

A *de novo* VPS16 missense variant blocks autophagic flux and extends DYT-VPS16 into infancy

Enrique Gonzalez Saez-Diez^{a,b}, Xutong Xue^a and Darius Ebrahimi-Fakhari^a

^aMovement Disorders Program, Department of Neurology, F.M. Kirby Neurobiology Center, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA; ^bMedical Faculty, Heidelberg University, Heidelberg, Germany

ABSTRACT

Autophagy terminates in the degradation of cargo delivered by fusion of the autophagosome with the lysosome, a step tethered by the homotypic fusion and vacuole protein sorting (HOPS) complex. Heterozygous loss-of-function variants in *VPS16*, which encodes a core HOPS subunit, cause autosomal dominant DYT-VPS16 dystonia. The pathogenicity of missense alleles has remained uncertain due to a lack of mechanistic data from patient-derived cells. We summarize our recent report of the youngest individual with DYT-VPS16 described to date. This child presented with infantile-onset generalized dystonia caused by a *de novo* *VPS16* missense variant, p. Ala466Thr. Patient-derived fibroblasts accumulate enlarged, stalled autolysosomes and show concurrent elevation of SQSTM1/p62 and the MAP1LC3B/LC3B-II:LC3B-I ratio, consistent with a block in autophagic flux. We discuss the consequences for variant interpretation, describe a previously unrecognized caudate-predominant neuroimaging correlate, and speculate on why striatal neurons are selectively vulnerable to HOPS dysfunction.

Abbreviations: HOPS: homotypic fusion and vacuole protein sorting; LOF: loss-of-function; MAP1LC3B/LC3B: microtubule associated protein 1 light chain 3 beta; SQSTM1: sequestosome 1.

ARTICLE HISTORY

Received 17 August 2026
Revised 27 August 2026
Accepted 31 August 2026

KEYWORDS

Autolysosome; autophagic flux; caudate nucleus; dystonia; DYT-VPS16; HOPS complex; MAP1LC3B; SQSTM1; VPS16

Punctum

Autophagy delivers cytoplasmic cargo to the lysosome for degradation, and the pathway is productive only if the autophagosome successfully fuses with a lysosome. This step requires different factors, including the six-subunit homotypic fusion and vacuole protein sorting (HOPS) complex, composed of VPS11, VPS16, VPS18, VPS33A, VPS39 and VPS41. It is recruited to late endosomes and lysosomes through Rab GTPases including RAB7A, and to the autophagosome through binding of its VPS33A-VPS16 module to STX17. There it tethers autophagosomes and lysosomes and drives membrane fusion by assembling the STX17- or the YKT6-containing trans-SNARE complex. Neurons are post-mitotic and cannot dilute undegraded material by division and therefore they are especially dependent on autophagy and as a consequence this machinery as well. Heterozygous loss-of-function (LOF) variants in *VPS16* cause autosomal dominant DYT-VPS16 dystonia, a recognizable syndrome of early-onset generalized dystonia. More than 80 individuals have been reported to date, almost all carrying truncating or splice-altering alleles. Whether missense variants can cause the same disease has remained unresolved as direct functional evidence from patient-derived cells has been lacking.

In our recent study, we describe a girl who presented at 9 months with failure to roll or sit independently, axial hypotonia, and emerging generalized dystonia involving the craniocervical region, limbs and trunk [1]. Because onset fell well below the range previously described for DYT-VPS16, approximately 2 years at the earliest, the diagnosis was delayed: a clinical gene panel, chromosomal microarray, and exome sequencing with mitochondrial DNA analysis were all non-diagnostic. Research trio short-read whole-genome sequencing identified a *de novo* heterozygous missense variant in *VPS16* (NM_022575.4:c.1396 G > A, p. Ala466Thr), classified as likely pathogenic under American College of Medical Genetics and Genomics/Association for

CONTACT Darius Ebrahimi-Fakhari  darius.ebrahimi-fakhari@childrens.harvard.edu  Department of Neurology, Boston Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA

© 2026 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.

Molecular Pathology criteria. This is the earliest presentation of DYT-*VPS16* reported to date. Brain magnetic resonance imaging showed symmetric, caudate-predominant striatal volume loss with ex vacuo dilatation of the frontal horns, without T2 signal abnormality, mineralization, or putaminal involvement. This represents a structural correlate not previously described in this disorder.

Ala466 is highly conserved and maps to the interface between the N-terminal β -propeller and the C-terminal α -solenoid of VPS16. The region is required for HOPS assembly and for positioning the Sec1/Munc18 module, formed by VPS16 and VPS33A, that coordinates SNARE priming during fusion. We therefore asked whether the allele behaves like the LOF variants that dominate the published cohort. Two independent readouts in patient-derived skin fibroblasts, compared with age- and passage-matched controls, indicate that it does. Transmission electron microscopy revealed markedly enlarged vacuolar structures morphologically consistent with stalled autolysosomes, replacing the compact endolysosomal compartments seen in controls. This ultrastructural phenotype closely resembles that reported for other confirmed LOF alleles. Additionally, immunoblotting showed significantly elevated steady-state SQSTM1/p62 together with an elevated MAP1LC3B/LC3B-II:LC3B-I ratio.

Importantly, a few experiments point to an inhibition in autophagy rather than an induction. An isolated increase in LC3B-II levels can reflect increased autophagosome formation, whereas the concurrent accumulation of SQSTM1, an autophagic substrate, indicates that cargo is not being cleared. SQSTM1 is itself a selective cargo receptor and can be transcriptionally induced, so steady-state levels alone are not conclusive; here they are accompanied by the ultrastructural accumulation of stalled autolysosomes. Together, these data suggest that p. Ala466Thr impairs autophagic flux, with prominent accumulation of stalled autolysosomal structures, recapitulating the mechanism documented for LOF alleles (Figure 1).

The most immediate consequence of our study is for the interpretation of the relevance of *VPS16* variants. Missense alleles in *VPS16* are frequently left as variants of uncertain significance in the

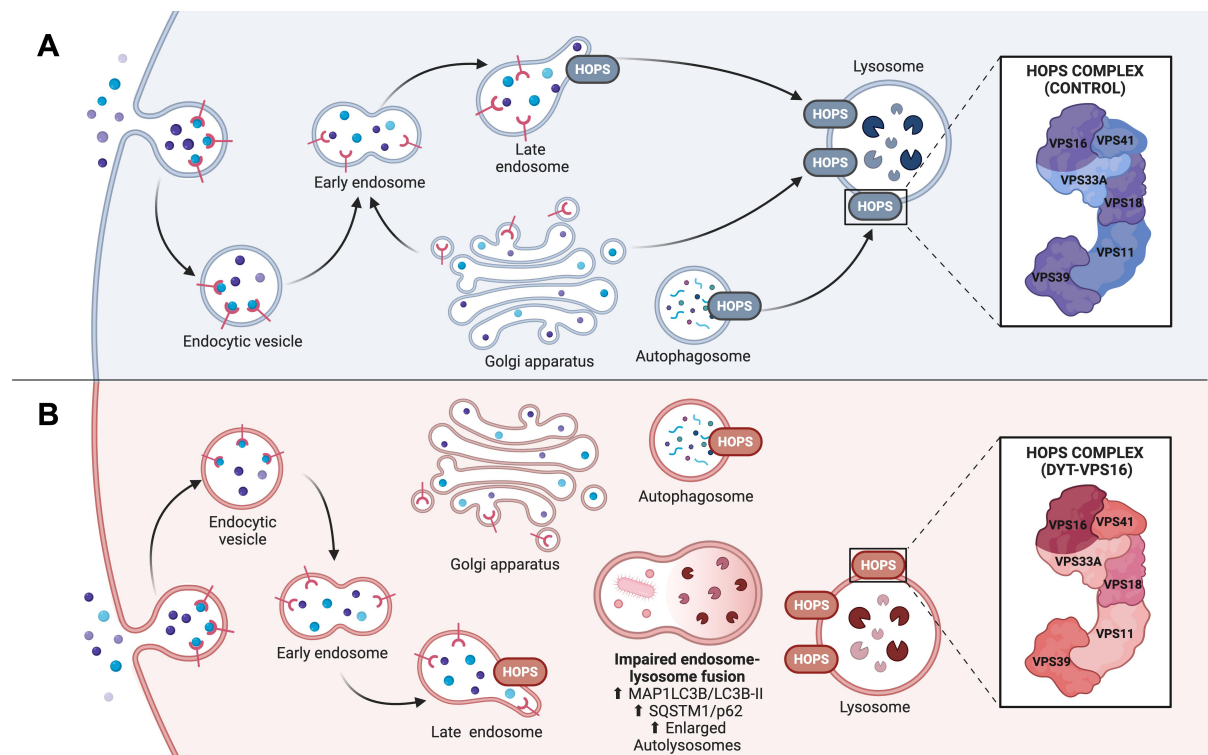


Figure 1. HOPS complex function in health and DYT-*VPS16* dystonia. (A) under physiological conditions, the HOPS complex (VPS11, VPS16, VPS18, VPS33A, VPS39, VPS41) tethers late endosomes and autophagosomes to lysosomes and promotes SNARE-mediated fusion, sustaining autophagic flux. (B) the heterozygous *VPS16* p. Ala466Thr variant yields a defective complex and impaired fusion of endosomes and autophagosomes with lysosomes. The resulting block in flux is evident in patient fibroblasts as they display increased levels of MAP1LC3B/LC3B-II and SQSTM1/p62 as well as the formation of enlarged, stalled autolysosomes. Created in BioRender. Grambs, M. (2026) <https://BioRender.com/15gpkha>.

absence of truncating evidence. Our data show that a single amino acid substitution at a structural interface can produce a cellular phenotype equivalent to that of confirmed LOF alleles in these assays. Steady-state SQSTM1 and MAP1LC3B/LC3B immunoblotting in patient-derived fibroblasts, combined with ultrastructural analysis, is technically straightforward and can provide robust functional evidence for pathogenicity. We would argue that this combination deserves a place among the functional assays used for the interpretation of HOPS-gene variants.

Why disease onset is so much earlier in this child than in most reported patients remains open, and simple haploinsufficiency would not obviously predict it. One possibility we find intriguing, and that the present data cannot rule out, is that an amino acid substitution at a domain interface is not equivalent to the absence of the protein: a mutant subunit that is still expressed and still incorporated may destabilize or mis-position the assembled complex, imposing a burden beyond a 50% reduction in dosage. Distinguishing the loss of a subunit from the presence of a defective one will require stoichiometry assessments on the HOPS complex *in vitro* and eventually *in vivo*.

The caudate-predominant volume loss described above, with ex vacuo enlargement of the frontal horns and no signal abnormality or mineralization, raises a related question about selective vulnerability. Why the caudate? Medium spiny neurons are long-lived, arborize extensively and carry a high basal autophagic burden. They are conspicuously affected across other endolysosomal movement disorders, including those caused by variants in *VPS11* and *VPS41*. Whether caudate-predominant atrophy is a general feature of HOPS dysfunction, and whether it tracks with disease progression, can only be determined through systematic re-review of neuroimaging across existing cohorts, an important question that merits further study.

VPS16 is also a core subunit of the class C core vacuole/endosome tethering complex, so the variant is likely to affect endosomal as well as autophagosomal tethering. Because autophagosomes commonly fuse with endosomes to form amphisomes before reaching the lysosome, a defect in endosome maturation would block flux upstream of the step we invoke and produce the same readouts. We therefore cannot exclude a block at more than one step of the pathway.

Several questions follow. Fibroblasts are a convenient but imperfect proxy for the affected cell type. Induced pluripotent stem cell-derived striatal neurons will be needed to determine whether the flux block is more severe in the neurons that degenerate. It is also unknown whether pharmacological enhancement of autophagy can restore flux in a HOPS-deficient background, especially because this defect lies downstream of the point of action of most available compounds. In the meantime, the molecular diagnosis carries immediate value: a recent multicenter analysis of deep brain stimulation of the internal globus pallidus in *VPS16*-related dystonia demonstrated significant benefit, which argues for early referral of young patients with refractory disease.

Author contributions

CRediT: **Enrique Gonzalez Saez-Diez**: Visualization, Writing – original draft; **Xutong Xue**: Writing – review & editing; **Darius Ebrahimi-Fakhari**: Writing – review & editing.

Data availability

No new data were generated in support of this Punctum. The data discussed are presented in full in the original publication [1].

Disclosure statement

The authors declare no conflicts of interest.

Funding

Research in the Ebrahimi-Fakhari Laboratory is supported by the National Institute of Neurological Disorders and Stroke [K08NS123552], [1U54NS148312], the Spastic Paraplegia Foundation, the Dystonia Medical Research Foundation, the Boston Children's Hospital Translational Research Program, the Boston Children's Hospital TIDO Accelerator Award. E.G. S.-D. is supported by a scholarship from the German Academic Scholarship Foundation.

ORCID

Enrique Gonzalez Saez-Diez  <http://orcid.org/0009-0008-0031-2756>

Xutong Xue  <http://orcid.org/0000-0001-9480-4226>

Darius Ebrahimi-Fakhari  <http://orcid.org/0000-0002-0026-4714>

Reference

1. Gonzalez Saez-Diez E, Xue X, Tam A, et al. De Novo VPS16 missense variant causes infantile-onset dystonia with defective autophagic flux. JCI Insight. 2026;11(16). doi: [10.1172/jci.insight.207998](https://doi.org/10.1172/jci.insight.207998)