

## Fundic Gland Cysts in *Atp4a*<sup>-/-</sup> Mice Mimic Fundic Gland Polyps in Humans

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**Abstract.** *Background:* Innumerable fundic gland polyps (FGPs) (that is massive FGP polyposis) may be found in the human stomach. Innumerable fundic gland cysts (FGCs) develop in mice lacking the gene *Atp4a* (*Atp4a*<sup>-/-</sup>), that encodes the enzyme ATPase. The aim was to monitor the evolution of FGCs in *Atp4a*<sup>-/-</sup> mice. *Materials and Methods:* Six pairs (male-female) of *Atp4a*<sup>-/-</sup> mice, 8, 12 and 20 months old, were investigated. Wild-type *Atp4a*<sup>+/+</sup> mice were used as controls. *Results:* In *Atp4a*<sup>-/-</sup> female mice, the mucosal thickness increased both at 8 months (943±72 mm) ( $p<0.02$ ) and at 12 months ( $p<0.0008$ ) and even further at 20 months ( $p<0.0001$ ). The FGC area was 13.183±7.218 mm<sup>2</sup> at 12 months and 73.361±50.591 mm<sup>2</sup> at 20 months ( $p<0.0008$ ). FGCs in *Atp4a*<sup>-/-</sup> mice were lined by parietal cells, mucous pit-foveolar cells and chief cells, thus mimicking FGPs in humans. *Conclusion:* Older female *Atp4a*<sup>-/-</sup> mice developed innumerable FGCs, a phenomenon very similar to massive FGPs seen in humans. In the light of these similarities, the *Atp4a*<sup>-/-</sup> mouse emerges as a suitable animal model to investigate the series of events taking place during the evolution of FGPs in humans.

Fundic gland polyps (FGPs) in humans are benign, circumscribed, slightly elevated lumps in the oxyntic mucosa characterized by a collection of mucosal cysts lined by parietal cells, chief cells and mucinous foveolar cells (1-3). The reported number of FGPs in individual patients varies in the literature from one to even hundreds (4-7). Recently, innumerable FGPs (that is massive FG polyposis) were found in a patient receiving protracted proton-pump inhibitor medication (8).

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Knock-out mice lacking the gene *Atp4a* (*Atp4a*<sup>-/-</sup>) that encodes the enzyme hydrogen potassium ATPase, the proton pump of acid secretion (9, 10), develop innumerable cysts in the gastric mucosa with increasing age. A recent review of histological sections in *Atp4a*<sup>-/-</sup> mice revealed that these mucosal cysts were very similar to those found in FGPs in humans (11).

Our aim was to study the evolution of fundic gland cysts (FGCs) in *Atp4a*<sup>-/-</sup> mice as well as to describe the histological similarities between FGCs in mice and FGPs in humans.

### Materials and Methods

All mice were housed in humidity and temperature controlled rooms, on a 12-hour light/dark cycle, with access to standard mouse chow and water *ad libitum*. The University of Cincinnati Institutional Animal Care and Use Committee approved animal protocols. Animal handlers were trained in an American Association of Assessment and Accreditation of Laboratory Animal Care facility. Mice were cared for and inspected daily (10).

*Atp4a*<sup>-/-</sup> mice, were produced as described previously (9, 10). Six pairs of mice were analyzed at each time point (8, 12 and 20 months). Wild-type animals *Atp4a*<sup>+/+</sup> were used as controls.

Euthanasia was carried out with carbon dioxide followed by nicking the diaphragm. The stomach was dissected immediately, opened along the greater curvature, rinsed free of food in phosphate-buffered saline (PBS) and fixed by immersion in a 2% paraformaldehyde/2.5% glutaraldehyde in PBS, or 4% paraformaldehyde in PBS for at least 24 h. Sections were stained with hematoxylin and eosin (H&E).

The thickness of the gastric mucosa was measured from the mucosal *lamina propria* border to the luminal border in a well-oriented area, using a calibrated ocular microscale.

Massive FGCs is herein defined as countless numbers of large, confluent mucosal cysts. Large FGCs were irregular in shape, due to the lateral forces of compression exerted by the expanding cysts. To by-pass the limitations of counting the actual number of FGCs in older female *Atp4a*<sup>-/-</sup> mice, the area with FGCs was calculated.

*Statistical analysis.* Data were analyzed using SAS V8.0 and means and standard errors of the means were determined using the general linear model. Results were considered significant when  $p<0.05$ .

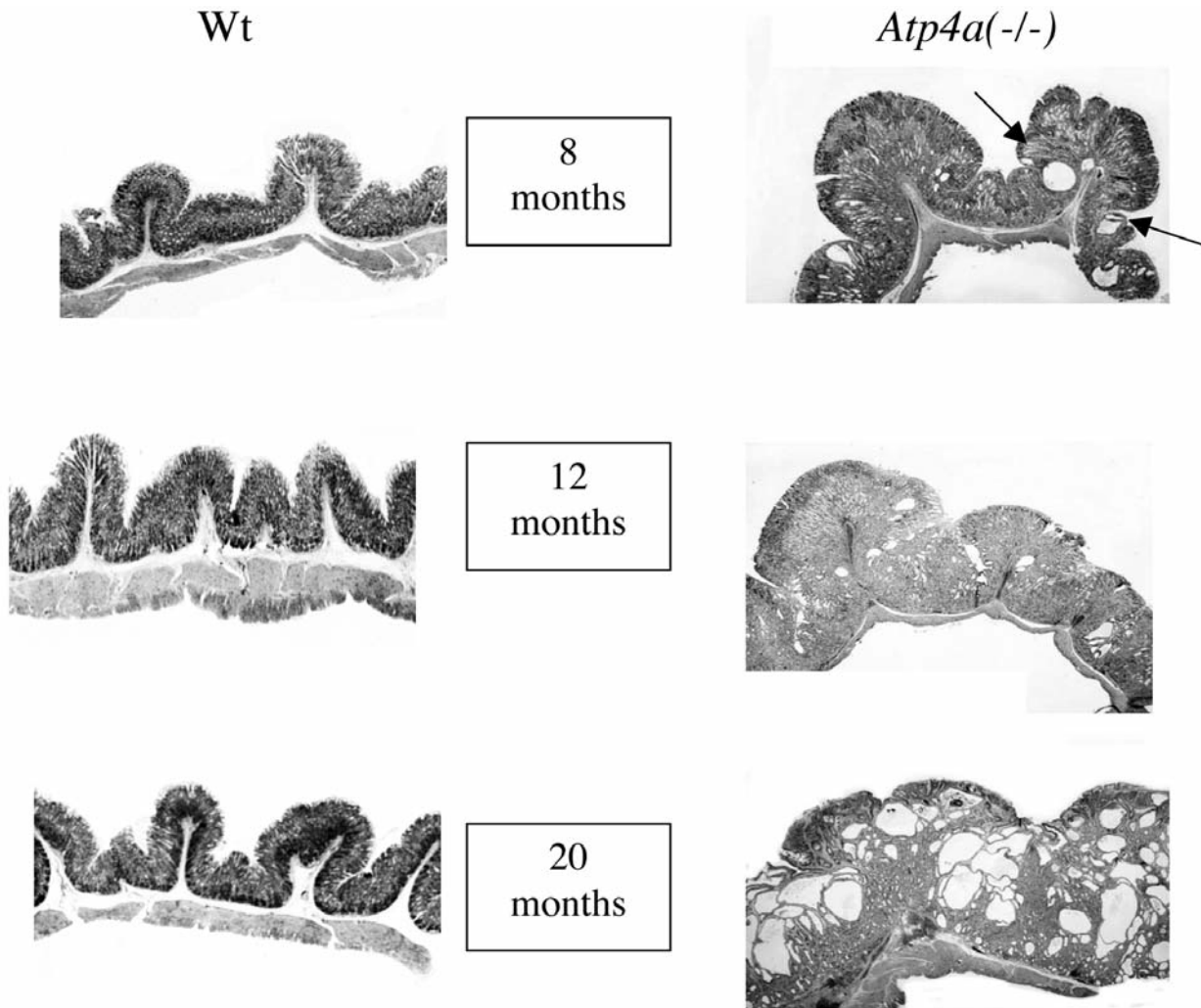


Figure 1. The gastric mucosa in 8-, 12- and 20-month-old wild-type  $Atp4a^{+/+}$  and in  $Atp4a^{-/-}$  female mice. Note the appearance of fundic gland cysts at 8 months (arrows) and the massive increase in the number of confluent large cysts at 20 months.

## Results

The FGCs in  $Atp4a^{-/-}$  mice were lined by parietal cells, had an expanded population of mucous pit-foveolar cells and chief cells.

The mucosal thickness increased both at 8 months ( $943 \pm 72$  mm) in  $Atp4a^{-/-}$  female mice, (it was  $504 \pm 52$  mm in 8-month-old wild-type female animals) ( $p < 0.02$ ) and at 12 months ( $p < 0.0008$ ), and even further at 20 months ( $p < 0.0001$ ).

The FGC area was  $73.361 \pm 50.591$  mm<sup>2</sup> in 20-month-old female  $Atp4a^{-/-}$  mice, compared to  $13.183 \pm 7.218$  mm<sup>2</sup> in 12-month-old female  $Atp4a^{-/-}$  mice ( $p < 0.0008$ ).

The area with large FGCs in 20-month-old female  $Atp4a^{-/-}$  mice was  $161.666 \pm 105.157$  mm<sup>2</sup> but only  $7.132.5 \pm 4.196$  mm<sup>2</sup> in male  $Atp4a^{-/-}$  mice of the same age ( $p < 0.0001$ ).

## Discussion

The polypoid profile found in histological sections from younger (8-month-old) female  $Atp4a^{-/-}$  mice had vanished in older (20 months)  $Atp4a^{-/-}$  animals, as the luminal mucosal outline became more linear following total mucosal expansion.

The increase in gastric mucosal thickness in female  $Atp4a^{-/-}$  mice concurred with the appearance of mucosal cysts in younger female mice. The mucosal cysts increased in number and became larger and confluent, particularly in older female  $Atp4a^{-/-}$  animals.

FGCs developed predominantly in female  $Atp4a^{-/-}$  mice. In humans, FGPs also develop predominantly in females (female:male in humans, 5:1). Notably, the contribution of female hormones to the evolution of GCGs in  $Atp4a^{-/-}$  mice and of FGPs in humans, has not yet been elucidated.

From the above, it is apparent that glandular secretions were retained in the gastric glands in *Atp4a*<sup>(-/-)</sup> mice, a process which started at a young age. In older animals, the glandular secretions were retained to a greater extent and affected more glands. The increase in the size of the cysts clearly indicate that the glandular secretions were no longer being discharged into the gastric cavity but that they were constantly accumulated within the dilated glands. Although the cause(s) leading to the cystic dilatations of the fundic glands have not yet been fully clarified in *Atp4a*<sup>(-/-)</sup> mice, it should be mentioned that the accumulation of glandular secretions in FGPs in humans is apparently due to the blocking of the glandular outflow by plugs of granular eosinophilic material, apparently derived from exfoliated, anucleated parietal cells (11).

Whatever the reason(s) for the accumulation of glandular secretions in massive FGCs in female *Atp4a*<sup>(-/-)</sup> mice, it should be stressed that casts of highly eosinophilic material were also found within FGCs in these animals (10). The amorphous, eosinophilic casts seemed to derive from the mucous pit foveolar cells, located above the fundic oxyntic glands. It is therefore not inconceivable that these eosinophilic casts had blocked the outlet of the subjacent glands in *Atp4a*<sup>(-/-)</sup> mice, resulting in the retention of the glandular secretions, with progressive cystic dilatation of the oxyntic glands.

In conclusion, female *Atp4a*<sup>(-/-)</sup> mice develop increased number of FGCs, with increasing age. Recently, a case with innumerable FGPs (that is, massive FG-polyposis) was found in a patient receiving protracted proton-pump inhibitor medication (8). The similarities between the FGCs in *Atp4a*<sup>(-/-)</sup> mice and FGPs in humans include the dilatation of fundic glands, the lining of the mucosal cysts by parietal cells, mucus pit foveolar cells and chief cells, the progressive accumulation of cellular secretions, the presence of anucleated eosinophilic material within glandular cysts and a female predominance in both species. In the light of these similarities, the *Atp4a*<sup>(-/-)</sup> mouse emerges as a suitable animal model to investigate the series of events taking place in the development of FGPs in humans.

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