouse model has published data with chronic in vivo exposure to ISR-modifying compounds.[62,80] In these cases, ISR-activating compounds were well-tolerated and showed corrective effects. More study is needed to understand when ISR-modifying treatments will work because there may be a developmentally sensitive window for certain interventions.[98] However, it is notable that in animal studies, ISR interventions show corrective effects both when delivered acutely to adult brain tissue (synaptic plasticity)[62,75] and also when delivered during a brief development window (DTI).[80] A better understanding of which phenotypes matter for dystonia expression will be helpful to provide guidance for clinical trial design. Fortunately, for translational efforts, the broad effects the ISR exerts on the proteome facilitate identifying biomarkers for patient stratification and determination of target engagement.[81]

Concluding Remarks

Recent, and rapidly accumulating, insights have introduced dysfunction in two core cellular biological processes, the EALP and the ISR, as causative factors for dystonia. In the preceding discussion, we presented the foundational human genetic evidence for EALP and ISR-related genes in dystonia and follow-on functional studies that validated dysfunction of these core processes in dystonia models. With this evidence to support focus on the biological pathway, we further speculated on mechanistic connections between other genetic dystonias and dystonia-implicated pathomechanisms that are predicted to impinge on processes regulated by EALP and ISR (summarized in Figs. 1 and 2). Finally, we proposed specific new therapeutic inroads that arise from these pathomechanisms and highlighted key knowledge gaps to fill to translate these insights to the clinic.

The degree of convergent evidence in human genetic and functional studies leads us to argue that the time is ripe to consider biological classifications in dystonia. As to exactly how many distinct dystonia molecular pathomechanisms there are and to what degree current biological groupings are inter-related, are two important questions to resolve. Moreover, further understanding of how defects of EALP and ISR cause varying clinical outcomes with predominant dystonic presentations in some individuals and completely different syndromes in other patients may provide important mechanistic insights with therapeutic implications. Future studies should address mechanisms of variable expressivity in these disorders, whereby a range of factors could play a role including epigenetics, genetic modifiers, environmental influences, and stochastic variation. Related to these efforts, a fourth critical question for future scientific efforts in dystonia is to understand how the pathomechanisms identified for rare monogenic forms relate to the more common, late-onset, idiopathic dystonias.

In this review, we provided extended discussion for two pathomechanisms to provide examples of the kinds of data contributing to such mechanistic groupings. Here, a cautionary note is to highlight some of the challenges in associating a dystonia with a mechanistic group. Diverse types of data may lend support, each with its own caveats. For genetic associations, these typically include the strength of the genetic association data, including replication, and gene annotations. For functional studies, there is much more diversity in the nature of experiments. Examples include pathway analysis of observed protein or transcriptional differences, specific biological process assays, and responses to pharmacological or genetic manipulations targeting a process. Gene and pathway annotations have limitations in that they are often based on overly simplistic functional annotations. Functional studies directly targeting specific processes are an important validation component; although each technical approach will have its own caveats and sources of false positives and negatives to consider. Even with these cautions in mind, emerging data support new molecular-genetic groupings in dystonia. As is generally true, recurrent and replicated findings across multiple lines of investigation strengthen the validity of the identified mechanism. The two processes we highlight provide examples of such recently accumulated evidence.

Knowledge of the EALP and ISR contributions to dystonia can further understanding of brain and circuit phenomena that have previously been associated with dystonia. For example, EALP functionally intersects with several processes that have independently been associated with dystonia pathogenesis. Aberrant trafficking to the lysosome, as caused by VPS gene mutations, may induce iron deposition or ultrastructural iron accumulation in vulnerable neurons.[40] Iron accumulation is a pathological hallmark of many dystonic disorders including those referred to as NBIA syndromes.[99] Impaired EALP function may also lead to mitochondrial dysfunction,[100] which in turn triggers the ISR[71,72] (Fig. 3). Roles for the ISR have been

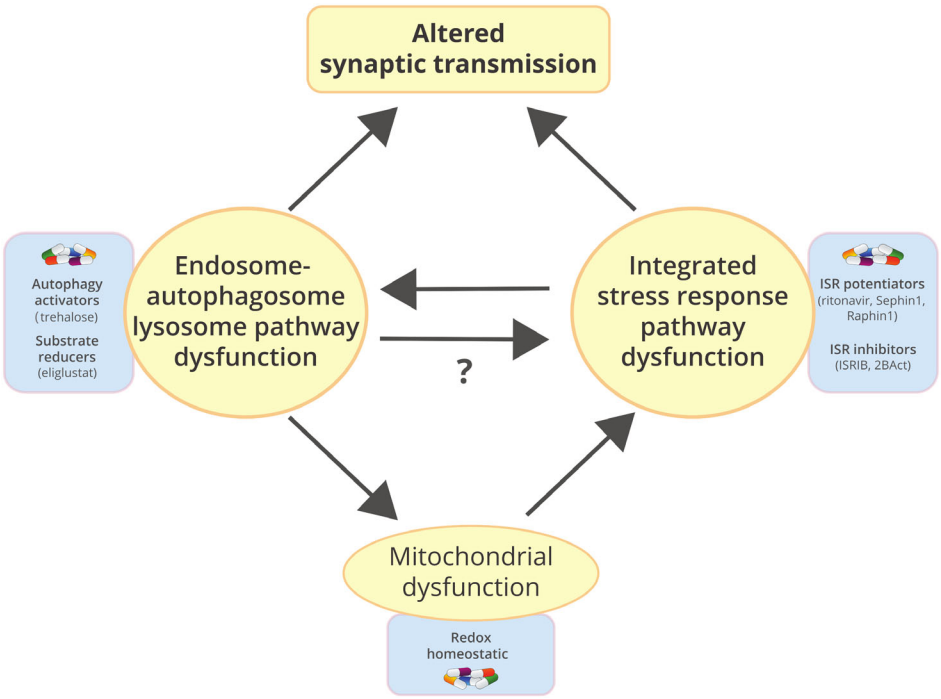

FIG. 3. Schematic highlighting potential mechanistic intersections and targeted therapeutics for endosome-autophagosome-lysosome pathway (EALP) and integrated stress response (ISR) disorders. Potential points of targeted treatment are displayed, with interventions in the stress-response, lysosomal, and mitochondrial pathways highlighted. Examples of dystonia-associated biological processes that intersect are indicated by black arrows. [Color figure can be viewed at wileyonlinelibrary.com]

directly established in several biological processes that are known to be disrupted in dystonia models (eg, synaptic plasticity, white matter ultrastructure, and cholinergic interneuron signaling). The ISR is a potent modifier of synaptic plasticity,[51,101] which has been separately identified as a dystonia patho-mechanism.[3,86] Striatal cholinergic interneurons and oligodendrocytes are cell types independently identified to be dysfunctional in dystonia,[6,83] that may also be particularly vulnerable cell types for ISR impairment.[53,95] Finally, it is likely that dysfunction of EALP and ISR may converge mechanistically to cause dystonia because many consequences of EALP dysfunction engage the ISR for resolution, including autophagy, mitochondrial integrity and iron homeostasis[37,41,71,72] (Fig. 3). Understanding whether and where dystonia biological processes intersect and final common pathways to dystonia pathogenesis is important work that lies ahead.

Harvesting the fruits of these efforts will bring many advantages for individuals with dystonia. Biological classifications can stratify patients with dystonia according to main underlying mechanisms of pathogenesis, supporting personalized care. In practice, grouping of patients on a molecular-genetic classification scheme could also guide careful assessment of broader phenotypic expressions, with a focus on identification of potentially treatable comorbidities that are known to be more often associated with the respective molecular pathology (eg, neurodevelopmental, behavioral or epileptic features in synapse dysfunction, possible systemic involvement in EALP defects, leukoencephalopathy in ISR etc.). Perhaps most importantly, the identification of a small number of common biological processes relevant to multiple dystonias will foster the development of mechanism-targeting therapeutics. In a rare disease, this is an important advantage because it increases the number of individuals that would be eligible for a clinical trial that might otherwise focus on more narrow genetic definitions. The genetic landscape of dystonia is too heterogeneous for efficient development of interventions at the specific gene (variant) level for all individual patients.

Notably, the biological classifications we have discussed already suggest the potential use of a number of small molecule therapeutic strategies. For EALP, small molecule modifiers of autophagy and lysosome may emerge as promising treatments. Studies have shown that the maintenance or upregulation of EALP activity may foster neuronal health and is effective in treating patients with motor symptoms (eg, in Parkinson's disease).[102] Enzymatic activity enhancement in the EALP, specific substrate reduction, and/or autophagic activation may be useful strategies and deserve potential consideration in future clinical trial designs. For the ISR, small molecules in mouse models for DYT1/TOR1A and DYT6/THAP1 models indicate that interventions

boosting the ISR are corrective.[62,75,80,81] Drug opportunities to boost the ISR include inhibition of its phosphatases (raphin, sephin, and guanabenz)[96,97,103] or novel activators like human immunodeficiency virus (HIV) protease inhibitors.[80,91] However, should some dystonias require ISR down modulation, there are also promising pharmacological interventions to reduce ISR activation (ISRIB and related molecules).[82] Interestingly, the same economy of scale that incentivizes therapeutic development for mechanistically connected groups by underlying pathways can also apply to genetic mutation type-specific therapies, for example, mutations causing premature stop codons could be regarded as a shared molecular target for development of effective stop-codon read-through compounds or biologics.[104]

Overall, we hope to convey that the time is ripe to consider molecular-genetic pathomechanisms and harvest those insights to pursue targetable interventions to transform therapeutic opportunity in dystonia. Today, most people with dystonia can expect lifelong persistence of the disorder and access to only symptomatic therapies. We believe that the amassing insights in dystonia research can lead us to a future with better diagnostics, earlier intervention and disease-modifying therapies, if not outright cures. ●

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Author Roles

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical analysis: A. Design, B. Execution, C. Review and critique; (3) Manuscript preparation: A. Writing of the first draft, B. Review and critique.

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