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Downregulation of V-ATPase V(0) Sector Induces Microvillus Atrophy Independently of Apical Trafficking in the Mammalian Intestine

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Aurélien Bidaud-Meynard, Anne Bourdais, Ophélie Nicolle, Maela Duclos, Jad Saleh, et al.. Downregulation of V-ATPase V(0) Sector Induces Microvillus Atrophy Independently of Apical Trafficking in the Mammalian Intestine. *Cellular and Molecular Gastroenterology and Hepatology*, 2024, 17 (6), pp.1072-1075. 10.1016/j.jcmgh.2024.02.011 . hal-04534129

HAL Id: hal-04534129

<https://hal.science/hal-04534129>

Submitted on 24 May 2024

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RESEARCH LETTER

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Microvillus inclusion disease (MVID) (OMIM 251850) is a rare genetic orphan condition associated with chronic intractable diarrhea and nutrient absorption defects that compromise the survival of newborns.¹ Mutations found in *MYO5B*, *STX3*, *STXBP2/MUNC18.2*, or *UNC45A* in MVID patients^{1,2} highlighted the role of apical trafficking of transporters and ion channels³ in the absorptive function. It also suggested that apical trafficking is involved in the maintenance of the enterocyte brush border (BB), whose atrophy is a typical feature of MVID.

We recently demonstrated that knockdown of V_0 sector subunits of the V-adenosine triphosphatase complex (V_0 -ATPase) induces an MVID-like phenotype in *Caenorhabditis elegans*.⁴ Here, we studied the function of V-ATPase in mammals by downregulating V_0 (*Atp6v0d1*, *Atp6v0c*) and V_1 (*Atp6v1e2*) subunits by inducible Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated protein 9 mutations in mouse intestinal organoids.⁵ Upon differentiation induction, this model can display a fully differentiated BB.⁶ We then analyzed the resulting phenotypes that we compared with that of *Myo5b* silencing, a bona fide MVID model⁷ (Supplementary Figure 1A).

Atp6v0d1 but not *Atp6v1e2* mutations induced a very severe BB atrophy with smaller, sparser, and slightly wider microvilli as observed by transmission electron microscopy (TEM), similarly to *Myo5b* mutations (Figure 1A and B and Supplementary

Figure 1B–D). F-actin staining also revealed the accumulation of cytoplasmic actin⁺ foci in both *Myo5b* and *Atp6v0d1* mutated organoids, reminiscent of microvillus inclusions,⁶ a typical phenotype of MVID¹ (Figure 1C). Super-resolution and TEM imaging indeed revealed the presence of microvillus inclusions lined with microvilli in *Myo5b*, *Atp6v0d1*, as well as in *Atp6v0c* mutated organoids (Figure 1D and Supplementary Figure 1E and F). Ultrastructural analysis showed that both *Atp6v0d1* and *Myo5b* mutations induced other MVID phenotypes, such as the accumulation of large vacuoles with heterogeneous content (mixed-organelles) and the formation of ectopic lumen between lateral membranes, indicating epithelial polarity defects (Figure 1E and Supplementary Figure 1G and H). Other MVID features such as defective lysosomes and digitations at the basolateral membrane also were observed upon *Atp6v1e2* mutation (Supplementary Figure 1B and I and Supplementary Table 1), which inhibits the acidification but not the vesicle fusion function of the V-ATPase.^{4,8} This indicates that some MVID hallmarks could be linked to a general defect in organelle pH or autophagy, as suggested previously.¹

MYO5B, *STX3*, *STXBP2/MUNC18.2*, and *UNC45A* encode factors implicated in the recycling of apical proteins through Ras-related protein Rab11⁺ endosomes.^{1,3} Consistently, *Myo5b*-mutated organoids displayed subapical accumulation of tubulovesicular compartments, as observed by TEM and periodic acid–Schiff staining, and of the apical transmembrane proteins CD10 and Dipeptidyl peptidase-4, or the trafficking factors Ras-related protein Rab11 and Syntaxin-3 (Figure 2A–C and Supplementary Figure 2). Surprisingly, although V_0 -ATPase controls the same recycling step in *C. elegans*,⁴ the V_0 -ATPase mutation in organoids was not associated with defective apical trafficking, suggesting that its function on apical membrane homeostasis differs from MVID factors (Figure 2A–C and Supplementary Figure 2).

To confirm these results in human beings, we analyzed small intestine resections from a patient suffering from osteopetrosis, a rare disease caused by mutations in *TCIRG1*, coding for the V_0 -ATPase $\alpha 3$ subunit⁹ (Supplementary Figure 3A). Immunohistochemistry against the BB marker phospho-ezrin revealed its basolateral mislocalization in this *TCIRG1* patient compared with control, but this BB polarity defect was not associated with a subapical accumulation of periodic acid–Schiff or apical proteins, unlike in a *MYO5B* patient, confirming our observations in organoids (Figure 2D and E and Supplementary Figure 3B, F, and G). Although immune cell infiltration might cause an enterocyte mispolarization, the specific mislocalization of P-ezrin in *TCIRG1* patients and organoids (Figure 2D and Supplementary Figure 3C–E), which also is mispolarized in MVID patients mutated in *MYO5B* gene,¹⁰ argues for a specific effect of V_0 -ATPase down-regulation on the BB. Thus, our data demonstrate that disruption of V_0 -ATPase function induces a microvillus atrophy that is uncoupled from apical trafficking defects. Similar to many patients with infantile osteopetrosis, this *TCIRG1* patient presented with a severe eating disorder requiring enteral feeding via gastrostomy for years. However, there was no obvious intestinal absorption failure, indicating that, in this patient, putative BB defects are not sufficient to provoke absorption failure.

Finally, to test the putative link between V_0 -ATPase and MVID, we studied the mutual requirement between *Myo5b* and *Atp6v0d1* for their apical localization. *Myo5b*, which localized at the cell cortex in control samples, partly accumulated intracellularly but remained apically localized upon *TCIRG1* mutations (Figure 2F). Contrarily, *Atp6v0d1* apical localization was dramatically affected in 2 MVID patients carrying *MYO5B* mutations (Figure 2G), suggesting that the microvillus atrophy associated with MVID could be owing to a loss of apical V_0 -ATPase. MVID therefore could be induced by the failure of partially independent processes: microvillus integrity and apical transport.

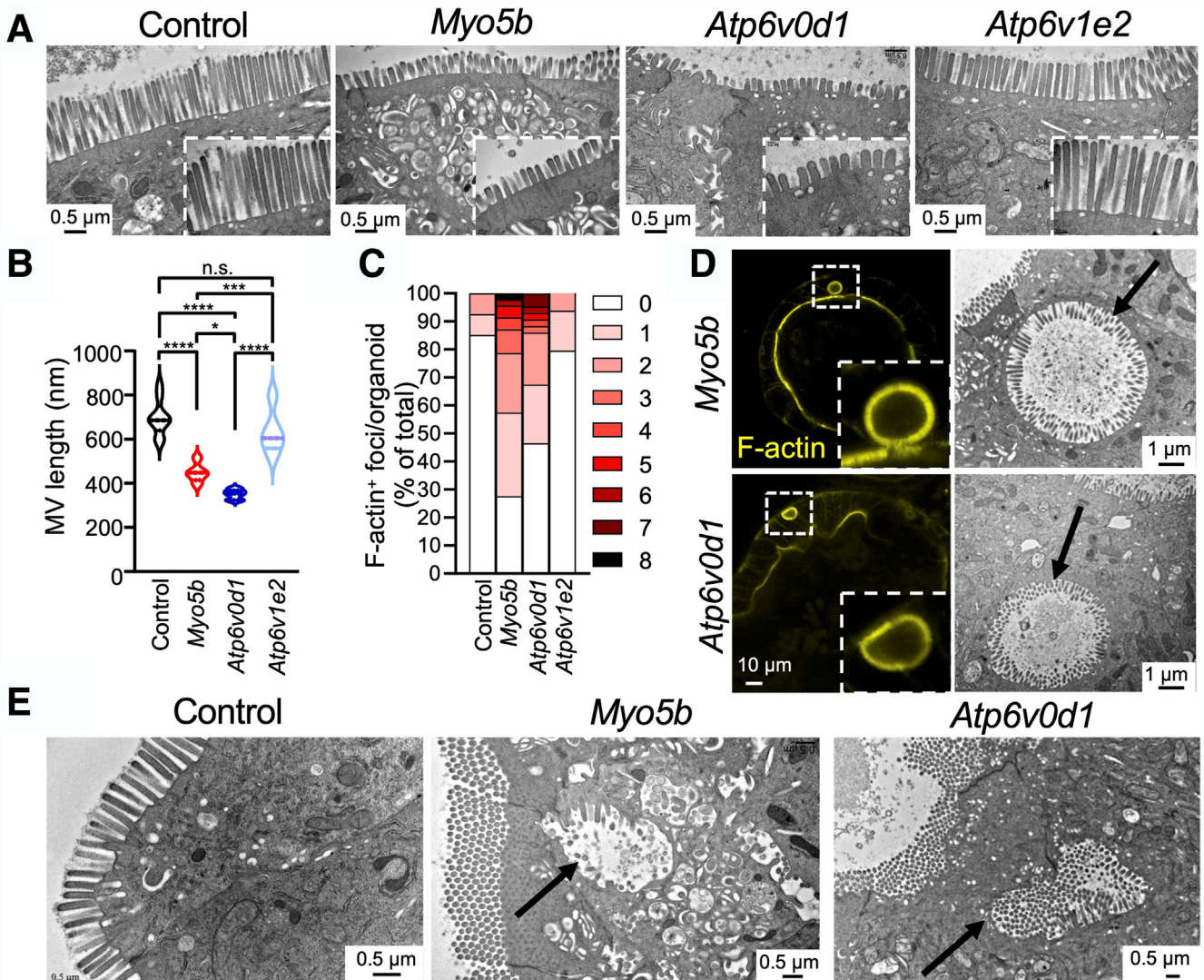


Figure 1. V₀-ATPase mutations recapitulate the microvillus inclusion disease-related structural defects. Control and *Myo5b*-, *Atp6v0d1*-, and *Atp6v1e2*-mutated organoids were analyzed by (A, B, D, and E) transmission electron microscopy (TEM) or (C and D) super-resolution microscopy of F-actin staining. (A) TEM analysis of the brush border in the indicated organoids. (B) Quantification of the microvilli (MV) length in the indicated genotypes. Data are mean MV length/organoid (N = 50 MV/organoid, 5–10 organoids). **P* < .05, ****P* < .001, *****P* < .0001, 1-way analysis of variance. (C) Quantification of the number of F-actin⁺ rounded foci on the full volume of an organoid. N = 27–49 organoids from 2 independent experiments. *Myo5b* and *Atp6v0d1* mutations induce microvillus inclusions, visualized by (D, left) F-actin staining and (D, right, arrows) TEM, as well as (E, arrows) ectopic lumen. (A and D) Insets are magnified images of the regions of interest.

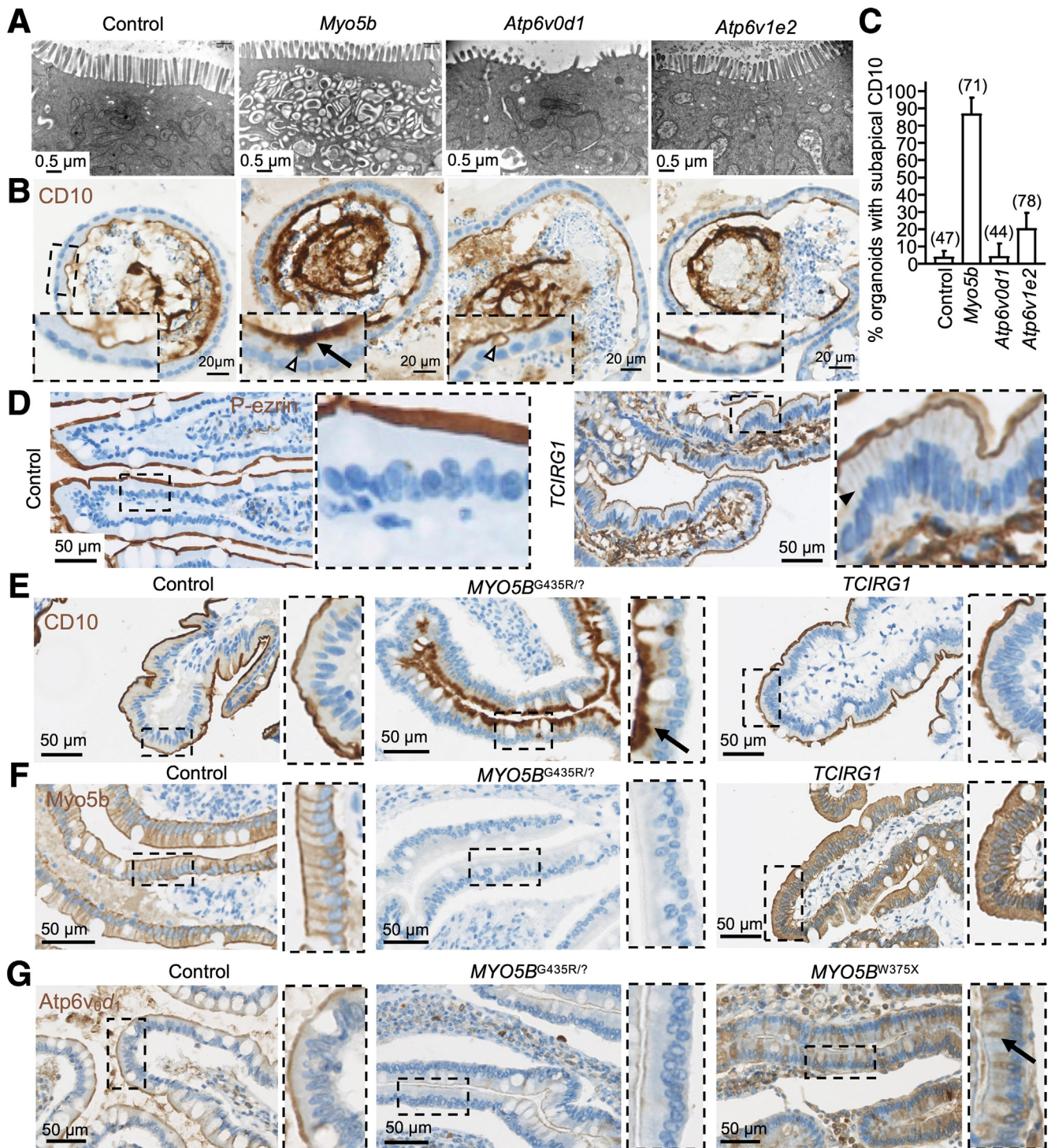


Figure 2. Comparison of Myosin Vb (Myo5b) and V₀ sector subunits of the V-adenosine triphosphatase complex function on brush border, polarity, and trafficking. (A) Transmission electron microscopy analysis of the subapical cytoplasmic content in the indicated organoids. Immunohistochemistry staining of (B, C, and E) CD10, (D) phospho-ezrin (P-ezrin), (F) Myo5b, and (G) Atp6v0d1 in (B–C) mouse intestinal organoids or (D–G) human duodenum samples. (C) Quantification of the number of organoids displaying a subapical accumulation of CD10. The histogram shows the means \pm SD. N = 3 independent experiments. The total number of organoids analyzed is indicated on the figure. Insets are magnified images. Arrows, open arrowheads, and closed arrowheads indicate the intracellular accumulation of markers, microvillus inclusions, and the basolateral appearance of markers, respectively.

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Supplementary Material

Note: To access the supplementary material accompanying this article, go to the full text version at <http://doi.org/10.1016/j.jcmgh.2024.02.011>

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Abbreviations used in this letter: BB, brush border; MVID, microvillus inclusion disease; TEM, transmission electron microscopy; V₀-ATPase, V₀ sector subunits of the V-adenosine triphosphatase complex.



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2352-345X
<https://doi.org/10.1016/j.jcmgh.2024.02.011>

Received July 10, 2023. Accepted February 12, 2024.

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Acknowledgments

The authors thank Hans Clevers and Calvin Kuo for Noggin and R-Spondin1-expressing cells, respectively. The authors are grateful to Marion Rabant and Claire Gandon for patient samples, Guillaume Halet for help with organoid culture setup, Caroline Poix for preliminary imaging, and members of the Dynamics of Epithelial Polarity laboratory for helpful discussions. Immunohistochemistry and imaging were performed at the Histo Pathology High Precision and the Microscopy Rennes Imaging Center (photon and electron services) facilities of the Unité Mixte de Service Biosit, a member of the national infrastructure France-BiImaging, supported by the French National Research Agency (ANR-10-INBS-04). Anne Bourdais and Ophélie Nicolle contributed equally to this work.

Conflicts of interest

The authors disclose no conflicts.

Funding

This work was supported by the European Union's Horizon 2020 research and innovation program under Marie Skłodowska-Curie grant agreement 844070 (A.B.M.), and Défis Scientifiques de l'Université de Rennes grant 17CQ436-S0 (A.B.M. and G.M.). Dynamics of Epithelial Polarity laboratory also received institutional funding from the Centre National de la Recherche Scientifique and the Université de Rennes.