

# The morphological and immunohistochemical spectrum of gastric biopsies of patients with absorptive hypercalciuria

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## ABSTRACT

**Objective:** Idiopathic hypercalciuria is characterised by renal stone formation and vertebral osteoporosis. The syndrome displays high clinical variability with patients almost equally distributed between fasting or renal type (prevalent calcium loss) and absorptive type (prevalent increase of intestinal absorption). Absorptive hypercalciuria (AH) is characterised by hypersensitivity of calcium-sensing receptors of antral G cells with normal fasting gastrinaemia and meal hypergastrinaemia. To the best of our knowledge, no study has been published about the morphological aspects of gastric biopsies of patients with AH and the immunohistochemical findings of gastrin-producing G cells. So we studied morphologically and immunohistochemically a group of 38 patients with AH, describing their gastric findings and associated lesion.

**Design:** All 38 patients had a clinical-laboratory diagnosis of AH with normal fasting hypergastrinaemia and an abnormal rise of gastrinaemia after a standardised meal test. Their 38 antral and 27 body-fundus biopsies, and 5 normal antral and body controls, were stained with H&E, Giemsa stain, polyclonal antiserum anti-Gastrin and a monoclonal antibody anti-Chromogranin A.

**Results:** Antral biopsies of all 38 patients showed a simple (15) or linear (23) hyperplasia of G cells, whereas only 2 of 27 body biopsies showed a nodular hyperplasia of endocrine cells. In five patients with AH, we found an association with fundic gland polyps (FGPs).

**Conclusions:** We found in all of the patients with AH a correlation between meal hypergastrinaemia and morphological antral G-cell hyperplasia. Moreover, in five patients, AH was associated with FGPs. We know from literature data that FGPs' development in Zollinger-Ellison syndrome is statistically associated with hypergastrinaemia. From our present data, we suggest that even in AH the stimulated hypergastrinaemia may have a role in polyp development.

## INTRODUCTION

Idiopathic hypercalciuria is found in up to 40% of stone formers, but has an incidence

## Summary box

### What is already known about this subject?

- ▶ Absorptive hypercalciuria (AH) is characterised by the hypersensitivity of calcium-sensing receptors of antral G cells.
- ▶ Patients with AH have normal fasting gastrinaemia and hypergastrinaemia stimulated by pepsines and calcium.
- ▶ At present, no morphological and immunohistochemical studies on gastric biopsies of patients with AH have been published.

### What are the new findings?

- ▶ Patients with AH showed morphological G-cell hyperplasia, independent from therapies or *Helicobacter pylori* infection.
- ▶ We found an unexpected association between AH and fundic gland polyps, with a 10-fold raised prevalence when compared with the general population, a difference extremely statistically significant.

### How might it impact on clinical practice in the foreseeable future?

- ▶ It is to be elucidated if even in patients with sporadic fundic gland polyps, not treated with proton pump inhibitors, hypergastrinaemia may play a role.
- ▶ Alternatively, somatic  $\beta$ -catenin mutation of sporadic fundic gland polyps may stimulate G-cell hyperplasia.

of less than 10% in the overall population.<sup>1</sup> The syndrome displays a large clinical variability with patients almost equally distributed between fasting or renal type (prevalent renal calcium loss) and absorptive type (prevalent increase of intestinal absorption).

Absorptive hypercalciuria (AH) is a clinical condition characterised by a large increase of calcium in the urine, formation of renal stones and osteoporosis in spite of

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normal levels in the blood. AH is characterised by hypersensitivity of calcium-sensing receptor (CaSR) of antral G cells, with normal fasting gastrinaemia and meal hypergastrinaemia.<sup>2</sup> G-cell CaSR is the predominant chemosensor mediating gastrin secretion.<sup>3</sup>

To the best of our knowledge, there are no published data about the morphology and immunohistochemistry of gastric biopsies of patients with AH.

So we studied the gastric biopsies of a group of 38 patients with an established diagnosis of AH,<sup>2</sup> analysing their morphology, immunohistochemical features and associated lesions.

## PATIENTS AND METHODS

Criteria for patient selection were published elsewhere.<sup>2</sup> All 38 patients had calcium urinary excretion higher than 250 mg/day (6.25 mmol/day, normal values, 2.5–6.25 mmol/day); they were on a free diet and were not taking calcium-sparing diuretics. After 1 month of a dietician-assisted calcium-free diet, they showed a reduction in daily calcium excretion (<100 mg/day or 2.5 mmol/day), and a decrease of calcium/creatinine values in fasting 2 h urine (<0.35 mmol Ca/mmol creatinine, normal values 0.1–0.2 mmol Ca/mmol creatinine), thus fulfilling the conventional criteria for AH diagnosis.

They were all free of severe dyspepsia, and none had taken antiacid therapy. Whereas fasting gastrinaemia was comparable to controls (60–70 ng/L), patients with AH responded to both the calcium load test (1 g calcium gluconate Calcium Sandoz fortissimo) and peptone load test (10 mg Liebig meal extract diluted in 250 mL of 0.9% saline) with an abnormal rise in gastrinaemia with almost doubling values when compared with controls.

The patients were referred to our institution with the principal aim of evaluating gastric antra and to find a possible correlation between meal hypergastrinaemia and the morphology of antral G cells.

Endoscopically, all patients did not show significant lesions, except for sparse antral microerosions or hyperaemia; in five patients, small body-fundic polyps were seen that were biopsied.

Five antral and body normal controls and biopsies, randomly taken from the antrum (in all 38 patients) and body-fundus (in 27 patients), plus the five body-fundus polyps were fixed in Bouin, embedded in paraffin, cut at 3  $\mu$  and stained with H&E and modified Giemsa. *Helicobacter pylori* were extensively searched for on modified Giemsa section at 40 $\times$  on both antral and body biopsies. Further sections were cut for the immunohistochemical study of endocrine population using anti-Gastrin polyclonal antiserum (Dako), diluted 1:300 for 30', room temperature, and an anti-Chromogranin A monoclonal antibodies cocktail (LK2H10 and PHE5 MoAbs, Neomarker) diluted 1:100 for 45' room temperature. We used four normal antral and body-fundic biopsies as controls.

Enterochromaffine like (ECL) cells (Chromogranin A+) were scored according to Solcia *et al.*<sup>4</sup>

We scored antral G cells as follows:<sup>5 6</sup>

- ▶ Normal: 1–2 G cells for each gland, with uneven distribution;
- ▶ Simple hyperplasia: 4–5 cells for each gland;
- ▶ Linear hyperplasia: continuous chain-like distribution of G cells (figure 1).

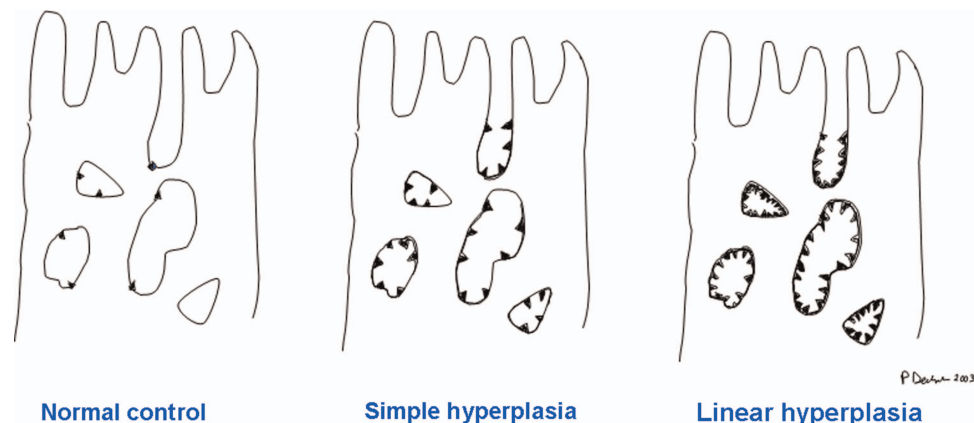
In a subset experiment, we counted all gastrin-positive cells in controls, simple hyperplasia and linear hyperplasia, evaluating at least 20 well-oriented gastric pits-neck-glands. Results were compared unpaired t test, calculated using the internet resource at <http://graphpad.com/quickcalcs/>

Gastritis was assessed following the approach by Owen, distinguishing chronic active and quiescent, superficial and deep (under the neck region) gastritis, assessing the presence of *H. pylori*, atrophy and intestinal metaplasia.<sup>7</sup>

We used strict criteria for the diagnosis of fundic gland polyps (FGPs),<sup>8–11</sup> that is:

- ▶ Shortened gastric pits;
- ▶ Presence of irregular dilations, both superficial and deep, bordered by all three cell types of body mucosa—mucous, parietal and chief cells.

All statistical analyses were performed using the GraphPad Quick calc resource at <http://graphpad.com/>



**Figure 1** Scoring system of antral G cells.<sup>5 6</sup>

**Table 1** Clinical features of the 38 patients with AH are resumed

AH patients	38							
♀	31							
♂	7							
Average age	58.2							
F/M	4.4/1							
<i>Helicobacter pylori</i>	Negative				Positive			
	12				26			
Antral G cells*	N	+	++	+++	N	+	++	+++
	–	4	8	–	–	11	15	–
Body ECL cells*	N	+	++	++++	N	+	++	+++
	3	1	–	–	11	–	–	2
Associated lesions	2 FGPs				3 FGPs			

Score key: N normal population, + simple hyperplasia, ++ linear hyperplasia, +++ micronodular hyperplasia.

\*The G-cell and ECL scores were evaluated before and after *H. pylori* eradication therapy, and they appeared unchanged.

AH, absorptive hypercalciuria; FGP, fundic gland polyp.

## RESULTS

### Clinical features

Table 1 shows anatomical and clinical features of the 38 patients with AH. They were mostly female (F/M: 4.4/1), with a median age of 58.2 years.

At least an antral biopsy was performed in all the patients, and body-fundic biopsies in 27 patients. None of the patients had been treated with proton pump inhibitor therapy previously.

### Histology

At first endoscopy, 26 patients showed active superficial gastritis with *H. pylori* colonisation. None of the 27 patients who had body-fundic biopsy showed atrophy or metaplastic change. Patients with *H. pylori* gastritis were treated with our standard eradication therapy (amoxicillin 1 g twice a day, clarithromycin 500 mg twice a day, omeprazole 20 mg twice a day, all drugs for 1 week). They underwent a second endoscopic examination with a mean interval of 20.6 months (minimum 4 to maximum 40 months). Their antral biopsies appeared *H. pylori* free with no residual significant inflammation.

The immunohistochemical staining for gastrin was performed on 5 controls and 38 patients and repeated again after eradication on the 26 patients originally with gastritis. The G-cell score of the first examination and the second after eradication appeared unchanged, thus not being affected by *H. pylori* infection or proton pump inhibitors (PPI) therapy. The five controls showed normal G-cell values. All 38 patients showed a G-cell hyperplasia (simple in 15 and linear in 23; figure 2). The 12 patients, originally *H. pylori* free, showed simple hyperplasia in 4 cases and linear hyperplasia in 8 cases; the 26 patients, retested after *H. pylori* eradication, showed simple and linear hyperplasia in 11 and 15 cases, respectively. The different distribution of simple and linear hyperplasia between the patients originally *H. pylori* negative and patients eradicated, judged with Fisher's exact test, was not statistically significant ( $p=0.7281$ ).

Evaluating at least 20 well-oriented antral glands in controls, cases with simple and linear hyperplasia, mean G cells per gland were scored (table 2), and the differences were evaluated with an unpaired t test. The difference between controls and simple hyperplasia was extremely statistically significant ( $p=0.0003$ ), whereas the difference between simple and linear hyperplasia was very statistically significant ( $p=0.0032$ ).

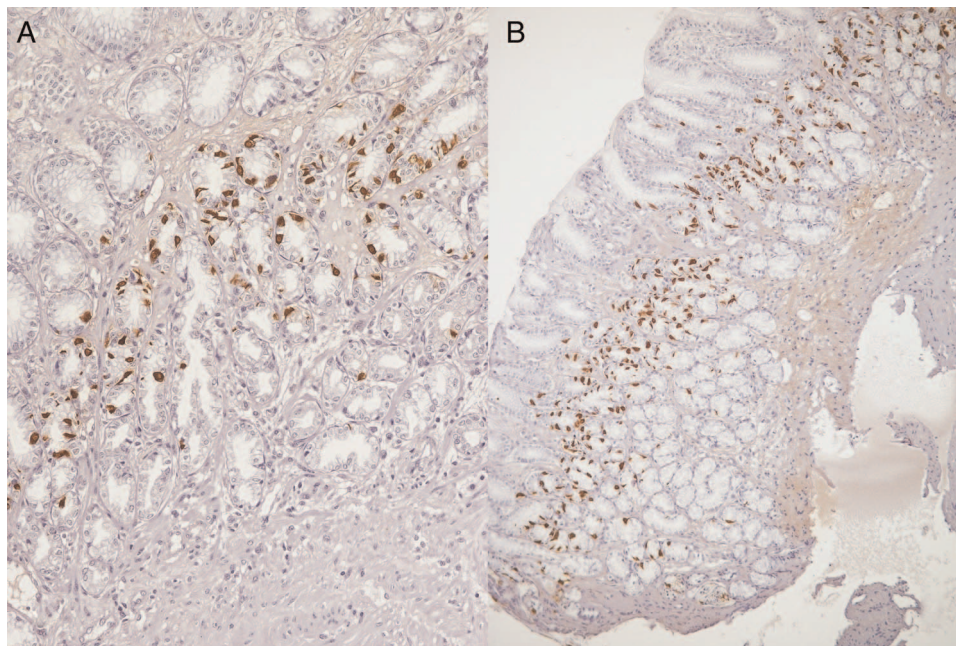
While studying the 27 body-fundic biopsies with an anti-Chromogranin antibody, we detected a normal ECL cell distribution in 25 cases, and a micronodular hyperplasia in only 2 cases. In both of them, the micronodular body hyperplasia was associated with linear G-cell hyperplasia. No signs of atrophy or parietal cell hypertrophy were present in all biopsies.

In five patients, we found an association with FGPs, that is, polyps characterised by shortened gastric pits and superficial and deep dilations bordered by mucous, parietal and chief cells (figure 3). No associated signs of parietal cell hypertrophy were seen, both in FGPs and in the 27 body-fundic biopsies. None of the polyps showed any sign of dysplasia.

**Patient 1**—A 59-year-old man, with antral active gastritis with *H. pylori* colonisation at the time of the first control, showed a normal body-fundic mucosa, without polyps or irregularities. Immunohistochemical stainings detected linear G-cell hyperplasia and a normal ECL cell population. On the next endoscopic control, 40 months after the eradication treatment, the gastric body presented some tiny sessile polyps (1–2 mm). The antral mucosa showed a mild hyperaemia *H. pylori* negative, and a persistent linear G-cell hyperplasia (figure 3).

**Patient 2**—A 59-year-old woman, with intense chronic active gastritis in the antral mucosa *H. pylori* positive, with simple G-cell hyperplasia associated to superficial active *H. pylori* positive gastritis in the body, normal endocrine population. The following posteradication control, 26 months after the first examination, detected a residual chronic quiescent *H. pylori* negative gastritis in the antral mucosa, with simple G-cell hyperplasia. Tiny





**Figure 2** Simple (A) and linear hyperplasia (B) highlighted by immunohistochemical stain for gastrin ((A) gastrin antiserum, 10x original magnification; (B) gastrin antiserum, 4x original magnification).

sessile polyps with the typical FGPs histological features were seen in the gastric body.

*Patient 3*—A 58-year-old woman showed at first chronic active *H. pylori* positive gastritis, with a linear G-cell hyperplasia, and a normal body-fundic mucosa. Thirty months later, the post-eradication endoscopic control evidenced a persistent linear G-cell hyperplasia in an already normal antral mucosa *H. pylori* negative, with FGPs in the body-fundus.

*Patient 4*—A 37-year-old woman had at the first endoscopic control normal antral mucosa *H. pylori* negative, with linear G-cell hyperplasia and associated FGPs.

*Patient 5*—A 60-year-old woman, with normal antral mucosa *H. pylori* negative at the first endoscopic control had a linear G-cell hyperplasia, and FGPs.

The prevalence of FGPs in patients with AH (13.1%) was confronted with FGPs' prevalence in our general endoscopic population (0.7%; 11) by mean of  $\chi^2$  test with Yates correction. The difference between the two

percentages was extremely statistically significant ( $p < 0.0001$ ).

## DISCUSSION

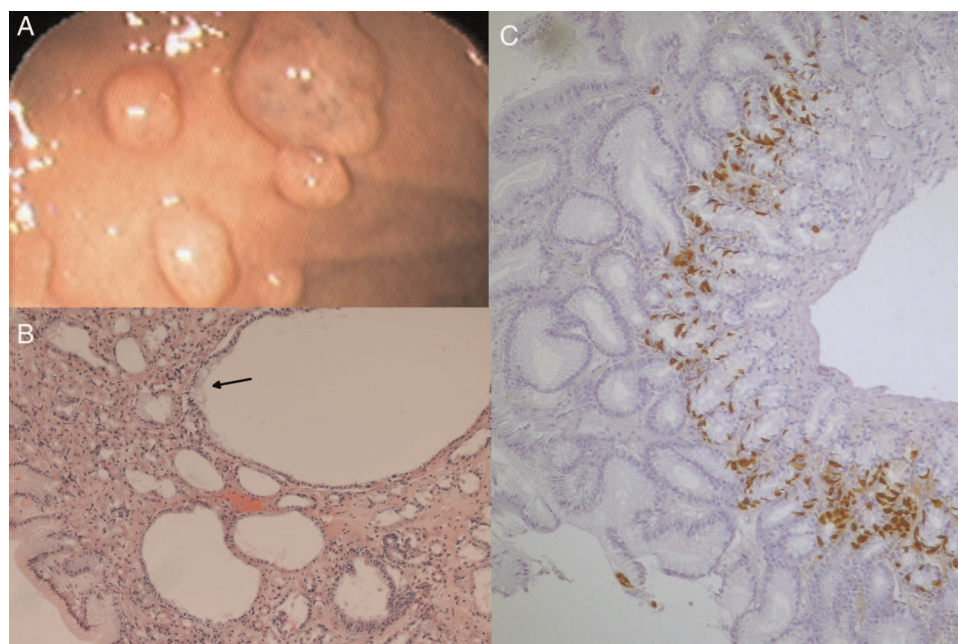
We studied morphologically and immunohistochemically the antral biopsies (and body-fundus biopsies in 27 cases) of 38 patients with AH. AH is characterised by a large increase of calcium in the urine, with renal stones and osteoporosis, with normal calcium levels in the blood, due to hypersensitivity of CaSRs located on the antral G-cell membrane. These patients show normal fasting gastrinaemia and meal hypergastrinaemia ( $\text{Ca}^{2+}$  and peptones) with median gastrin levels almost doubled compared with normal controls.<sup>2</sup> It is now known that gastric CaSR and gastrin-secreting G cells are both activated by  $\text{Ca}^{2+}$ , amino acids and elevated pH. This association strongly suggests an important role of CaSRs in the meal-stimulated gastrin secretion and regulation.<sup>3</sup>

The first aim of our histological and immunohistochemical work was to define if patients with AH and meal hypergastrinaemia had morphological antral G-cell alterations. None of the 38 patients had a history of significant dyspepsia or had received antisecretory therapies.

According to literature data,<sup>12</sup> and our own experience, normal controls showed 1–2 G cells per gland, with irregular distribution. All our 38 patients presented G-cell hyperplasia, either simple (4–5 cells per gland, uniformly distributed) or linear G-cell hyperplasia (continuous chain-like distribution). In a subset experiment, we scored the mean G cell per gland in our five controls, simple and linear hyperplasia (1.18; 3.48; 7.12, respectively), and we found that the difference between

**Table 2** The mean number of G cells per gland was scored in controls, simple and linear hyperplasia and the differences evaluated with unpaired t test were considered very statistically significant

	Controls	Simple hyperplasia	Linear hyperplasia
Mean G cells per gland	1.18	3.48	7.12
SD	1.37	2.32	4.61
	$p=0.0003$	$p=0.0032$	



**Figure 3** Tiny polyps covered by pink glistening mucosa in the body-fundus were apparent at endoscopy (A); histologically, they showed superficial and deep cystic dilation, covered by mucus (arrow), parietal and chief cells, diagnostic for fundic gland polyps (B); antigastrin immunostaining evidence a persistent linear hyperplasia after the *Helicobacter pylori* eradication (C) ((B) H&E,  $\times 10$  original magnification; (C) gastrin antiserum,  $\times 4$  original magnification).

controls and simple hyperplasia was extremely statistically significant ( $p=0.0003$ ), and the difference between simple and linear hyperplasia was very statistically significant ( $p=0.0032$ ).

Twenty-six patients initially presented with *H. pylori* gastritis. As *H. pylori* infection may induce a G-cell reactive hyperplasia,<sup>13</sup> immunohistochemical stainings were performed again after eradication therapy, with a very long mean interval (20.6 months), with identical results in all cases. This very long interval seems to rule out a possible influence of both *H. pylori* and PPI therapy. None of the patients had shown significant body atrophy, another possible confounding condition for G-cell hyperplasia. Moreover, comparing the percentages of simple and linear hyperplasia between the 12 patients originally *H. pylori* negative and the 26 patients eradicated, there was not any statistically significant difference. This control between the two groups appears as a further proof of the primitivity of G-cell hyperplasia in patients with AH.

So we could conclude that the observed G-cell hyperplasia was primitive and that it represented the morphological counterpart of meal hypergastrinaemia in AH.

On the other hand, only 2 of 27 patients with a body biopsy had a micronodular endocrine cell hyperplasia. Evidently, in patients with AH, the meal hypergastrinaemia is not high enough to produce marked pictures of endocrine hyperplasia in the body-fundic mucosa, as seen in the Zollinger-Ellison syndrome.<sup>14</sup>

Unexpectedly, we found in five patients with AH an association with FGPs. FGPs are tiny sessile polyps of the gastric body-fundic mucosa, so far described as sporadic, without other gastrointestinal associations,<sup>8, 10</sup> syndromic

forms associated with familial adenomatous polyposis, classic or attenuated,<sup>15, 16</sup> with Zollinger-Ellison syndrome,<sup>14, 17</sup> and recently as part of the gastric adenocarcinoma-associated proximal polyposis of the stomach (GAPPS).<sup>6, 18</sup> In 2 of our 12 patients, with gastric antrum *H. pylori* negative, the polyps were already present at the first endoscopic examination. In the other three patients, FGPs appeared in the second endoscopic control, after *H. pylori* eradication. So even in patients with AH, we found an inverse relationship between polyps and *H. pylori* infection.<sup>19, 20</sup>

The prevalence of FGPs associated with patients with AH was more than 10-fold that of sporadic FGPs (13, 1% vs 0, 7% of sporadic FGPs), and this difference was extremely statistically significant. Aprile *et al*<sup>14</sup> demonstrated in a multivariate analysis that in 106 patients with Zollinger-Ellison syndrome there was a statistically significant association between polyp formation and levels of gastrinaemia. Similarly, gastrin may have a role in the increased prevalence of FGPs in patients with AH.

In a preliminary experiment, we stained with a gastrin antiserum the gastric antrum biopsies of 79 patients with sporadic FGPs that had never been treated with PPI previously; we found that 75.9% of these patients presented a G-cell hyperplasia morphologically similar to patients with AH.<sup>5</sup> Furthermore, similar findings were shown by Worthley *et al*<sup>18</sup> in the gastric antrum of patients with GAPPS.<sup>6</sup> Apart from this finding, our patients with AH had a negative family history for gastric or colonic cancer, and none of the FGPs showed any dysplastic change.

Another important question to be addressed is to verify if among sporadic FGPs may exist a subset of patients with abnormal calcium excretion, stone formation or abnormal response to calcium or peptone load. At present, we have no data about this interesting question.

In conclusion, we have seen that patients with AH, with sporadic FGPs and with GAPPS share in common an antral G-cell hyperplasia. This fact may represent a promising field of future research.

**Contributors** DP conceived the study design and wrote the draft. BJ gave a substantial contribution in draft preparation. TE and BS contributed substantially to the study design. OB, BA, AI and DM collected cases and also assisted in a critical reading of the manuscript.

**Competing interests** None.

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**Data sharing statement** No additional data are available.

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