UPDATE

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

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Abstract

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 etiopathogenesis 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 HOPS-associated Neurological Disorders (HOPSANDs) this group of diseases, 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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Running title: HOPS-associated neurological disorders

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

Abbreviations: Hypomyelinating Leukodystrophy 12 = HLD12; homotypic fusion and protein sorting complex = HOPS complex; HOPSANDs = HOPS-associated Neurological Disorders; lysosomal storage disorders = LSDs; Mucopolysaccharidosis-Plus Syndrome = MPSPS; neurodegeneration with brain iron accumulation = NBIA; whole-exome sequencing = WES.

Introduction

Dystonia is a movement disorder defined by the presence of sustained or intermittent muscle contractions causing 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] 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 also non-degenerative isolated dystonias may display a progressive course.[2] Neuroimaging may support the diagnosis of the neurodegenerative group, by showing reduced volume or altered signal of the basal ganglia.[3] The most significant example is represented by neurodegeneration with brain iron accumulation (NBIA), 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 system 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 et cetera).[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 of *WDR45*[6], *ATP13A2*[7], *VAC14*[8,9], *IRF2BPL*[10], and *SQSTM1*[11] genes.[5] 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 re-formed.[13] Autophagy is a self-degradative cellular process 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] HOPS complex is composed by the four "Vps-C core" proteins (i.e., Vps11, Vps16, Vps18, and Vps33a) and two additional subunits (i.e., Vps39 and Vps41).[15]

Genetic and clinical findings

Recent publications linked mutations in genes encoding for the HOPS complex with the etiopathogenesis 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.[18,16,20,21] *VPS16* mutations were found with different genetic strategies. The homozygous mutation was found to cosegregate with juvenile-onset progressive generalized dystonia in a large consanguineous family from China using a combined approach of whole-exome sequencing (WES) 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 WES data derived from 138 patients with generalized dystonia. Several of these deleterious variants were then confirmed to cosegregate with dystonia in multigenerational families displaying a dominant pattern of inheritance with incomplete penetrance.[16] Two additional heterozygous carriers have since been identified through a screening of *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. Four of these patients showed on brain MRI 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.[17,16] Firstly, compound heterozygous mutations were found through WES 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] Very 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]

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 WES analysis.[19] Interestingly, brain MRI showed bilateral hypointensity in the globus pallidus in Fast Field Echo (FFE) sequence.[19] Biallelic *VPS11* mutations were already associated with a severe infantile neurogenetic disorder, called Hypomyelinating Leukodystrophy 12 (HLD12), 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 generalized dystonia patients, suggesting the existence of population-specific founder effects.

The fact that brain MRI of some patients carrying HOPS-associated genes mutations displays involvement of basal ganglia, possibly compatible with brain iron accumulation, is very 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 Figure 2A and B.

Biallelic mutations of *VPS33A*, encoding for one of the remaining HOPS complex subunit, have been already associated with a human disease known as Mucopolysaccharidosis-Plus Syndrome (MPSPS) which presents with an early lethal phenotype characterized by severe neurological impairment, respiratory and cardiac issues, anaemia, dysostosis multiplex and renal involvement. *VPS18* and *VPS39* genes have not been associated with a human genetic disorder yet. Despite a candidate gene approach was used to search for rare deleterious variants in these two genes in available dystonia genetic databases, no pathogenic variants were found.[16] Interestingly, 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 of these pathways.[17,16,19] In addition, a marked increase of lysosomal enzymes quantity and activity was observed in *VPS11*-mutated fibroblasts.[19] Interestingly, the activity of the same lysosomal hydrolases was raised also at the plasma membrane level, suggesting a possible exocytosis of these accumulated enzymes.[19] Moreover, in these same *VPS11*-mutated fibroblasts, an increased expression of 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 the evidence combined from genetic and functional studies supports the hypothesis that the identified mutations are loss-of-function, damaging the function of HOPS complex hence impairing the fusion of late endosomes and autophagosomes with the lysosomes.

Notably, also cultured fibroblasts of MPSPS patients (*VPS33A* mutation) 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 hydrolases activity in 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 the lysosomal-autophagic dysfunction due to HOPS complex disruption and the dysfunction/degeneration of basal ganglia will shed light on the etiology and potential therapeutic interventions in 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 for 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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Figure legends

Figure 1 Cartoon model of disease mechanism. Mutations of *VPS16*, *VPS41* an *VPS11* cause a dysfunction of the HOPS complex leading to a defect of 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).

Figure 2 Schematic representations of *VPS16*, *VPS41*, and *VPS11* mutations identified in dystonia patients to date. (**A**) Graphical view of reported dystonia-causing variants in *VPS16*[18,16,20,21] (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 (in two families in a homozygous state and in one family in compound heterozygosity with an additional pathogenic allele).[22] The positions of functional protein domains annotated in the UniProt database are also shown.

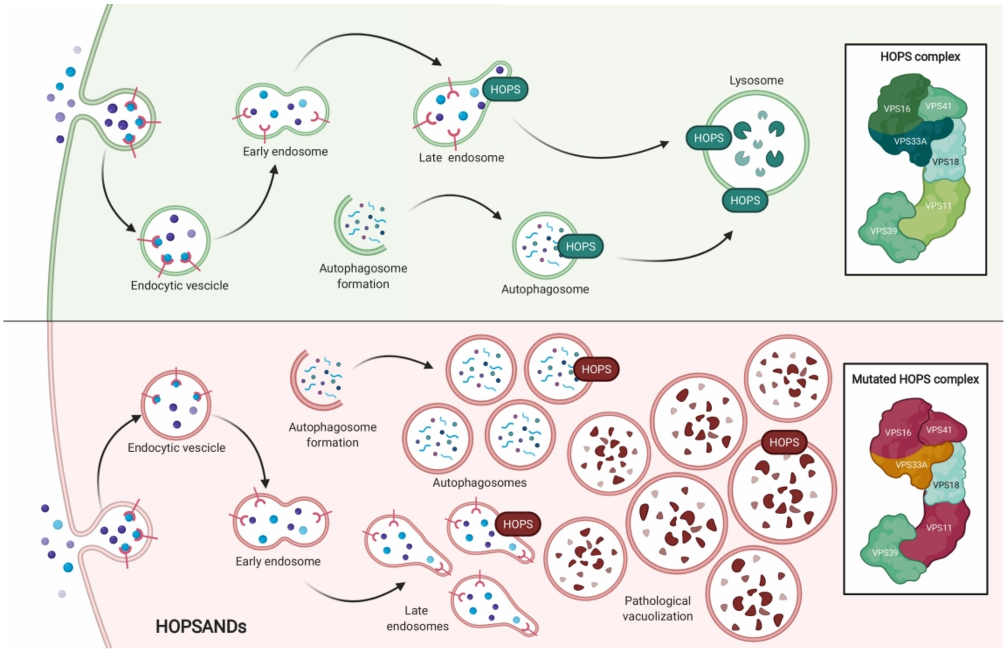

Figure 1: Cartoon model of disease mechanism: Mutations of VPS16, VPS41 an VPS11 cause a dysfunction of the HOPS complex leading to a defect of the fusion of lysosomes with autophagosomes and accumulation of abnormal lysosomal and autophagic vesicles. Adapted from the template "Mutation of HOPS Complex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

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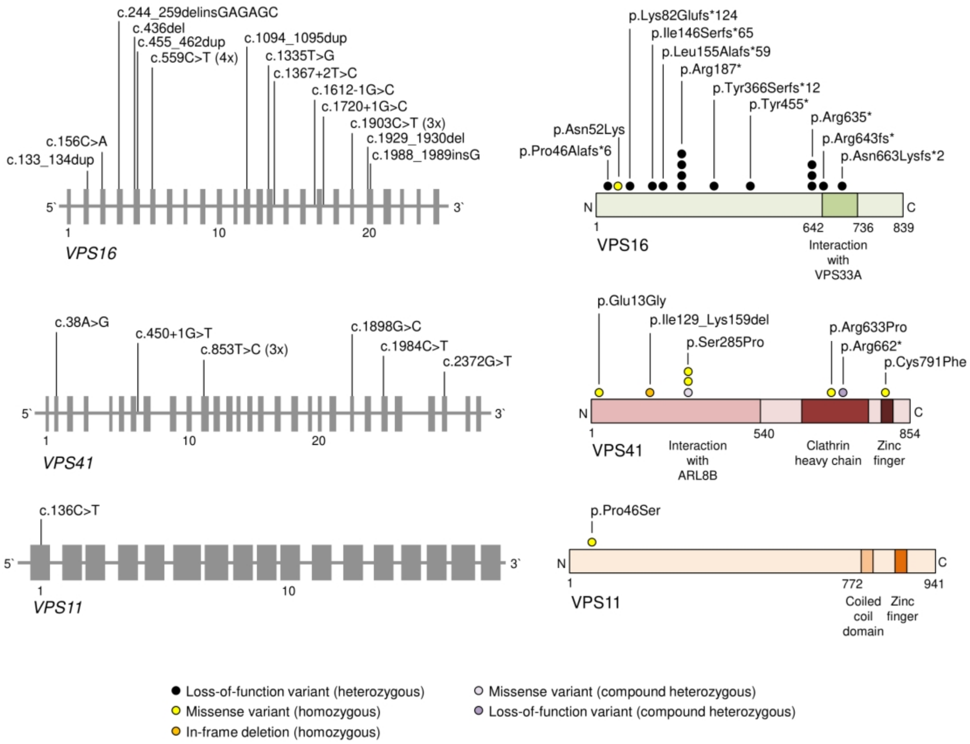

Figure 2 Schematic representations of VPS16, VPS41, and VPS11 mutations identified in dystonia patients to date. (A) Graphical view of reported dystonia-causing variants in VPS1618,16,20,21 (including unpublished data), VPS4116,17,22,30, and VPS11.19 Three heterozygous VPS16 splice-site mutations, whose 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 (in two families in a homozygous state and in one family in compound heterozygosity with an additional pathogenic allele).22 The positions of functional protein domains annotated in the UniProt database are also shown.

705x529mm (72 x 72 DPI)

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	T2 hypointensity of globi pallidi; mild brain atrophy	Abnormal vacuolation
VPS16	Dystonia (AD)	19	Adolescence	Dystonia	Absent to moderate	None	Psychiatric/behavioural abnormalities	T2 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; T2 hypointensity of globi pallidi in some cases	Abnormal vacuolation

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