BRAIN UPDATE

HOPS-associated neurological disorders (HOPSANDs): linking endolysosomal dysfunction to the pathogenesis of dystonia

Edoardo Monfrini,[1,2] Michael Zech,[3,4] Dora Steel,[5,6] Manju A. Kurian,[5,6] Juliane Winkelmann[3,4,7,8] and Alessio Di Fonzo[2]

The homotypic fusion and protein sorting (HOPS) complex is the structural bridge necessary for the fusion of late endosomes and autophagosomes with lysosomes.

Recent publications linked mutations in genes encoding HOPS complex proteins with the aetiopathogenesis of inherited dystonias (i.e. *VPS16*, *VPS41*, and *VPS11*). Functional and microstructural studies conducted on patient-derived fibroblasts carrying mutations of HOPS complex subunits displayed clear abnormalities of the lysosomal and autophagic compartments.

We propose to name this group of diseases HOPS-associated neurological disorders (HOPSANDs), which are mainly characterized by dystonic presentations. The delineation of HOPSANDs further confirms the connection of lysosomal and autophagic dysfunction with the pathogenesis of dystonia, prompting researchers to find innovative therapies targeting this pathway.

1 Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation, University of Milan, Milan 20122, Italy
2 Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit, Milan 20122, Italy
3 Institute of Neurogenomics, Helmholtz Zentrum München, Munich 85764, Germany
4 Institute of Human Genetics, Technical University of Munich, Munich 81675, Germany
5 Developmental Neurosciences, UCL Great Ormond Street Institute of Child Health, London WC1N 1EH, UK
6 Department of Neurology, Great Ormond Street Hospital, London WC1N 3JH, UK
7 Lehrstuhl für Neurogenetik, Technische Universität München, Munich 81675, Germany
8 Munich Cluster for Systems Neurology, SyNergy, Munich, 81377, Germany

Correspondence to: Dr Alessio Di Fonzo
Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico
Via Francesco Sforza 35, 20122 Milan, Italy
E-mail: alessio.difonzo@policlinico.mi.it

Keywords: dystonia genetics; HOPS; HOPSANDs; lysosome; autophagy

Abbreviation: HOPS = homotypic fusion and protein sorting

Received February 12, 2021. Revised April 02, 2021. Accepted April 08, 2021. Advance access publication April 19, 2021

Introduction

Dystonia is a movement disorder defined by the presence of sustained or intermittent muscle contractions that cause abnormal movements and postures.[1] Dystonia appears in the setting of non-degenerative syndromes, affecting a neural network involving basal ganglia, cerebellum, and other brain structures, or as a manifestation of several neurodegenerative disorders.[2] The temporal pattern can distinguish between progressive and static dystonias.[1] Disease progression can be measured in terms of dystonia intensity and/or involvement of other muscles groups.[1] Typically, neurodegenerative dystonias are progressive but non-degenerative isolated dystonias may also display a progressive course.[2] Neuroimaging may support the diagnosis of the neurodegenerative group, by showing reduced volume or altered signals of the basal ganglia.[3] The most significant example is represented by neurodegeneration with brain iron accumulation, a group of genetic disorders displaying progressive iron accumulation in the basal ganglia, which present with dystonia as one of the most prominent clinical features often in combination with other neurological signs (e.g. parkinsonism, pyramidal signs and chorea).[4] Nevertheless, not all the neurodegenerative dystonias have specific brain imaging hallmarks.

Several lines of evidence suggest that dysregulation of the endolysosomal and autophagic systems is linked to the pathogenesis of dystonia.[5] Dystonic features are part of the clinical presentation of many lysosomal storage disorders (e.g. Niemann-Pick type C, neuronal ceroid lipofuscinosis, gangliosidosis, fucosidosis, etc).[5] In addition, genetic defects affecting proteins of the endolysosomal and autophagic pathways can cause neurological diseases mainly characterized by dystonia. Notable examples of this group are complex dystonia syndromes caused by mutations in *WDR45*,[6] *ATP13A2*,[7] *VAC14*,[8,9] *IRF2BPL*[10] and *SQSTM1*.[11] Remarkably, the endolysosomal-autophagic pathway is already known to play a critical role in the pathogenesis of other neurodegenerative movement disorders. The most notable example is Parkinson's disease, which can be associated with mutations of the lysosomal genes *GBA*, *VPS35* and *ATP13A2*.[12]

Lysosomes are dynamic cytoplasmic organelles at the crossroad of endocytic, autophagic and phagocytic trafficking pathways. Fusion with these other organelles results in the formation of hybrid structures, in which the degradation of macromolecules and wasted cellular components occurs and from which lysosomes are reformed.[13] Autophagy is a self-degradative cellular process that is critical for balancing energy supplies in response to nutrient deprivation. It also plays a housekeeping role in removing misfolded or aggregated proteins and clearing damaged organelles.[14] The homotypic fusion and protein sorting (HOPS) complex is the structural bridge necessary for the fusion of late endosomes and autophagosomes with the lysosomes in the cytoplasm.[15] The HOPS complex is composed of the four 'Vps-C core' proteins (VPS11, VPS16, VPS18 and VPS33A) and two additional subunits (VPS39 and VPS41) (Fig. 1).[15]

Genetic and clinical findings

Recent publications linked mutations in genes encoding the HOPS complex with the aetiopathogenesis of inherited dystonias (i.e. *VPS16*, *VPS41* and *VPS11*).[16–19]

A single homozygous and several heterozygous *VPS16* mutations were identified in patients affected by dystonia.[16,18,20,21]

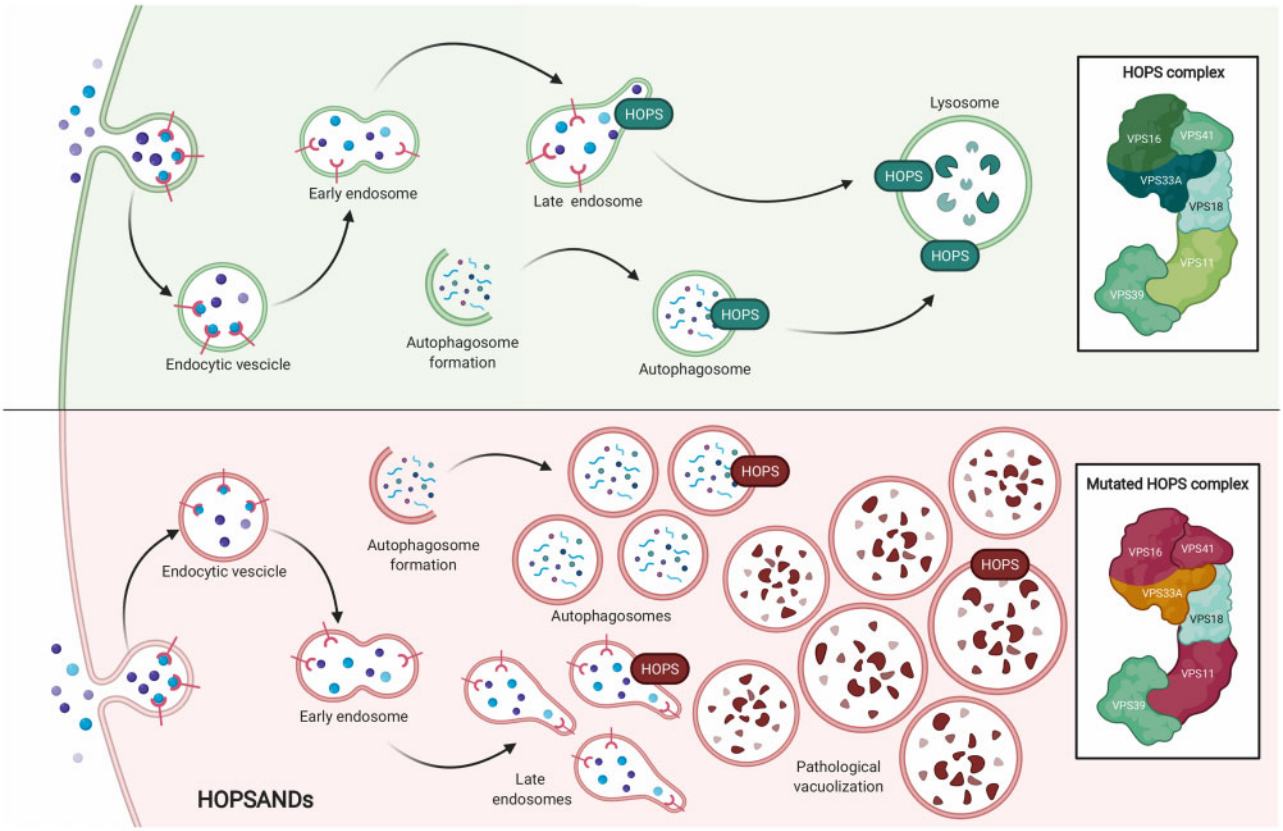

Figure 1 Model of the disease mechanism. Mutations of *VPS16*, *VPS41 and VPS11* cause dysfunction of the HOPS complex, leading to a defect in the fusion of lysosomes with autophagosomes and accumulation of abnormal lysosomal and autophagic vesicles. Adapted from the template 'Mutation of HOPS Complex Subunits', by BioRender.com (2020).

VPS16 mutations were found using different genetic strategies. The homozygous mutation was found to co-segregate with juvenile-onset progressive generalized dystonia in a large consanguineous family from China using a combined approach of whole-exome sequencing and homozygosity mapping.[18] In contrast, the heterozygous *VPS16* mutations were initially identified in 19 dystonic patients from 14 families, starting from a weighted burden analysis of whole-exome sequencing data derived from 138 patients with generalized dystonia. Several of these deleterious variants were then confirmed to co-segregate with dystonia in multigenerational families displaying a dominant pattern of inheritance with incomplete penetrance.[16] Two additional heterozygous carriers have since been identified through screening of the *VPS16* gene by two different groups.[20,21] Clinically, the majority of subjects harbouring a *VPS16* mutation display early-onset dystonia with prominent oromandibular, bulbar, cervical and upper limb involvement, followed by progressive generalization. The course of the diseases was slowly progressive in most patients, who retained the ability to walk in adulthood. Interestingly, some patients responded favourably to deep brain stimulation. On brain MRIs, four of these patients showed bilateral and symmetrical hypointensity of the globus pallidus in T_2-weighted sequences, suggesting possible iron deposition.[16]

Biallelic *VPS41* mutations were initially found independently by two different groups reporting three patients affected by dystonia in more complex phenotypes.[16,17] First, compound heterozygous mutations were found through whole-exome sequencing in two siblings displaying dystonia, ataxia and retinal dystrophy.[17] Another patient carrying a homozygous splicing disrupting *VPS41* mutation was identified through screening of genes encoding for a selected group of HOPS proteins (i.e. *VPS18*, *VPS39* and *VPS41*).[16] He presented with global developmental delay, generalized dystonia, optic atrophy and axonal neuropathy.[16] Brain MRI of all subjects showed progressive cerebellar atrophy and thinning of the corpus callosum. Interestingly, bilateral T_2-weighted hypointensity in the globus pallidus appeared in a subsequent brain MRI of one of the two siblings, possibly indicating neurodegeneration with brain iron accumulation.[17] Recently, nine affected individuals from five unrelated families were found to carry deleterious *VPS41* homozygous variants. All these patients presented with a progressive neurodevelopmental disorder characterized by cognitive impairment, cerebellar atrophy and motor dysfunction with dystonia and ataxia.[22]

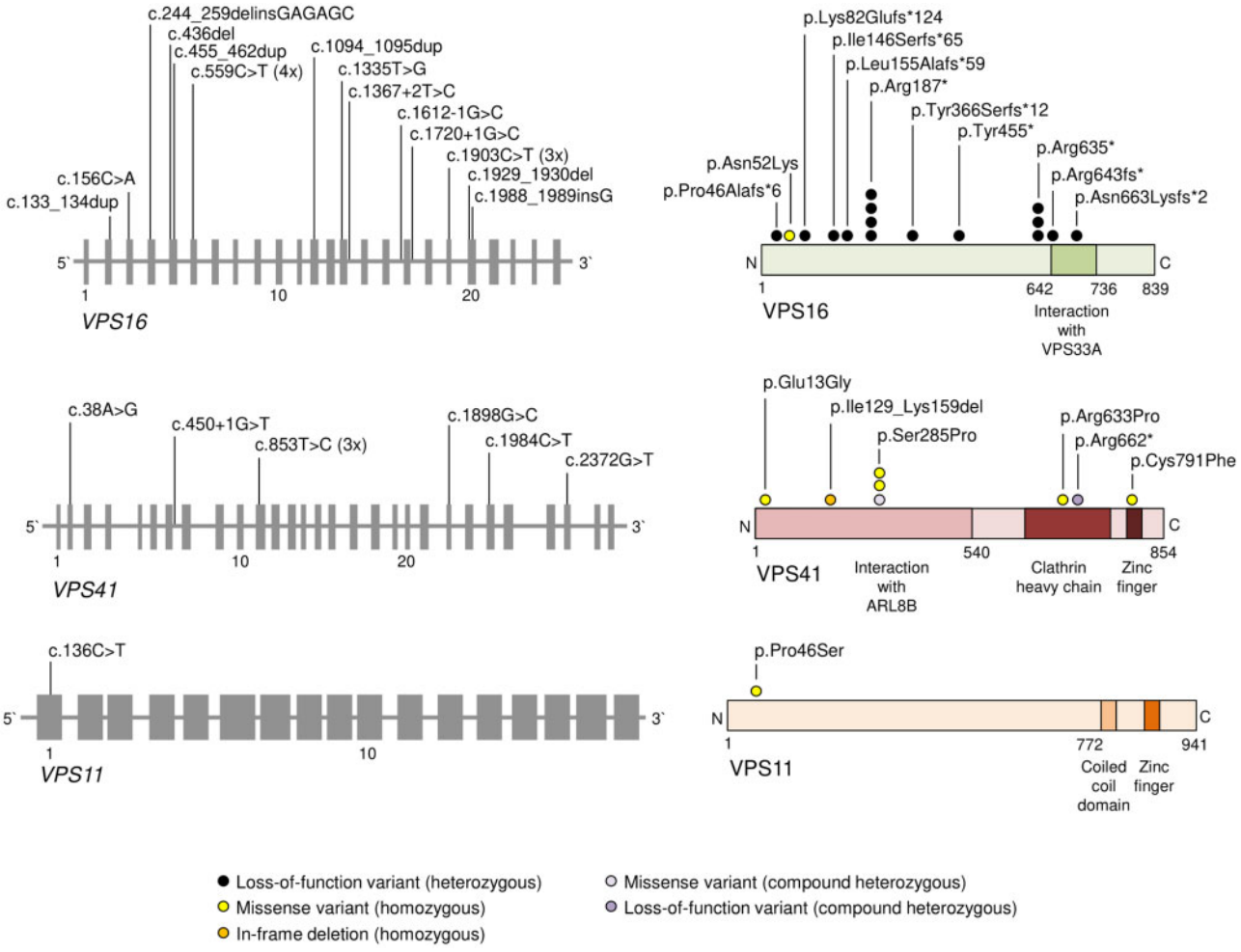

Figure 2 Schematic representations of *VPS16*, *VPS41* and *VPS11* mutations identified in dystonia patients to date. Graphical view of reported dystonia-causing variants in *VPS16*[16,18,20,21] (including unpublished data), *VPS41*[16,17,22,30] and *VPS11*.[19] Three heterozygous *VPS16* splice-site mutations, whose effect was not determined at the protein level, are only shown in the gene structure graphic. A *VPS16*-involving microdeletion[16] is not illustrated. The heterozygous *VPS16* p.Arg187* and p.Arg635* mutations were identified in four and three independent families, respectively (including unpublished data).[16] The *VPS41* p.Ser285Pro mutation was found in three unrelated families (two homozygous families and one compound heterozygous family with an additional pathogenic allele).[22] The positions of the functional protein domains annotated in the UniProt database are also shown.

Table 1 Clinical phenotypes of genes encoding HOPS complex associated with dystonia

Gene	Disease phenotype (inheritance)	No. of families reported	Typical age of onset	Motor disorder	Intellectual disability	Other features found in all or most	Other clinical features occasionally reported	Typical MRI findings	Histopathology
VPS11	Hypomyelinating leukodystrophy (AR)	8	Infancy	Spasticity; opisthotonic posturing	Profound	Epilepsy; optic neuropathy	Autonomic dysfunction; hearing loss	Hypomyelination; cerebellar atrophy; thin corpus callosum	Abnormal vacuolation
	Dystonia (AR)	1	Adulthood	Dystonia	Absent	None	None	T_2 hypointensity of globi pallidi; mild brain atrophy	Abnormal vacuolation
VPS16	Dystonia (AD)	19	Adolescence	Dystonia	Absent to moderate	None	Psychiatric/ behavioural abnormalities	T_2 hypointensity of globi pallidi in some cases; mild brain atrophy	Subtly increased vacuoles
	Dystonia (AR)	1	Adolescence	Dystonia	Absent	None	None	Normal	Unknown
VPS41	Neurological disorder (AR)	7	Infancy	Dystonia; ataxia	Severe	Optic neuropathy	Peripheral neuropathy; epilepsy	Cerebellar atrophy; thin corpus callosum; T_2 hypointensity of globi pallidi in some cases	Abnormal vacuolation

AD = autosomal dominant; AR = autosomal recessive.

A novel homozygous *VPS11* variant was found in a single patient with adult-onset progressive generalized dystonia and prominent bulbar involvement from a consanguineous family through a combined approach of homozygosity mapping and whole-exome sequencing analysis.[19] Interestingly, brain MRI showed bilateral hypointensity in the globus pallidus in a fast-field echo sequence.[19] Biallelic *VPS11* mutations were already associated with a severe infantile neurogenetic disorder called hypomyelinating leukodystrophy 12, indicating that at least two different phenotypes are associated with mutations of this gene.[23]

Both *VPS41*- and *VPS11*-associated diseases seem to be very rare in large unselected cohorts of whole-exome-sequenced individuals with dystonia,[24] whereas *VPS16*-associated disease accounts for up to 4% of cases in some cohorts of genetically unresolved generalized dystonia.[25] At least two disease-causing *VPS16* alleles (p.Arg187* and p.Arg635*) were found recurrently among European patients with generalized dystonia, suggesting the existence of population-specific founder effects.

The fact that brain MRI of some patients carrying HOPS-associated gene mutations display basal ganglia involvement, possibly compatible with brain iron accumulation, is intriguing. However, brain MRI imaging of future identified patients with the same genetic lesions are warranted to corroborate this observation. Moreover, neuropathological studies will be necessary to definitively establish the nature of the observed MRI abnormalities in this specific group of neurological disorders. A summary of the genetic and clinical characteristics of these dystonic disorders is presented in Fig. 2 and Table 1.

Biallelic mutations of *VPS33A*, encoding one of the remaining HOPS complex subunits, have already been associated with a human disease known as mucopolysaccharidosis-plus syndrome (MPSPS), which presents with an early lethal phenotype characterized by severe neurological impairments, respiratory and cardiac issues, anaemia, dysostosis multiplex and renal involvement. The *VPS18* and *VPS39* genes have not been associated with a human genetic disorder yet. Despite a candidate gene approach being used to search for rare deleterious variants in these two genes in available dystonia genetic databases, no pathogenic variants were found.[16] Interestingly, the VPS18 conditional knock-out mouse showed severe neurodegeneration and neuronal migration defects, with evidence of autophagy block and lysosomal abnormalities.[26] Phenotypically, neural-specific VPS18-deficient mice displayed severe postnatal growth retardation and died prematurely. No dystonic features were reported.[26] Similarly, neither *Vps16* nor *Vps41* mutant mice displayed dystonia, suggesting that the human dystonic phenotype may not be fully recapitulated by these models.[17,18]

Disease mechanisms

Functional studies conducted on patient-derived fibroblasts carrying *VPS16*, *VPS41* and *VPS11* mutations displayed clear overlapping abnormalities of the lysosomal and autophagic compartments.[16,19] Electron microscopy of *VPS16*-, *VPS41*- and *VPS11*-mutated fibroblasts showed large clustered vacuolar structures, with or without inclusions, suggestive of an alteration in these pathways.[16,17,19] In addition, a marked increase in lysosomal enzyme quantity and activity was observed in *VPS11*-mutated fibroblasts.[19] Interestingly, the activity of the same lysosomal hydrolases was also raised at the plasma membrane level, suggesting a possible exocytosis of these accumulated enzymes.[19] Moreover, in these same *VPS11*-mutated fibroblasts, increased expression of the autophagic proteins p62 and LC3B, without a proportional raise of beclin-1 levels, indicated an accumulation of autophagosomes without autophagy induction, suggesting an impairment of the autophagy flux.[19] All

of the evidence combined from genetic and functional studies supports the hypothesis that the identified mutations are loss-of-function, damaging the function of the HOPS complex and hence impairing the fusion of late endosomes and autophagosomes with the lysosomes (Fig. 1).

Notably, cultured fibroblasts of MPSPS patients (*VPS33A* mutation) also displayed the typical vacuolations of HOPS-related disorders.[27] Moreover, plasma lysosomal enzymatic activities in these patients were raised above the reference range, in line with the observed increase of lysosomal enzymatic activity in *VPS11*-mutated fibroblasts.[19] In view of this, the possible use of lysosomal hydrolase activity in the plasma as a possible diagnostic and prognostic biomarker in HOPS-related disorders should be investigated in future studies.

Final remarks

It remains to be elucidated whether HOPS-associated phenotypes are the result of neurodegeneration or whether they might also be related to disordered early neurodevelopmental processes. Future studies aimed at understanding the exact mechanism linking lysosomal-autophagic dysfunction due to HOPS complex disruption and the dysfunction/degeneration of basal ganglia will shed light on the aetiology and potential therapeutic interventions for these disorders. Possible therapeutic approaches may include autophagy inducers, small-molecule chaperones and/or substrate-reducing molecules, which are already under study for other lysosome-associated disorders.[28,29]

In conclusion, mutations in genes encoding HOPS complex subunits are associated with a novel group of inherited dystonias, which we propose to name HOPS-associated neurological disorders (HOPSANDs). This group of inherited disorders confirms and deepens the connection between the pathogenesis of dystonias and the dysfunction of lysosomes and autophagy, prompting researchers to find innovative therapies targeting these pathways.

Funding

No funding was received towards this work.

Competing interests

The authors report no competing interests.

References

1. Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: A consensus update. *Mov Disord*. 2013;28(7):863–873.
2. Phukan J, Albanese A, Gasser T, Warner T. Primary dystonia and dystonia-plus syndromes: Clinical characteristics, diagnosis, and pathogenesis. *Lancet Neurol*. 2011;10(12):1074–1085.
3. Bekiesinska-Figatowska M, Mierzewska H, Jurkiewicz E. Basal ganglia lesions in children and adults. *Eur J Radiol*. 2013;82(5):837–849.
4. Klein C, Lohmann K, Marras C, Münchau A. Hereditary dystonia overview. In: Adam MP, Ardinger HH, Pagon RH et al., eds. *GeneReviews®*. University of Washington; 2017. https://www.ncbi.nlm.nih.gov/sites/books/NBK1155/
5. Gonzalez-Latapi P, Marotta N, Mencacci NE. Emerging and converging molecular mechanisms in dystonia. *J Neural Transm (Vienna)*. 2021;128(4):483–498.
6. Seibler P, Burbulla LF, Dulovic M, et al. Iron overload is accompanied by mitochondrial and lysosomal dysfunction in WDR45 mutant cells. *Brain*. 2018;141(10):3052–3064.
7. Usenovic M, Tresse E, Mazzulli JR, Taylor JP, Krainc D. Deficiency of ATP13A2 leads to lysosomal dysfunction, α-synuclein accumulation, and neurotoxicity. *J Neurosci*. 2012;32(12):4240–4246.
8. Schulze U, Vollenbröker B, Braun DA, et al. The Vac14-interaction network is linked to regulators of the endolysosomal and autophagic pathway. *Mol Cell Proteomics*. 2014;13(6):1397–1411.
9. Stutterd C, Diakumis P, Bahlo M, et al. Neuropathology of childhood-onset basal ganglia degeneration caused by mutation of VAC14. *Ann Clin Transl Neurol*. 2017;4(12):859–864.
10. Ginevrino M, Battini R, Nuovo S, et al. A novel IRF2BPL truncating variant is associated with endolysosomal storage. *Mol Biol Rep*. 2020;47(1):711–714.
11. Haack TB, Ignatius E, Calvo-Garrido J, et al. Absence of the autophagy adaptor SQSTM1/p62 causes childhood-onset neurodegeneration with ataxia, dystonia, and gaze palsy. *Am J Hum Genet*. 2016;99(3):735–743.
12. Klein AD, Mazzulli JR. Is Parkinson's disease a lysosomal disorder? *Brain*. 2018;141(8):2255–2262.
13. Luzio JP, Pryor PR, Bright NA. Lysosomes: Fusion and function. *Nat Rev Mol Cell Biol*. 2007;8(8):622–632.
14. Glick D, Barth S, Macleod KF. Autophagy: Cellular and molecular mechanisms. *J Pathol*. 2010;221(1):3–12.
15. Ostrowicz CW, Bröcker C, Ahnert F, et al. Defined subunit arrangement and rab interactions are required for functionality of the HOPS tethering complex. *Traffic*. 2010;11(10):1334–1346.
16. Steel D, Zech M, Zhao C, et al.; Genomics England Research Consortium. Loss-of-function variants in HOPS complex genes VPS16 and VPS41 cause early onset dystonia associated with lysosomal abnormalities. *Ann Neurol*. 2020;88(5):867–877.
17. van der Welle R, Jobling R, Burns C, et al. VPS41 recessive mutation causes ataxia and dystonia with retinal dystrophy and mental retardation by inhibiting HOPS function and MTORC1 signaling. *bioRxiv*. [Preprint] doi:10.1101/2019.12.18.867333
18. Cai X, Chen X, Wu S, et al. Homozygous mutation of VPS16 gene is responsible for an autosomal recessive adolescent-onset primary dystonia. *Sci Rep*. 2016;6(1):25834.
19. Monfrini E, Cogiamanian F, Salani S, et al. A novel homozygous VPS11 variant may cause generalized dystonia. *Ann Neurol*. 2021;89(4):834–839.
20. Pott H, Brüggemann N, Reese R, et al. Truncating VPS16 mutations are rare in early onset dystonia. *Ann Neurol*. 2021;89(3):625–626.
21. Li L-X, Jiang L-T, Liu Y, et al. Mutation screening of VPS16 gene in patients with isolated dystonia. *Parkinson Relat Disord*. 2021;83:63–65.
22. Sanderson LE, Lanko K, Alsagob M, et al. Bi-allelic variants in HOPS complex subunit VPS41 cause cerebellar ataxia and abnormal membrane trafficking. *Brain*. 2021;144(3):769–780.
23. Edvardson S, Gerhard F, Jalas C, et al. Hypomyelination and developmental delay associated with VPS11 mutation in Ashkenazi-Jewish patients. *J Med Genet*. 2015;52(11):749–753.
24. Zech M, Jech R, Boesch S, et al. Monogenic variants in dystonia: An exome-wide sequencing study. *Lancet Neurol*. 2020;19(11):908–918.
25. Zech M, Steel D, Kurian MA, Winkelmann J. Reply to "Truncating VPS16 mutations are rare in early-onset dystonia". *Ann Neurol*. 2021;89(3):626.
26. Peng C, Ye J, Yan S, et al. Ablation of vacuole protein sorting 18 (Vps18) gene leads to neurodegeneration and impaired neuronal migration by disrupting multiple vesicle transport pathways to lysosomes. *J Biol Chem*. 2012;287(39):32861–32873.

27. Pavlova EV, Shatunov A, Wartosch L, et al. The lysosomal disease caused by mutant VPS33A. *Hum Mol Genet*. 2019;28(15):2514–2530.
28. Marques ARA, Saftig P. Lysosomal storage disorders - challenges, concepts and avenues for therapy: Beyond rare diseases. *J Cell Sci*. 2019;132(2):jcs221739.
29. Bonam SR, Wang F, Muller S. Lysosomes as a therapeutic target. *Nat Rev Drug Discov*. 2019;18(12):923–948.
30. Li X-Y, Wang L, Guo Y, Wan X-H. Mutations in the VPS16 gene in 56 early-onset dystonia patients. *Mov Disord*. 2021;36(3):780–781.