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

Dora Steel, MD, Michael Zech, MD, Chen Zhao, PhD, Katy E. S. Barwick, PhD, Derek Burke, PhD, Diane Demailly, MD, Kishore R. Kumar, FRACP, Giovanna Zorzi, MD, Nardo Nardocci, MD, Raun A. Khayrzhansh, MD, Matias Wagner, MD, Arcangelo Iuso, PhD, Riccardo Berutti, PhD, Matej Škorvánek, MD, PhD, Ján Nespal, MD, Ryan Davis, PhD, Sarah Wiethoff, MD, Kishitii Mankad, FRCR, Sniya Sudhakar, Arianna Ferrini, PhD, Suvasini Sharma, DM, Erik-Jan Kamsteeg, PhD, Marina A. Tijssen, MD, PhD, Corien Verschuuren, MD, Martje E. van Egmond, MD, PhD, Joanna M. Flowers, PhD, Meriel McEntagart, MD, Arianna Tucci, PhD, Philippe Coubes, MD, PhD, Bernabe I. Bustos, PhD, Paulina Gonzalez-Latapi, MD, Stephen Tisch, FRACP, Paul Darveniza, FRACP, Kathleen M. Gorman, MD, Kathryn J. Peall, MB, BCH, Kai Bötzel, MD, Jan C. Koch, MD, Tomas Kmiec, MD, PhD, Barbara Plecko, MD, Professor of Pediatrics, Sylvia Boesch, MD, Bernhard Haslinger, MD, Robert Jech, MD, Barbara Garavaglia, PhD, Nick Wood, PhD, Henry Houlden, MD, Paul Gissen, MD, PhD, Steven J. Lubbe, PhD, Carolyn M. Sue, MB, BS PhD, Laura Cif, MD, PhD, Niccolò E. Mencacci, MD, PhD, Glenn Anderson, FIBMS, Manju A. Kurian, PhD, and Juliane Winkelmann, MD, Genomics England Research Consortium

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Address correspondence to Dr Manju Kurian, NIHR Research Professor and UCL Professor of Neurogenetics, UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, United Kingdom. E-mail: manju.kurian@ucl.ac.uk Dr Juliane Winkelmann, Institute of Neurogenomics, Helmholtz Zentrum Munich, Chair of Neurogenetics, Technical University of Munich, TU00000 Technische Universität München, 80333 München, Arcisstr. 21, Germany. E-mail: juliane.winkelmann@tum.de

† These authors contributed equally to this work.
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 criteria\(^3\) at movement disorder specialty centers in Austria, Czechia, and Germany. The clinical characteristics of the cohort are...
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. 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), a robust method for detecting gene-disease associations. 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 × 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: (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 GeneMatcher 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 Reynolds lead citrate. The 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 \( 6.9 \times 10^{-8} \) (Fig 1, Supplementary Table S2). In addition to 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. 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).

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 \( p \) value of \( < 1.25 \times 10^{-8} \) (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]
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
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 complex and protection of the trans-SNARE complex from disassembly once formed (Fig 6).

Our observation of incomplete penetrance in VPS16-related disease (a common hindrance to gene discovery) suggests that, like most other genetic dystonias, 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, 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
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 patients. Whether this reflects a common pathophysiological mechanism 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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age of dystonia onset, yr</th>
<th>Initial movement disorder</th>
<th>Current movement disorder</th>
<th>Ambulant?</th>
<th>Helpful interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Axial</td>
<td>Limb</td>
<td>Facial/ bulbar</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>Cervical dystonia</td>
<td>Y</td>
<td>Y (R &gt; L)</td>
<td>Y (severe speech involvement)</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>Writer’s cramp</td>
<td>Y (mild)</td>
<td>Y (U &gt; Lo)</td>
<td>N</td>
</tr>
<tr>
<td>3 P</td>
<td>17</td>
<td>Upper limb dystonia (R)</td>
<td>Y</td>
<td>Y (U only)</td>
<td>Y (severe speech involvement)</td>
</tr>
<tr>
<td>3 Aunt</td>
<td>4</td>
<td>Upper limb dystonia (R)</td>
<td>Y</td>
<td>Y (U only)</td>
<td>Yes (speech involvement)</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>Speech involvement then cervical dystonia</td>
<td>Y</td>
<td>Y (U only)</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>Cervical dystonia</td>
<td>Y</td>
<td>Y (U only)</td>
<td>Y</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>Upper limb dystonia (R)</td>
<td>Y</td>
<td>Y (U &gt; Lo)</td>
<td>Y</td>
</tr>
<tr>
<td>7 M</td>
<td>19</td>
<td>Lower limb dystonia (R)</td>
<td>Y</td>
<td>Y (U &gt; Lo)</td>
<td>Y (lost in adulthood)</td>
</tr>
<tr>
<td>7 C</td>
<td>11</td>
<td>Facial + cervical dystonia</td>
<td>Y</td>
<td>Y (mild; U &gt; Lo)</td>
<td>Y (speech involvement)</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>Cervical + upper + lower limb (R) dystonia</td>
<td>Y</td>
<td>Y (U and Lo)</td>
<td>Y (marked dysphonia)</td>
</tr>
<tr>
<td>9 F</td>
<td>14</td>
<td>Oromandibular dystonia</td>
<td>Y (cervical)</td>
<td>Y (U and Lo)</td>
<td>Y (dysphonia; blepharospasm)</td>
</tr>
<tr>
<td>9 C</td>
<td>Unknown</td>
<td>Oromandibular dystonia</td>
<td>Y (cervical)</td>
<td>N</td>
<td>Y (blepharospasm; oromandibular)</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>Oromandibular dystonia</td>
<td>Y (cervical)</td>
<td>Y (mild; U only)</td>
<td>Y (laryngeal)</td>
</tr>
<tr>
<td>11 S1</td>
<td>8</td>
<td>Writer’s cramp</td>
<td>Y (cervical)</td>
<td>Y (U &gt; Lo)</td>
<td>Y (severe dysphonia)</td>
</tr>
<tr>
<td>11 S2</td>
<td>7</td>
<td>Writer’s cramp</td>
<td>N</td>
<td>Y (Lo &gt; U)</td>
<td>Y (dysphonia; oromandibular)</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>Speech involvement</td>
<td>Y</td>
<td>Y</td>
<td>Yes (anarthria)</td>
</tr>
<tr>
<td>13 F</td>
<td>50</td>
<td>Cervical dystonia</td>
<td>Y</td>
<td>Y (U &gt; Lo)</td>
<td>N</td>
</tr>
<tr>
<td>13 C</td>
<td>9</td>
<td>Lower limb dystonia (R)</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>Cervical dystonia + speech involvement</td>
<td>Y</td>
<td>Y (U &gt; Lo)</td>
<td>Y</td>
</tr>
</tbody>
</table>

Botox = botulinum toxin type A; DBS = deep brain stimulation; L = left; Lo = lower; R = right; TXY = trihexyphenidyl; U = upper.
an additional family where 2 siblings with homozygous missense variants in \textit{VPS41} were affected by dystonia and ataxia, with similar MRI findings to our proband, and lysosomal abnormalities in patient-derived fibroblasts.\textsuperscript{16} Thus, our study further supports the emerging role of biallelic LOF \textit{VPS41} mutations in early-onset movement disorders.

The microscopic vacuolar changes we observed in both \textit{VPS16} and \textit{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 \textit{VPS33A}, another subunit of the HOPS complex.\textsuperscript{17} These observations are in keeping with in vitro studies on human cell lines, where depletion of both \textit{VPS16}\textsuperscript{18} and \textit{VPS41}\textsuperscript{19} have been separately shown to impair endosomal-lysosomal fusion. Furthermore, the accumulation of vacuoles has been observed in \textit{Drosophila} pigment cells in a \textit{dVps16A} knockdown model\textsuperscript{20} and yeast cells expressing mutant \textit{vps41} protein are reported to contain many small membrane-bound compartments.\textsuperscript{21} It has been suggested that \textit{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 \textit{VPS41} has been shown

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Features of proband with \textit{VPS41}-related condition. (A) Pedigree of the \textit{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 \textit{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 \textit{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]}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{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 \textit{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.}
\end{figure}
to be protective in Caenorhabditis elegans models of both Parkinson’s and Alzheimer’s diseases.

Although other components of the HOPS complex have been reported in human disease (specifically VPS33A in mucopolysaccharidosis-plus syndrome\(^{17}\) 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**


**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

**References**


