



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

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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.

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

Abbreviation: HOPS = homotypic fusion and protein sorting

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}

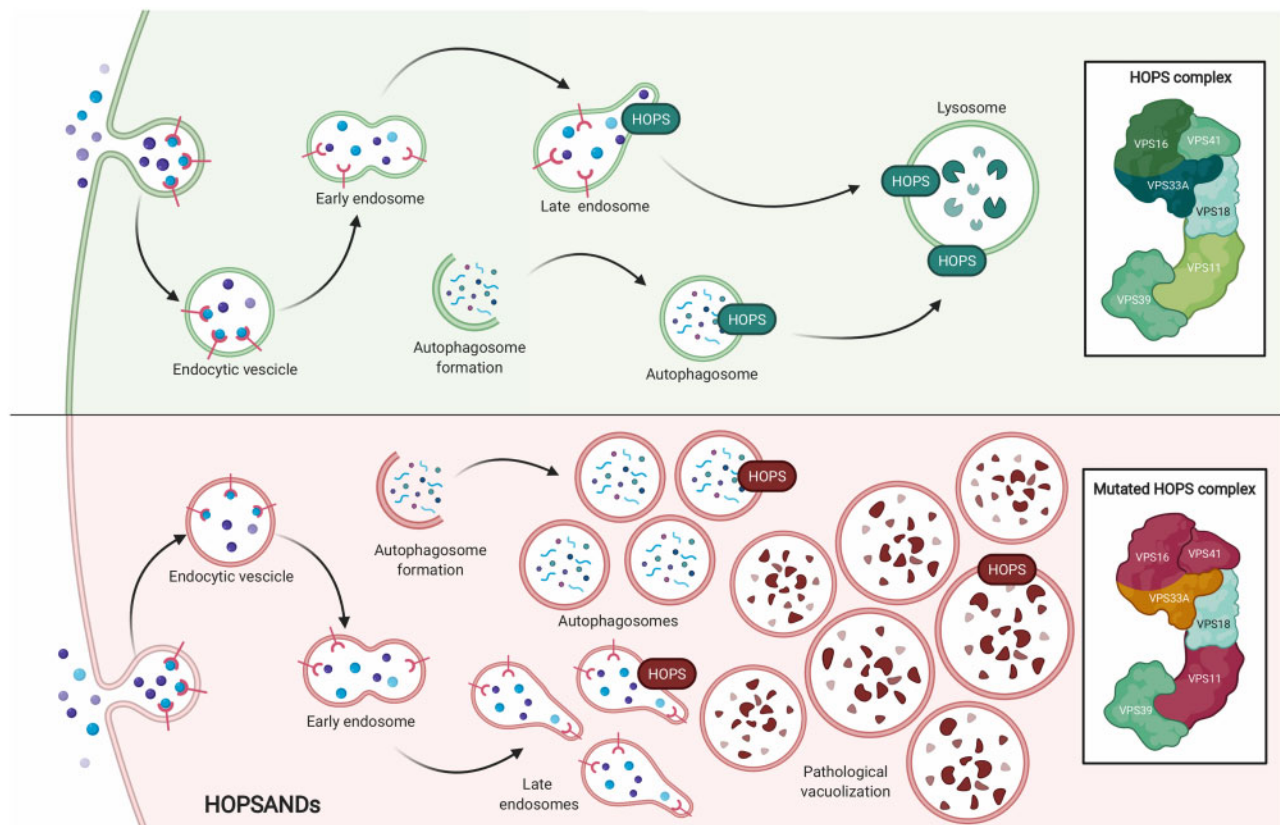


Figure 1 Model of the disease mechanism. Mutations of *VPS16*, *VPS41* and *VPS11* cause dysfunction of the HOPS complex, leading to a defect in the fusion of lysosomes with autophagosomes and accumulation of abnormal lysosomal and autophagic vesicles. Adapted from the template 'Mutation of HOPS Complex 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 T₂-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 T₂-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

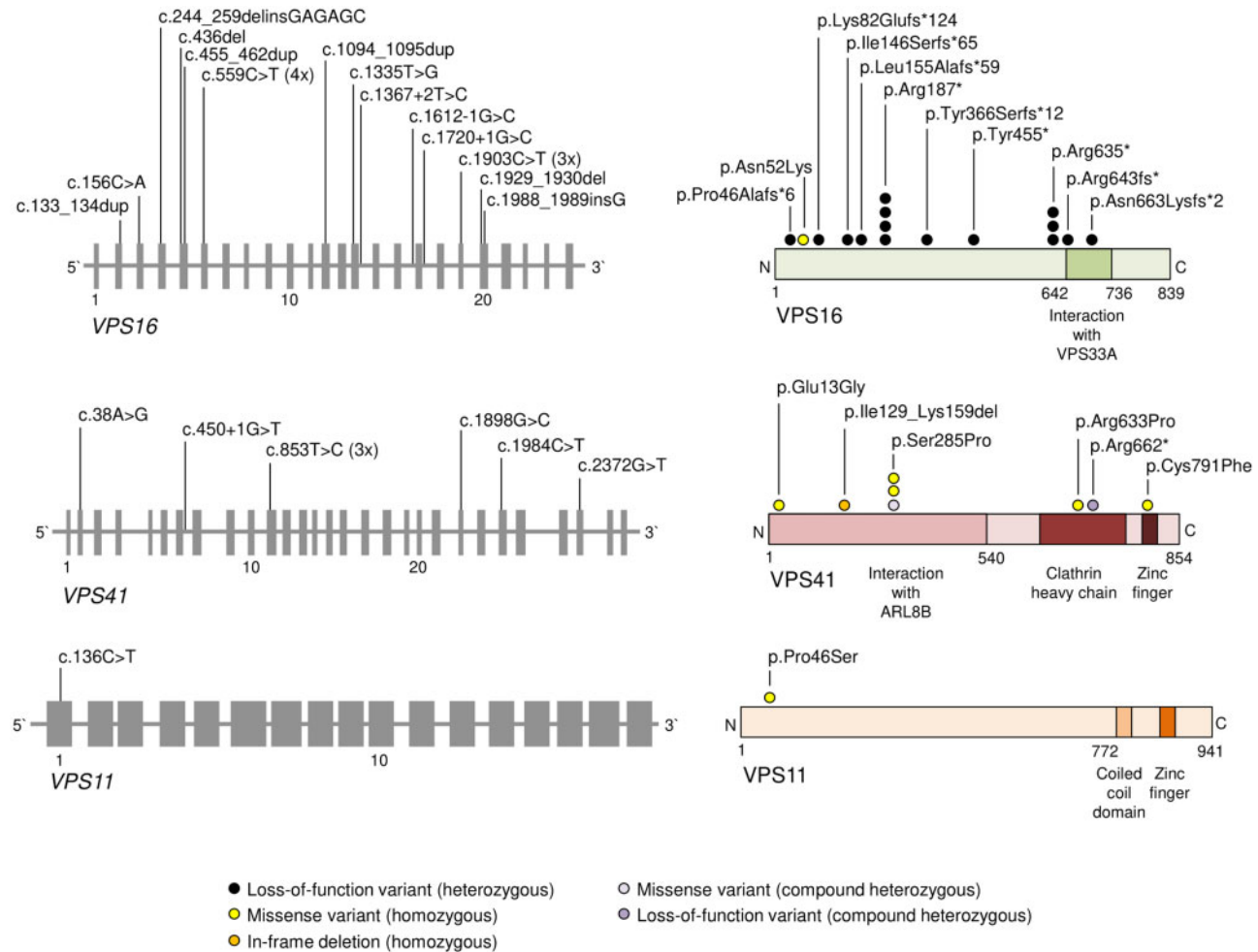


Figure 2 Schematic representations of VPS16, VPS41 and VPS11 mutations identified in dystonia patients to date. Graphical view of reported dystonia-causing variants in VPS16^{16,18,20,21} (including unpublished data), VPS41^{16,17,22,30} and VPS11.¹⁹ Three heterozygous VPS16 splice-site mutations, whose effect was not determined at the protein level, are only shown in the gene structure graphic. A VPS16-involving microdeletion¹⁶ 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
VPS41	Dystonia (AR)	1	Adolescence	Dystonia	Absent	None	None	Normal	Unknown
	Neurological disorder (AR)	7	Infancy	Dystonia; ataxia	Severe	Optic neuropathy	Peripheric 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.

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