

Loss-of-Function Variants in HOPS Complex Genes *VPS16* and *VPS41* Cause Early Onset Dystonia Associated with Lysosomal Abnormalities

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Objectives: The majority of people with suspected genetic dystonia remain undiagnosed after maximal investigation, implying that a number of causative genes have not yet been recognized. We aimed to investigate this paucity of diagnoses.

Methods: We undertook weighted burden analysis of whole-exome sequencing (WES) data from 138 individuals with unresolved generalized dystonia of suspected genetic etiology, followed by additional case-finding from international databases, first for the gene implicated by the burden analysis (*VPS16*), and then for other functionally related genes. Electron microscopy was performed on patient-derived cells.

Results: Analysis revealed a significant burden for *VPS16* (Fisher's exact test p value, 6.9×10^9). *VPS16* encodes a subunit of the homotypic fusion and vacuole protein sorting (HOPS) complex, which plays a key role in autophagosome-lysosome fusion. A total of 18 individuals harboring heterozygous loss-of-function *VPS16* variants, and one with a microdeletion, were identified. These individuals experienced early onset progressive dystonia with predominant cervical, bulbar, orofacial, and upper limb involvement. Some patients had a more complex phenotype with additional neuropsychiatric and/or developmental comorbidities. We also identified biallelic loss-of-function variants in *VPS41*, another HOPS-complex encoding gene, in an individual with infantile-onset generalized dystonia. Electron microscopy of patient-derived lymphocytes and fibroblasts from both patients with *VPS16* and *VPS41* showed vacuolar abnormalities suggestive of impaired lysosomal function.

Interpretation: Our study strongly supports a role for HOPS complex dysfunction in the pathogenesis of dystonia, although variants in different subunits display different phenotypic and inheritance characteristics.

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Dystonia is a common movement disorder associated with significant disability and increased risk of mortality.^{1,2} It is characterized by sustained or episodic muscle contractions, which cause abnormal, often repetitive movements and twisting postures affecting the limbs, trunk, neck, and face.³ Despite significant advances in next-generation sequencing technologies, over 85% of people with suspected genetic dystonia remain undiagnosed after whole-genome sequencing,⁴ implying that the majority of genetic dystonias currently remain unrecognized. The reasons for this are multifactorial, attributed to locus heterogeneity, incomplete disease penetrance, and the current limitations of next-generation sequencing technologies.

Here, we report a cohort of individuals with loss-of-function (LOF) mutations in 2 components of the homotypic fusion and vacuole protein sorting (HOPS) complex, a

highly conserved complex required for endosome-lysosome and autophagosome-lysosome fusion.⁵ We describe a series of patients with generalized dystonia associated with heterozygous LOF variants in *VPS16* and also report biallelic LOF variants in a second HOPS complex gene, *VPS41*, in a child with a severe infant-onset dystonic disorder.

Subjects and Methods

Generalized Dystonia Cohort for Burden Analysis

A consecutive series of 138 unrelated individuals with generalized dystonia (57 men and 81 women, all self-identifying as European) was recruited into the study. Diagnoses were established in accordance with the dystonia consensus criteria³ at movement disorder specialty centers in Austria, Czechia, and Germany. The clinical characteristics of the cohort are

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Additional supporting information can be found in the online version of this article.

summarized in Supplementary Table S1. We excluded patients from the cohort who had (i) a known genetic diagnosis or (ii) an acquired form of the disease.

Whole-Exome Sequencing

The generalized dystonia cohort underwent whole-exome sequencing (WES) at the Helmholtz Center Munich (Munich, Germany) according to previously described methods.⁶ In brief, the exonic portions of genomic DNA were enriched in solution and indexed with Agilent (Agilent Technologies) SureSelect Human All Exon kits, version 5 and 6. Sequencing was carried out as 100-bp paired-end runs with HiSeq2500/4000 equipment (Illumina). Read processing and variant annotation used an in-house pipeline based on BWA, SAMtools, PINDEL, GATK, ExomeDepth, and custom scripts (Helmholtz Center Munich and Technical University of Munich). Variant filtering was done per standard pipeline analyses, integrating data from online repositories (1000 Genomes Project, gnomAD, dbSNP, ClinVar, and HGMD) and in-house control-exome collections. For the 138 exomes, we obtained on average 13.6 Gb of sequence, resulting in a mean read depth of 143.6-fold with 98.6% of the target nucleotides covered at least 20-fold. Sequences were visualized with IGV. Across the cohort, the exome data were used to exclude causative variants in known disease genes, as described.⁶

Case–Control Rare-Variant Collapsing Analysis (Burden Test)

Gene-based collapsing analysis of rare variants in patients with dystonia versus controls was performed using TRAPD (Test Rare vAriants with Public Data),⁷ a robust method for detecting gene-disease associations.⁸ We searched for genes with excess mutational burden by comparing genotype counts from 138 generalized dystonia cases with those from gnomAD control subjects (non-Finish European [NFE] cohort, $N = 64,603$). We coded case and control subjects according to the presence or absence of at least one qualifying variant in any of the ~20,000 consensus coding sequence (CCDS) genes and focused on the following genetic models: (1) dominant LOF, in which qualifying variants were defined as stop-gain, frameshift, and splice-site-altering (± 2 nucleotides of exon boundary) alleles; and (2) dominant non-synonymous, in which qualifying variants were defined as LOF and missense alleles. The minor allele frequency (MAF) threshold of qualifying variants was set at < 0.0005 , with the frequency of minor alleles determined from gnomAD (NFE cohort) and ~4,000 non-neurological in-house control exomes for variants present in dystonia case subjects, and in gnomAD (NFE cohort) for variants present in control subjects. To detect differences in the carrier rate of qualifying variants between case and control subjects, we used a one-sided Fisher's exact test. Exome-wide significance was defined at a p value of $< 1.25 \times 10^{-6}$, correcting for ~20,000 CCDS genes studied in two individual case-control comparisons. To avoid spurious results, we undertook extensive quality control and harmonization analyses, as described earlier^{7,8}: (i) variants overlapping low-complexity regions were filtered out; (ii) sites with a read depth of < 10 -fold in either of the 2 cohorts were ignored; and (iii) rare synonymous variant burden

testing was conducted. On the basis of the latter, only the top 85% of sites in terms of quality-by-depth (QD) scores for the case sequencing cohort and the top 95% of sites in terms of QD scores for gnomAD were included in the analysis.

Identification of Additional Cases

Using GeneMatcher⁹ and direct communications, international collaborators were requested to screen their genomic databases for additional cases. Details of the WES/whole genome sequencing (WGS) methods used differed slightly among each center and can be provided on request. Cases were identified from the UCL Great Ormond Street Institute of Child Health neurogenetic movement disorders cohort (London, UK); the Kolling Institute of Medical Research (Sydney, Australia); the Carlo Besta Neurological Institute (Milan, Italy); the Koios Database of the Queen Square Genomics Group at University College London (London, UK); the Genomics England 100 K Genomes Project dataset (UK), and Radboud University Medical Centre (Nijmegen, The Netherlands). Databases from Cardiff (Wales) and Dublin (Ireland) were also checked but no additional cases were found there. Variants identified through WGS or WES and familial segregation were verified by Sanger sequencing. Details of protocols, reagents, and primer sequences are available on request. All variants are given with reference to the GRCh38 build.

We subsequently undertook a targeted search of the databases above for any additional individuals with mutations affecting other HOPS complex genes not previously associated with disease, namely *VPS18*, *VPS39*, and *VPS41*.

Electron Microscopy

Whole blood samples were obtained in EDTA and centrifuged to produce a buffy coat. Patient fibroblasts were obtained from skin biopsies and cultured in Ham's F10 medium with 12% fetal calf serum. Penicillin and streptomycin were added to the medium for transfer of fibroblasts. Following culture, cells were disaggregated using 0.2% trypsin for microscopy, then centrifuged to form solid clusters. Clusters were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer followed by secondary fixation in osmium tetroxide. Tissues were dehydrated in graded ethanol, transferred to an intermediate reagent, propylene oxide, and then infiltrated and embedded in Agar 100 epoxy resin. Polymerization was undertaken at 60°C for 48 hours. Ninety (90) nm ultrathin sections were cut using a Diatome diamond knife on a Leica Ultracut UCT microtome. Sections were transferred to copper grids and stained with alcoholic uranyl acetate and Reynolds lead citrate. The fibroblasts were examined using a JEOL 1400 transmission electron microscope.

Ethics

Ethical approval for genetic research was obtained by each center separately as follows: Great Ormond Street (Family 7): approved by the London Bloomsbury Research Ethics Committee (ref: 13

/LO/0168); Generalized dystonia cohort including Families 1–6: all subjects provided written informed consent, and the study protocol was approved by the institutional ethics review boards at the Technical University of Munich, Medical

University Innsbruck, and Charles University in Prague; Kolling Institute of Medical Research, Northern Sydney Local Health District (Families 8 and 9); reference number RESP/15/314, HREC/15/HAWKE/434; UCL Queen's Square Institute of Neurology, London (Family 10); other families recruited from the Carlos Besta Neurology Institute, Milan (Families 11 and 12); Radboud University Medical Centre, Nijmegen (Family 13); and Genomics England (the 100 K Genomes Project; Family 14) under ethical approval gained by those institutions.

Written informed consent was obtained from patients or their legal guardians for participation with separate consent for publication of recognizable images/videos or invasive procedures, such as skin biopsy where appropriate.

Results

Weighted Burden Analysis and Case Identification

The weighted burden analysis of 138 individuals with etiologically unresolved generalized dystonia (Supplementary Table S1) identified a single study-wide significant signal, *VPS16*, with a Fisher's exact test p value of 6.9×10^{-9} (Fig 1, Supplementary Table S2). In addition to

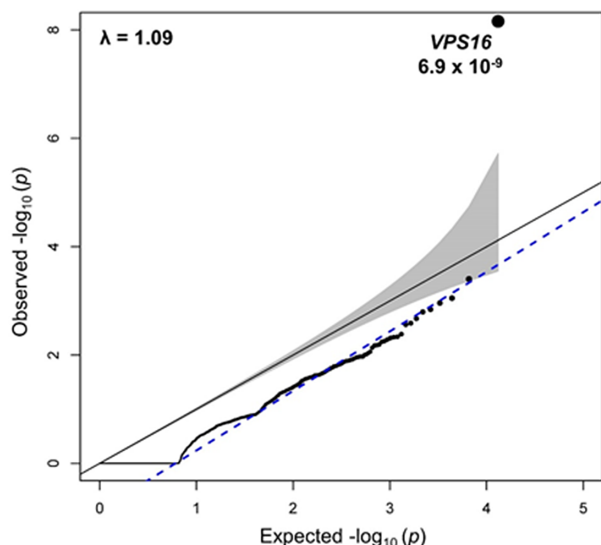


FIGURE 1: Weighted burden analysis. Expected versus observed p values of the loss-of-function model are shown for exome-wide gene collapsing analysis in a cohort of 138 individuals with generalized dystonia and gnomAD controls (64,603 non-Finish European subjects). A Fisher's exact test was used to determine differences in the carrier rate of qualifying variants between cases and controls. Qualifying variants were defined as stop-gain, frameshift, and essential splice-site variants with a minor allele frequency of < 0.0005 , whereas exome-wide significance was set to a p value of $< 1.25 \times 10^{-6}$ (Bonferroni-corrected threshold, see Methods). We observed a significant mutational burden (minimal genomic inflation) for *VPS16*, in which 5 individuals with dystonia had a qualifying variant. The signal and corresponding Fisher's exact test p value for *VPS16* is indicated. [Color figure can be viewed at www.annalsofneurology.org]

5 heterozygous LOF alleles uncovered in the burden test (carrier rate of 3.6% in case subjects), we found one individual with a *VPS16*-encompassing microdeletion in the cohort. Through international collaboration, an additional 13 cases with heterozygous LOF variants in *VPS16* were identified. All 19 patients (from 14 families) had *VPS16* variants predicted to result in haploinsufficiency (Table 1, Supplementary Table S3). One proband (Patient 14) had a second, non-truncating *VPS16* variant but phasing of the variants could not be established as parental samples were unavailable. For the other 18 probands, detailed genomic analysis did not identify a second potentially pathogenic *VPS16* variant. Moreover, 9 individuals from 5 multigenerational families (Families 3, 7, 8, 9, and 13) confirmed a clearly dominant pattern of disease inheritance (Fig 2). Segregation analysis was possible in 9 families: of these, de novo occurrence was confirmed in 1 family; inheritance from a symptomatic parent was found in 4 families; and inheritance from an apparently nonmanifesting parent in 4 families, indicating incomplete penetrance.

Clinical Features of Patients With *VPS16*

Affected individuals presented with a progressive early onset dystonia (median age 12 years, range 3–50 years), with prominent oromandibular, bulbar, cervical, and upper limb involvement (Fig 3A, Tables 2 and 3). Progressive generalization ensued in most cases, although most remained ambulant, and only a minority (16%) lost the ability to walk in adulthood (Supplementary Videos S1–S3). Additional clinical features of mild to moderate intellectual disability and neuropsychiatric symptoms were present in approximately one-third of patients, and 50% of families had a positive family history of dystonia (Supplementary Table S4). A degree of interfamilial and intrafamilial phenotypic variability was evident, both with regard to age of symptom onset and dystonia severity. Routine diagnostic testing was unremarkable. In 4 individuals, magnetic resonance imaging (MRI) showed bilateral and symmetrical hypointensity of the globi pallidi and sometimes also the midbrain and dentate nuclei on MRI sequences known to demonstrate susceptibility (T2-weighted, T2*-weighted, and susceptibility-weighted datasets), suggestive of iron deposition.¹⁰ Mild generalized cerebral atrophy was also apparent in 4 individuals. Although not grossly abnormal, caudate nuclei and putamina appeared relatively small and bright on T2 (Fig 3B, see Supplementary Table S4). Some patients had a partial response to levodopa, trihexyphenidyl, and/or botulinum toxin type A injections. Deep-brain stimulation was also beneficial for some, but not all patients; sustained improvement in motor and disability scores for the Burke-Fahn-Marsden Dystonia Rating Scale were observed for Patient 7C (Supplementary Video S4, Supplementary Table S5).

TABLE 1. Genomic Characteristics of VPS16 Variants Identified

Genomic position	cDNA variant	Effect on protein	Variant type	Family number
chr20:2835462–3974387	NA	Microdeletion	CNV	6
chr20:2860515	c.436del	p.Ile146Serfs*65	Frameshift	9
chr20:2860534–2860541	c.455_462dup	p.Leu155Alafs*59	Frameshift	13
chr20:2860792	c.559C > T	p.Arg187*	Stop-gain	4, 10
chr20:2862601–2862602	c.1094_1095dup	p.Tyr366Serfs*12	Frameshift	7
chr20:2863068	c.1335 T > G	p.Tyr455*	Stop-gain	3
chr20:2863102	c.1367 + 2 T > C	p.?	Splice site loss	8
chr20:2864178	c.1612-1G > C	p.?	Splice site loss	14
chr20:2864288	c.1720 + 1G > C	p.?	Splice site loss	11
chr20:2864631	c.1903C > T	p.Arg635*	Stop-gain	1, 2, 12
chr20:2865039–2865040	c.1988_1989insG	p.Asn663Lysfs*2	Frameshift	5

CNV = copy number variant; NA = all variants are described with reference to build GRCh38, transcript NM_022575.3.

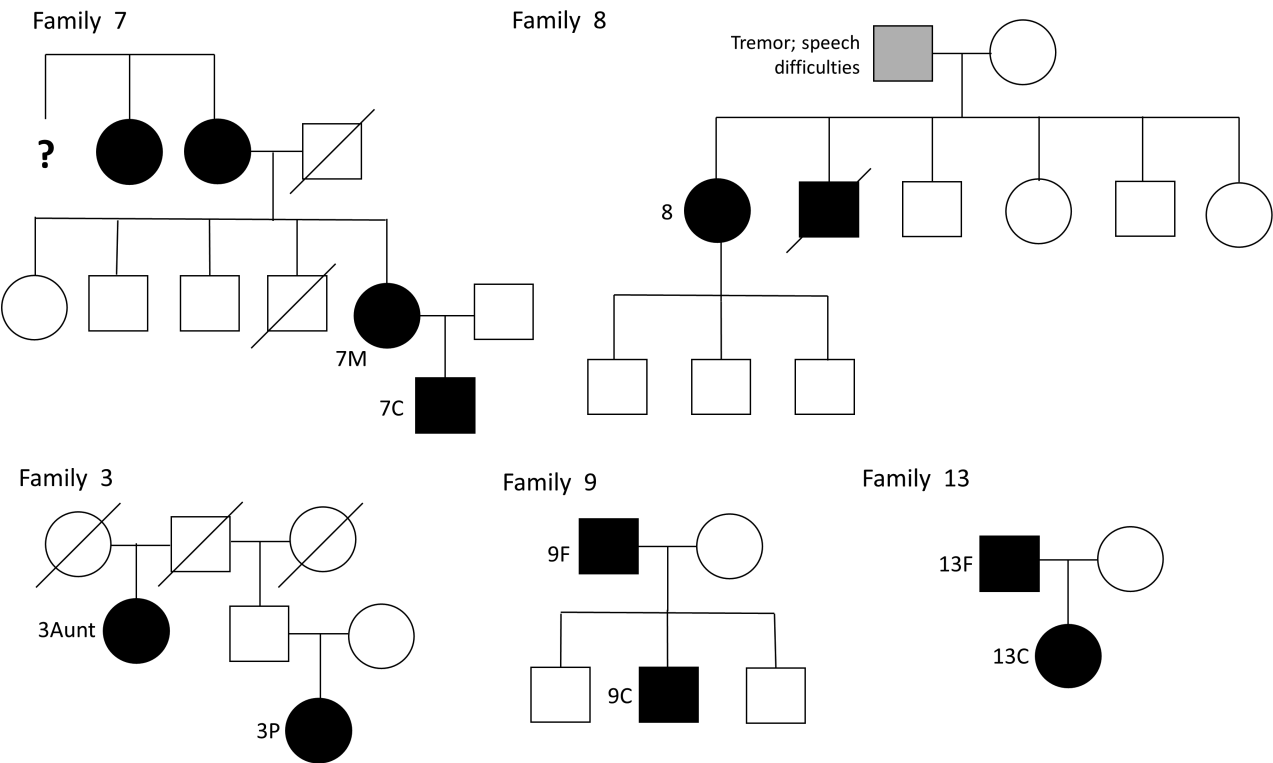


FIGURE 2: Pedigrees of families affected by VPS16 dystonia, showing autosomal dominant inheritance. Key: circle = female; square = male; black-filled shape = individual affected by dystonia; grey-filled shape = individual may have been affected by dystonia; diagonal slash = individual deceased; ? = number/status of additional siblings not known.

Identification and Characterization of Individuals With Biallelic VPS41 Variants

Screening databases for potentially pathogenic variants in HOPS complex genes not previously implicated in disease

(VPS18, VPS39, and VPS41) identified one proband from a consanguineous family with a homozygous canonical splicing variant (NM_014396.3:c.450 + 1G > T) in VPS41, resulting in exon 7 skipping and loss of 22 amino acid

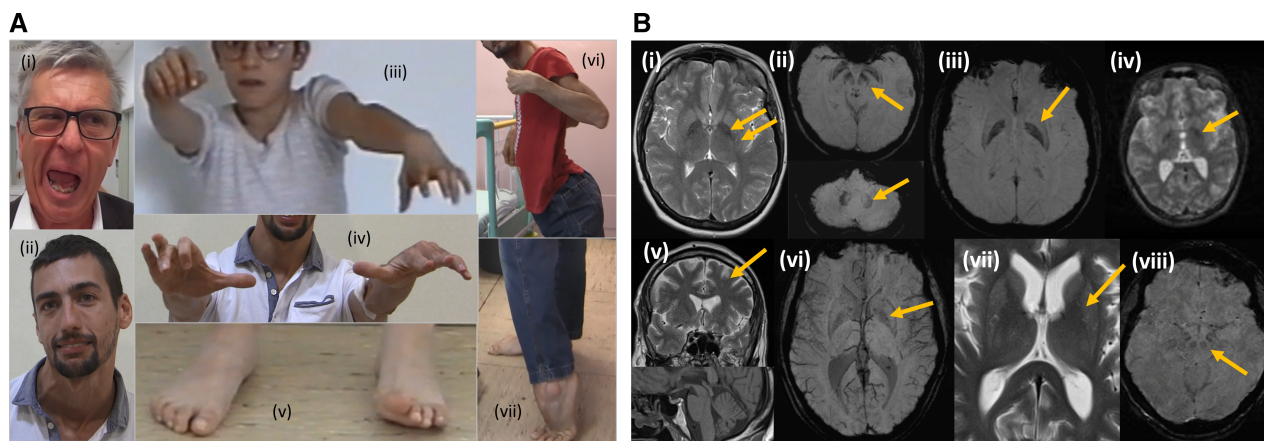


FIGURE 3: Clinical photographs showing dystonic posturing in *VPS16* patient cohort. (A) (i) Patient 9 F demonstrating orofacial dystonia elicited during speech; (ii) Patient 11 S1 showing cervical dystonia; (iii) Patient 12 showing upper limb posturing; (iv) Patient 11 S1 showing hand posturing; (v) Patient 12 showing spontaneous striatal toe on the left; (vi) Patient 12 as an adult, standing, showing exaggerated lumbar lordosis; and (vii) Patient 12 as an adult, standing, showing involuntary plantar flexion/tiptoe posture. (B) Selected MRI brain images for patients with *VPS16* dystonia. Abnormalities indicated by white arrows. (i) Axial T2 image from Patient 7 M (aged 34 years, pre-DBS) shows hypointensity consistent with iron deposition in the globi pallidi; (ii) susceptibility-weighted images (SWIs) from Patient 7 M showing hypointensity in the midbrain nuclei (above) and dentate nucleus of the cerebellum (below); (iii) axial SWI from Patient 7 M showing hypointensity of the globi pallidi; (iv) axial T2 image from Patient 1 (aged 10 years) showing hypointensity of the globi pallidi; (v) subtle generalized atrophy in Patient 9 F, demonstrated in a coronal T2 image of the cerebrum (above) and a sagittal T1 image of the cerebellum (below); (vi) axial SWI from Patient 10, aged 32 years, showing hypointensity in the globi pallidi; (vii) enlarged axial T2 image from Patient 13 F, aged 55 years, showing relatively small, bright caudates, and putamina; and (viii) axial SWI image from Patient 3 P (aged 21 years) showing hypointensity of the midbrain nuclei. [Color figure can be viewed at www.annalsofneurology.org]

residues (p.Ile129_Lys150del; Fig 4). This patient presented in infancy with global developmental delay and generalized dystonia. He attained a few words of speech and voluntary limb movements but never sat unsupported. He had pale optic discs and an axonal neuropathy. From 6 years of age, his condition began to deteriorate, with reduced motor abilities and alertness. An MRI of the brain showed atrophy of the superior cerebellar vermis and slimming of the posterior limb of the corpus callosum (Fig 4).

Electron Microscopy

Electron microscopy (EM) was performed on patient-derived fibroblasts and peripheral lymphocytes from patients with both *VPS16* and *VPS41* mutations in order to determine the impact on lysosomal and vacuolar morphology. When compared to age-matched controls, *VPS16* patient cells (fibroblasts $n = 6$; and lymphocytes $n = 4$) contained increased clusters of vacuoles, with some containing inclusions in the form of particulate or laminated material (Fig 5, see Supplementary Fig S1). EM analysis of patient-derived fibroblasts and lymphocytes from the patient with *VPS41* showed numerous membrane-bound vacuoles containing granular material and, in some cases, fine electron-dense laminated strands. A large number of small pinocytic vesicles arising from the plasma membrane were also seen. In both *VPS16*-related and *VPS41*-related disease, the EM changes seen in patient-derived tissue were consistent with lysosomal dysfunction.

Discussion

We report a cohort of 20 individuals with mutations in 2 related genes, *VPS16* and *VPS41*, which encode vacuolar protein sorting-associated proteins 16 and 41, respectively, both key components of the HOPS complex. The HOPS complex mediates autophagosome-lysosome and endosome-lysosome fusion through several different interactions with SNARE proteins, including catalyzing the formation of the SNARE complex¹¹ and protection of the trans-SNARE complex from disassembly once formed (Fig 6).¹²

Our observation of incomplete penetrance in *VPS16*-related disease (a common hindrance to gene discovery) suggests that, like most other genetic dystonias,¹³ additional genetic, epigenetic, and/or environmental factors are likely to play an important role in disease manifestation. Indeed, weighted burden analysis suggests a wider role for *VPS16* in conferring genetic susceptibility in a broader group of patients with dystonia. Although adolescent-onset dystonia has been reported in a single family harboring a homozygous missense mutation in *VPS16*,¹⁴ our data suggest that *VPS16* haploinsufficiency (dominant inheritance with incomplete penetrance) is a much more common genetic mechanism for *VPS16*-related disease.

Autosomal dominant *VPS16*-related disease appears to be an early-onset, progressively generalizing dystonia, which may occur in isolation or in combination with neuropsychiatric and neurodevelopmental features. In this

TABLE 2. Probands' Variants and Demographic Characteristics

Family	Patient	Mutation	Inheritance*	Sex	Current age, yr
1	1	p.Arg635*	NK	M	27
2	2	p.Arg635*	NK	F	42
3	3P	p.Tyr455*	I-AP	F	24
	3Aunt			F	50
4	4	p.Arg187*	I-AP	M	26
5	5	p.Asn663 Lysfs*2	NK	M	38
6	6	Micro-deletion: chr20:2835462–3974387	DN	F	21
7	7 M	p.Tyr366 Serfs*12	I-SP	F	45
	7C			M	26
8	8	c.1367 + 2 T > C	I-SP	F	69
9	9F	p.Ile146 Serfs*65	I-SP	M	62
	9C			M	30
10	10	p.Arg187*	NK	M	32
11	11S1	c.1720 + 1 G > C	I-AP	M	30
	11S2			M	24
12	12	p.Arg635*	I-AP	M	33
13	13F	p.Leu155Alafs*59	I-SP	M	58
	13C			F	17
14	14	c.1612–1 G > C	NK	M	57

Patient identifiers: XC = child; XF = father; XM = mother; XP = proband; XS = sibling.

DN = de novo; F = female; I-AP = inherited from asymptomatic parent; I-SP = inheritance from symptomatic parent; M = male; NK = not known.

*For multigenerational families, inheritance refers to the younger affected generation.

study, it clinically resembles other “classic” genetic dystonias, such as those related to *KMT2B* or *TOR1A*: indeed, at least 1 family (Family 7) in our study had initially been referred for *KMT2B* testing. Radiologically, too, there is a degree of overlap with *KMT2B* disease, with basal ganglia hypointensity seen on T2 (and other related MRI sequences) in a proportion of patients¹⁵: whether this reflects a common pathophysiological mechanistic end point remains to be determined. Our series does not identify any therapeutic option as reliably beneficial for all patients with *VPS16*-related disease but it is notable that some patients did derive significant benefit from deep brain stimulation (DBS), a treatment that has also proved very useful for both *TOR1A* and *KMT2B*-affected patients. Three patients also reported some degree of levodopa responsiveness, which, although far from conclusive, may be worth pursuing for mutation-positive patients.

There are clear differences between *VPS16*-related and *VPS41*-related disease, although both involve subunits of the HOPS complex and manifest with dystonia as a prominent symptom. Whereas the cases of *VPS16*-related dystonia we report involve monoallelic variants, predicted to cause haploinsufficiency, the child with *VPS41*-related disease has biallelic LOF mutations. He also has a correspondingly more profound phenotype, with very early onset of symptoms (presentation in infancy compared to the *VPS16* patient cohort, median age of presentation 12 years), severe neurodevelopmental impairment, and evidence of clinical deterioration from during childhood. The differing MRI findings (cerebellar vermis atrophy in *VPS41* vs subtle basal ganglia changes in *VPS16*) also suggest some divergence of pathophysiological pathways. Corroborating our findings, we note that a paper not yet published but recently deposited with bioRxiv describes

TABLE 3. Features of Probands' Dystonia

Patient	Age of dystonia onset, yr	Initial movement disorder	Current movement disorder				Ambulant?	Helpful interventions
			Axial	Limb	Facial/ bulbar			
1	10	Cervical dystonia	Y	Y (R > L)	Y (severe speech involvement)	Y		DBS (partial improvement)
2	13	Writer's cramp	Y (mild)	Y (U > Lo)	N	Y		N
3 P	17	Upper limb dystonia (R)	Y	Y	Y (severe speech involvement)	N (lost in adulthood)		N
3 Aunt	4	Upper limb dystonia (R)	Y	Y (U only)	Yes (speech involvement)	Y		Levodopa (minor improvement); sensory trick
4	15	Speech involvement then cervical dystonia	Y	Y (U only)	Y	Y		N
5	30	Cervical dystonia	Y	Y (U only)	Y	Y		N
6	11	Upper limb dystonia (R)	Y	Y (U > Lo)	Y	Y		N
7 M	19	Lower limb dystonia (R)	Y	Y	Y	N (lost in adulthood)		DBS (significant improvement)
7 C	11	Facial + cervical dystonia	Y	Y (mild; U > Lo)	Y	Y (with difficulty)		DBS (significant improvement)
8	7	Cervical + upper + lower limb (R) dystonia	Y	Y (U and Lo)	Y (marked dysphonia)	Y		Levodopa; Botox Sensory trick
9 F	14	Oromandibular dystonia	Y (cervical)	Y (U and Lo)	Y (dysphonia; blepharospasm)	Y		Levodopa; Botox Sensory trick
9 C	Unknown	Oromandibular dystonia	Y (cervical)	N	Y (blepharospasm; oromandibular)	Y		N
10	19	Oromandibular dystonia	Y (cervical)	Y (mild; U only)	Y (laryngeal)	Y		TXY; Botox Sensory trick
11 S1	8	Writer's cramp	Y (cervical)	Y (U > Lo)	Y (severe dysphonia)	Y		Botox (larynx)
11 S2	7	Writer's cramp	N	Y (Lo > U)	Y (dysphonia; oromandibular)	Y		N (DBS no use)
12	3	Speech involvement	Y	Y	Yes (anarthria)	N (lost age 20y)		N (DBS no use)
13 F	50	Cervical dystonia	Y	Y (U > Lo)	N	Y (with difficulty)		
13 C	9	Lower limb dystonia (R)	N	Y	N	Y		TXY
14	14	Cervical dystonia + speech involvement	Y	Y (U > Lo)	Y	Y		Botox Sensory trick

Botox = botulinum toxin type A; DBS = deep brain stimulation; L = left; Lo = lower; R = right; TXY = trihexyphenidyl; U = upper.

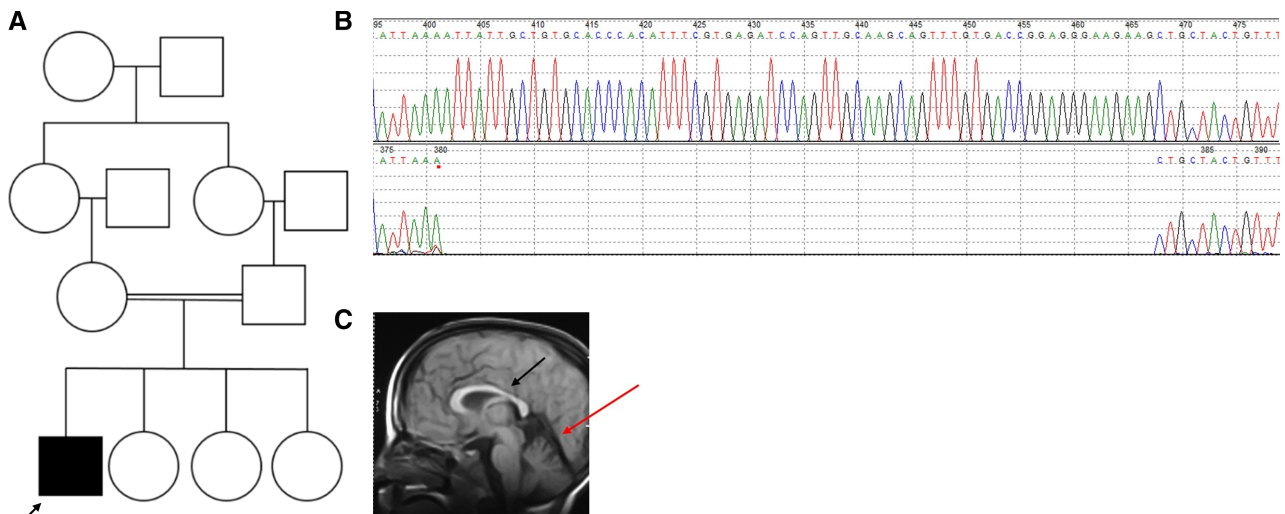


FIGURE 4: Features of proband with *VPS41*-related condition. (A) Pedigree of the *VPS41* patient's family: the proband is indicated with an arrow. Key: circle = female; square = male; filled shape = affected individual; double horizontal line = consanguineous union. (B) Sequencing chromatogram for cDNA of *VPS41*: top row shows wild-type reference sequence and second row shows results from Sanger sequencing of patient cDNA. Bases corresponding to Exon 7 are absent in the patient. (C) T2-weighted midline sagittal MRI brain scan from *VPS41* proband. Note thinning of posterior aspect of corpus callosum (black arrow) and atrophy of superior cerebellar vermis (white arrow). [Color figure can be viewed at www.annalsofneurology.org]

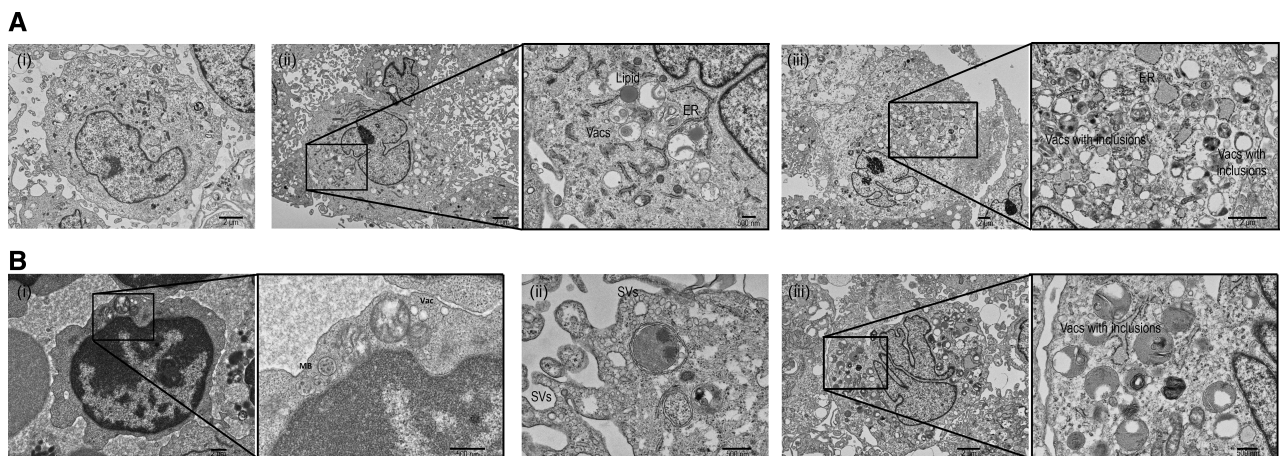


FIGURE 5: Representative electron microscopy images of patient-derived and control cells. (A) (i) Control fibroblast from a healthy individual. (ii) From Patient 8; (iii) from Patient 11 S2; black rectangles in (ii) and (iii) indicate the region enlarged in the following image. (B) Representative electron microscopy images of *VPS41* patient-derived cells. (i) Lymphocyte, showing small vacuoles and multivesicular bodies; (ii) fibroblast showing numerous small intracellular and membrane-abutting vesicles; (iii) fibroblast showing vacuoles containing inclusions. Black rectangles in (i) and (iii) indicate the region enlarged in the following image. ER = endoplasmic reticulum; MB = multivesicular bodies; SVs = small vesicles PV: pinocytic vesicles; Vacs = vacuoles.

an additional family where 2 siblings with homozygous missense variants in *VPS41* were affected by dystonia and ataxia, with similar MRI findings to our proband, and lysosomal abnormalities in patient-derived fibroblasts.¹⁶ Thus, our study further supports the emerging role of biallelic LOF *VPS41* mutations in early-onset movement disorders.

The microscopic vacuolar changes we observed in both *VPS16* and *VPS41*-patient-derived cells are consistent with lysosomal dysfunction. Vacuolar changes have also been observed in fibroblasts from patients with mucopolysaccharidosis-plus syndrome due to biallelic variants in *VPS33A*, another subunit of the HOPS complex.¹⁷

These observations are in keeping with in vitro studies on human cell lines, where depletion of both *VPS16*¹⁸ and *VPS41*¹⁹ have been separately shown to impair endosomal-lysosomal fusion. Furthermore, the accumulation of vacuoles has been observed in *Drosophila* pigment cells in a *dVps16A* knockdown model²⁰ and yeast cells expressing mutant *vps41* protein are reported to contain many small membrane-bound compartments.²¹ It has been suggested that *VPS41*, through its contribution to autophagocytosis, plays a role in suppression of neurodegenerative processes, especially those mediated by toxic accumulation of aberrant proteins: overexpression of human *VPS41* has been shown

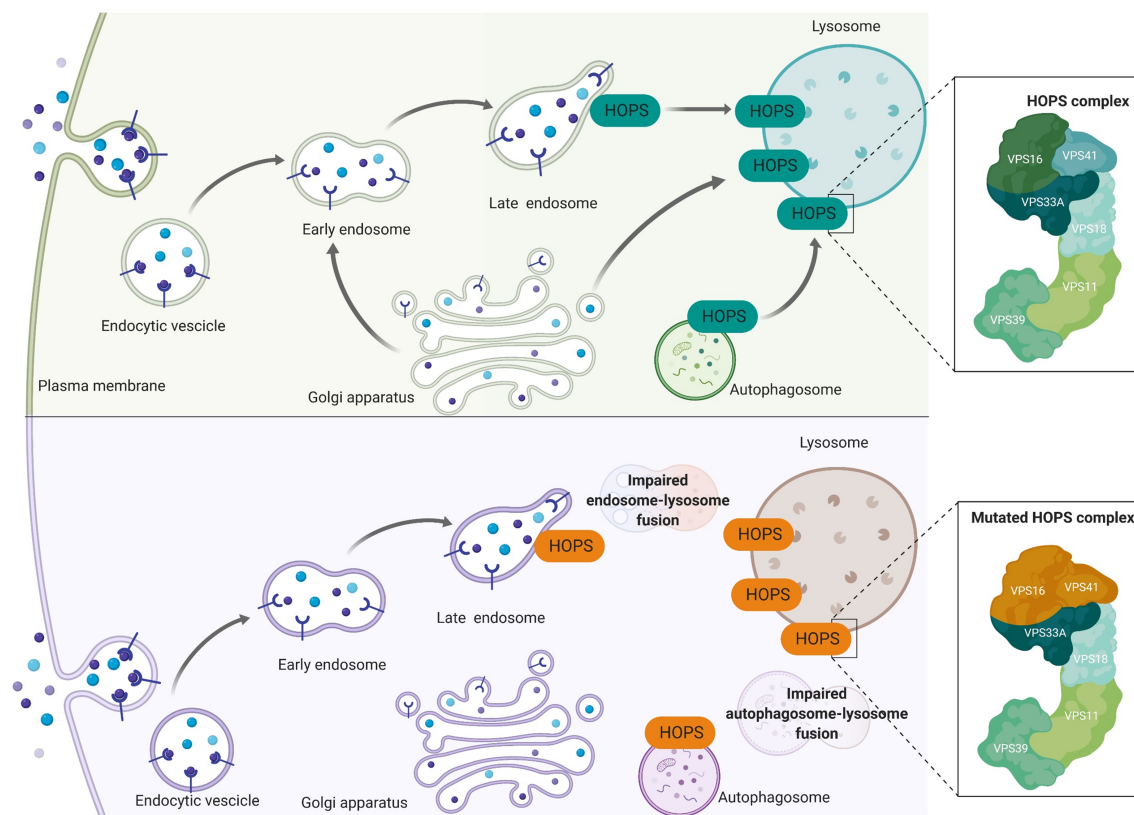


FIGURE 6: Schematic showing the role of the HOPS complex (right) in fusion of endosomes and autophagosomes with lysosomes, in health (above) and disease (below). [Color figure can be viewed at www.annalsofneurology.org]

to be protective in *Caenorhabditis elegans* models of both Parkinson's²² and Alzheimer's diseases.²³

Although other components of the HOPS complex have been reported in human disease (specifically *VPS33A* in mucopolysaccharidosis-plus syndrome¹⁷ and *VPS11* in hypomyelinating leukodystrophy type 12)²⁴ unlike *VPS16* and *VPS41*, none have been associated with dystonia phenotypes, thereby identifying a new pathway in dystonia pathogenesis. We postulate that impairment of endosomal-lysosomal fusion may hinder key cellular processes within core neural networks governing motor control (see Fig 6). Overall, our study provides compelling evidence for the role of *VPS16* and *VPS41* in the physiological control of movement, mediated through its role in the HOPS complex and lysosomal function.

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Author Contributions

M.A.K., J.W., D.S., and M.Z. contributed to the conception and design of the study. D.S., M.Z., C.Z., K.E.S.B., D.B., D.D., K.R.K., G.Z., N.N., R.K., M.W., A.I., R.B., M.S., J.N., R.D., S.W., K.M., S.S., A.F., S.Sh., E.J.K., M.A.T., C.V., M.E.v.E., J.M.F., M.M., A.T., P.C., B.I.B., P.G.L., S.T., P.D., K.M.G., K.J.P., K.B., J.C.K., T.K., B.P., S.B., B.H., B.G., R.J., N.W., H.H., P.G., S.J.L., C.M.S., L.C., N.E.M., G.A., M.A.K., and J.W. contributed to the acquisition and analysis of data. D.S., M.Z., M.A.K., J.W., C.Z., G.A., A.F., K.M., and S.S. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

None of the authors has any relevant conflict of interest to declare.

URLs

CADD (Combined Annotation Dependent Depletion): <https://cadd.gs.washington.edu/snv>
gnomAD: <https://gnomad.broadinstitute.org/>
MutationTaster: <http://www.mutationtaster.org/>
PROVEAN: <http://provean.jcvi.org/index.php>

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