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

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Downregulation of V-ATPase V₀ Sector Induces Microvillus Atrophy Independently of Apical Trafficking in the Mammalian Intestine

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. Mutations found in MYO5B, STX3, STXB2/MUNC18.2, or UNC45A in MVID patients highlighted the role of apical trafficking of transporters and ion channels 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 V₀ sector subunits of the V-adenosine triphosphatase complex (V₀-ATPase) induces an MVID-like phenotype in Caenorhabditis elegans. Here, we studied the function of V-ATPase in mammals by down-regulating V₀ (Atp6v0d1, Atp6v0c) and V₁ (Atp6v1e2) subunits by inducible Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated protein 9 mutations in mouse intestinal organoids. Upon differentiation induction, this model can display a fully differentiated BB. We then analyzed the resulting phenotypes that we compared with that of Myo5b silencing, a bona fide MVID model (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, a typical phenotype of MVID (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 were also 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. This indicates that some MVID hallmarks could be linked to a general defect in organelle pH or autophagy, as suggested previously. MYO5B, STX3, STXB2/MUNC18.2, and UNC45A encode factors implicated in the recycling of apical proteins through Ras-related protein Rab11-dependent processes: microvillus integrity (Figure 2A). Mutations in Rab11 and Syntaxin-3 (Figure 2A and Supplementary Figure 2), which is also mislocalized in MVID patients mutated in MYO5B gene, argues for a specific effect of V₀-ATPase down-regulation on the BB. Thus, our data demonstrate that disruption of V₀-ATPase function induces a microvillus atrophy that is uncoupled from apical trafficking defects. Similar to many patients with infantile osteopetrosis, this TCIIRG1 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.

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 V₀-ATPase a₃ subunit (Supplementary Figure 3A). Immunohistochemistry against the BB marker phospho-ezrin revealed its basolateral mislocalization in this TCIIRG1 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 Supplementary Figure 3B, F, and G). Although immune cell infiltration might cause an enterocyte mispolarization, the specific mislocalization of P-ezrin in TCIIRG1 patients and organoids (Figure 2D and Supplementary Figure 3C–E), which also is mislocalized in MVID patients mutated in MYO5B gene, argues for a specific effect of V₀-ATPase down-regulation on the BB. Thus, our data demonstrate that disruption of V₀-ATPase function induces a microvillus atrophy that is uncoupled from apical trafficking defects. Similar to many patients with infantile osteopetrosis, this TCIIRG1 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 V₀-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 TCIIRG1 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 V₀-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 Atp6vle2–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 (Myc5b) 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 ± 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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**Conflicts of interest**

The authors disclose no conflicts.

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