Density of Musashi-1-positive stem cells in the stomach of patients with irritable bowel syndrome

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Abstract. Irritable bowel syndrome (IBS) affects ~12% of the global population. Although the etiology of IBS is not completely understood, several factors are known to serve a pivotal role in its pathophysiology, including genetic factors, diet, the intestinal microbiota, gastrointestinal endocrine cells and low-grade inflammation. Musashi-1 is expressed by stem cells and their early progeny, and is used as a stem cell marker. The low density of intestinal endocrine cells in patients with IBS is thought to be caused by decreased numbers of intestinal stem cells and their differentiation into enteroendocrine cells. The present study employed Musashi-1 as a marker to detect stem cells in the stomach of 54 patients with IBS and 51 healthy subjects. The patients and controls underwent standard gastroscopy, and biopsy samples were taken from the corpus and antrum. Immunohistochemical staining of gastrin, somatostatin and Mushasi-1 was carried out and semi-quantified by computerized image analysis. The density (number of positive cells/mm^2 epithelium) of gastrin-positive cells in the controls and patients with IBS were 337.9±560 and 531.0±908 (median ± range; P<0.0001), respectively. For somatostatin-positive cells, the density reached 364.4±526.0 in the healthy controls and 150.7±514.0 in patients with IBS (P<0.0001). The density of Musashi-1-positive cells was defined as the number of cells per gastric or pyloric gland neck. In the corpus, Musashi-1-positive cells density reached 3.0±7.0 in the corpus of the healthy controls and 3.8±7.7 in the patients with IBS. Moreover, the corresponding values in the antrum were 6.0±6.0 and 6.0±6.0, respectively. The Musashi-1-positive cell density did not differ significantly between the controls and patients with IBS in the corpus or antrum (P=0.4 and 0.3, respectively). These findings indicated that changes in the stomach endocrine cells observed in patients with IBS may not be explained by an abnormality in stem cells like those found in the small and large intestines of these patients.

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Introduction

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder with unclear pathophysiology (1,2). However, several factors are known to contribute to the pathophysiology of this condition, including genetic factors, diet, the intestinal microbiota, enteroendocrine cells and low-grade inflammation (1-4).

Gastrointestinal endocrine cells produce >10 different hormones that regulate several functions of the gastrointestinal tract, such as gastrointestinal motility, visceral sensitivity, absorption, secretion, proliferation, local immune defense and appetite (1,5). The gastrointestinal endocrine cells interact and integrate with each other, and with the enteric, autonomic and central nervous systems (6). In IBS, gastrointestinal motility and secretion are abnormal and patients exhibit visceral hypersensitivity (5). The density of enteroendocrine cells in patients with IBS has been reported to be lower than healthy subjects, which may contribute to the pathophysiology of IBS (2,5,7-15).

Previous studies have suggested that the intestinal density of Musashi-1- and neurogenin-3-positive cells were reduced in patients with IBS (7,13,16). Musashi-1 is a marker for intestinal stem cells and their early progeny (17-21). Neurogenin-3 is expressed by an early progenitor for endocrine cells (22,23). It has been proposed that the low density of enteroendocrine cells seen in patients with IBS could be caused by this abnormality in stem cells and their differentiation into enteroendocrine cells (6,7,9-13,16,24-26). Furthermore, the role of genetic factors, diet, intestinal microbiota, enteroendocrine cells and low-grade inflammation in the pathophysiology of IBS is likely mediated through intestinal stem cells (27).

The density of endocrine cells in the stomach corpus is similar in patients with IBS and healthy subjects (28,29). However, the density of gastrin-positive cells in the stomach antrum has been reported to be higher, whereas the density of somatostatin-positive cells has been reported to be lower in patients with IBS, compared with healthy subjects (30). These differences may explain the high incidence of erosive esophagitis observed in patients with IBS (31), as gastrin stimulates acid secretion and somatostatin inhibits acid secretion (6).

The aim of the present study was to investigate whether the changes in the density of gastrin- and somatostatin-positive in the stomach of patients with IBS were associated with alterations in stem cells.

Materials and methods

Patients and controls. Patients with IBS (n=54), according to the Rome IV criteria (32), were recruited from an outpatient clinic at Stord Hospital (Stord, Norway). A control group was formed from healthy subjects (n=51) without any gastrointestinal complaints recruited through an announcement in the local newspaper. The controls comprised 38 females and 13 males with a mean age of 38 years (range, 20-67 years).The characteristics of the patients with IBS are provided in Table I. Co-existence of other functional gastrointestinal disorders was not recorded in the patients with IBS included in the study. The present study was approved by The Regional Committee for Medical and Health Research Ethics West (approval no. 2017/1197/REK Vest). All participants gave oral and written consent to participate.

Gastroscopy and histopathology. Both the patients and controls underwent standard gastroscopy after an overnight fast. Three biopsy samples were taken from the antrum and three from the corpus (major curvature) of the stomach. Two additional biopsies were taken from the antrum and analyzed in a rapid urease test for *Helicobacter pylori* (HelicotecUT Plus, Strong Biotech Corp.). The two biopsy samples were placed in polystyrene gel containing urea substrate for 1 h at room temperature. In the presence of *Helicobacter pylori* urease hydrolysis urea to produce ammonia and CO_2, which changes the pH and is detected as a change in the color of the gel from yellow to red. The biopsy samples were fixed in 4% buffered paraformaldehyde at room temperature overnight, embedded in paraffin wax, then cut into 5-μm sections that were stained with hematoxylin for 20 sec and eosin at room temperature for 3-4 min and were examined under a light microscope (Olympus BX43, Olympus Corporation).

Immunohistochemistry and computerized image analysis. The sections were immunostained using the avidin-biotin-complex (ABC) method with the Vectastain® Elite ABC-HRP kit (cat. no. PK-6200, Vector Laboratories, Inc.). The sections were incubated for 20 min with the blocking normal horse serum then with the primary antibodies at room temperature overnight. The sections were incubated with the secondary biotinylated horse anti-rabbit IgG antibody for 30 min at room temperature followed by the Avidin-bioytin complex for 30 min at room temperature. The following polyclonal rabbit primary antibodies were used: Anti-human gastrin-17 (cat. no. IR519; 1:500; Dako; Agilent Technologies, Inc.); anti-synthetic cyclic (amino-acid sequence 1-14) somatostatin (cat. no. A0566; 1:200; Dako; Agilent Technologies, Inc.); and an antibody raised against a synthetic peptide derived from human Musashi-1 (amino acids 1-100; internal sequence) conjugated to keyhole limpet haemocyanin (cat. no. ab21628; 1:100; Abcam).

The density of Musashi-1-positive cells was semi-quantified on a computer linked to a BX43 light microscope equipped with a DP26 digital camera using Olympus cellSens imaging software (version 1.7; all from Olympus

Corporation). The number of Musashi-1-immunoreactive cells, and the number of gastric or pyloric gland necks in each field were counted manually by pointing and clicking the computer mouse in ten randomly chosen fields. A x400 magnification was used and each field on the monitor represented a tissue area of 0.14 mm². The data from the fields were tabulated, and the cell density was expressed as the number of cells per gastric or pyloric gland neck. The number of gastrin- or somatostatin-immunoreactive cells, and the area of epithelial cells were measured using the same system in 10 randomly chosen fields. The density of gastrin- and somatostatin-positive cells were expressed as number of cells/mm² epithelium. Immunostained sections from the patients with IBS and controls were coded and mixed, with measurements made by an investigator who was blind to the identity of the sections.

Statistical analysis. The χ^2 test was used to assess differences between the patients and controls with regards to sex and the incidence of *H. pylori* infection. Differences in age, and in the densities of gastrin-, somatostatin-, and Musashi-1-positive cells were tested using the Mann-Whitney nonparametric test. The statistical analyses were performed using GraphPad Prism software (version 8; GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Patients and controls. The sex and age distributions did not differ significantly between the patients with IBS and the controls (P=0.9 and 0.8, respectively). The urease test and histopathological examination indicated *H. pylori* infection in three patients and two controls, also with no significant difference between the groups (P=0.6; Table I).

Gastroscopy and histopathology. Gastroscopy suggested that the esophagus, stomach and duodenum were all macroscopically normal in both groups. A histopathological examination of the corpus and antrum indicated normal histology, with the exception of individuals with *H. pylori* infection, in whom metaplasia was found in the antrum.

Immunohistochemistry and computerized image analysis. The gastrin-positive cell density in the controls and patients with IBS were 303.0±560.0 and 623.0±668.0 (median ± range; P<0.0001), respectively (Fig. 1). The corresponding figures for somatostatin-positive cell density were 302.0±526.0 and 140.0±514.0 (P<0.0001; Fig. 2).

Musashi-1-immunoreactive cells were concentrated at the necks of the gastric glands of the corpus and of the pyloric glands of the antrum, where they were evident mostly in a clonogenic proliferation or differentiation form (Fig. 3). The density of Musashi-1-positive cells in the corpus was 6.0±6.0 in the controls and 6.0±6.0 in the patients with IBS (P=0.4); the corresponding values in the antrum were 3.0±7.0 and, 3.8±7.7 respectively (P=0.3; Fig. 4). A subgroup analysis based on the phenotype of the disorder (diarrhea predominant, constipation predominant or mixed type) was not performed, as the number of patients in these subgroups was too small to allow reliable analysis.

Table I. Characteristics of the controls and patients with IBS.

Clinicopathological variable	Control group, n=51	IBS group, n=54
Age, mean (range) years	36 (20-64)	32 (18-52)
Sex, n		
Male	41	44
Female	10	10
IBS subtype, n		
IBS-D	0	20
IBS-C	0	21
IBS-M	0	13
IBS duration, years (mean ± SD)	0	15.5±8.0
H. pylori infection	2	3
Medication, n (%)		
PPI medication	2 (3.9)	53 (98.2)
Birth control medication	32 (62.8)	42 (77.8)
Antimigraine medication	0 (0.0)	2 (3.7)
Medication against asthma/allergies	0 (0.0)	3 (5.6)
Medication with laevothyroxine	0 (0.0)	1 (1.9)
Medication with heart/vascular drugs	1 (2.0)	0 (0.0)

IBS, irritable bowel syndrome; PPI, proton pump inhibitor; D, diarrhea predominant; C, constipation predominant; M, mixed type.
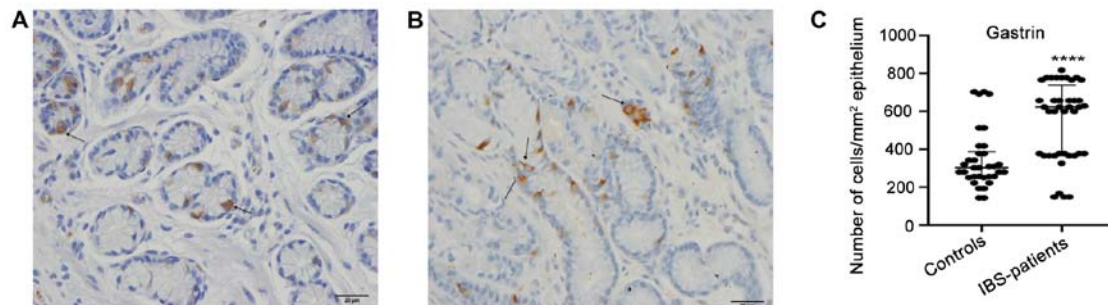

Figure 1. Gastrin-positive-cells (arrows) in (A) a healthy control and in (B) a patient with IBS. (C) Densities of gastrin immunoreactive cells in the epithelium of patients with IBS and healthy controls. Scale, 20 μm. ****$P<0.0001$. Data are presented as the median and interquartile range. IBS, irritable bowel syndrome.
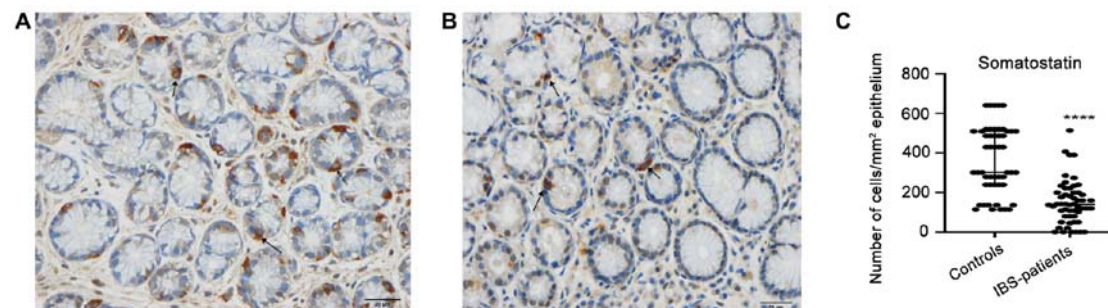

Figure 2. Somatostatin-positive-cells (arrows) in (A) a healthy control and in (B) a patient with IBS. (C) Densities of somatostatin-immunoreactive cells in the epithelium of patients with IBS and healthy controls. Scale, 20 μm. ****$P<0.0001$. Data are presented as the median ± interquartile range. IBS, irritable bowel syndrome.

Discussion

The stomach is divided anatomically into the corpus and antrum, each comprising different cells and functions (33).

The gastric glands of the corpus contain mucous neck cells, parietal cells, chief cells and endocrine cells. In the antrum, endocrine cells and mucous cells are the main cell types in the pyloric glands (33,34). While serotonin- and
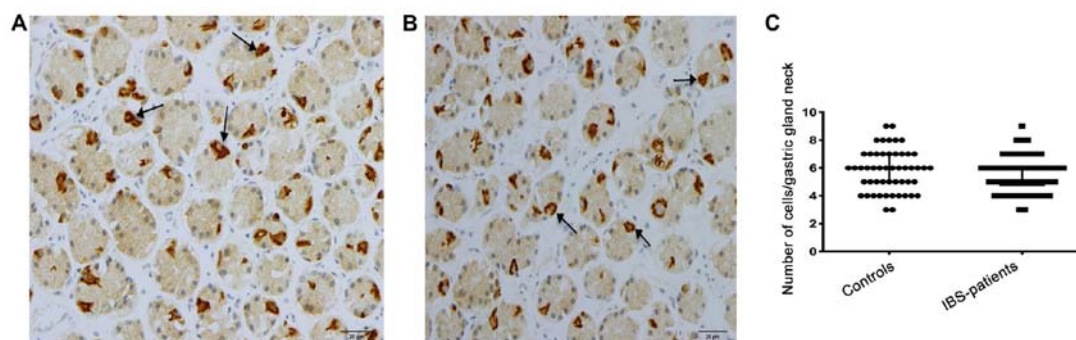
Figure 3. Musashi-1-positive cells (arrows) in the necks of the gastric glands of the corpus in (A) a patient with irritable bowel syndrome and (B) in a healthy control. (C) Densities of Musashi-1-positive cells in the corpus of patients with IBS and healthy controls. Scale, 20 μm. Data are presented as the median and interquartile range. IBS, irritable bowel syndrome.
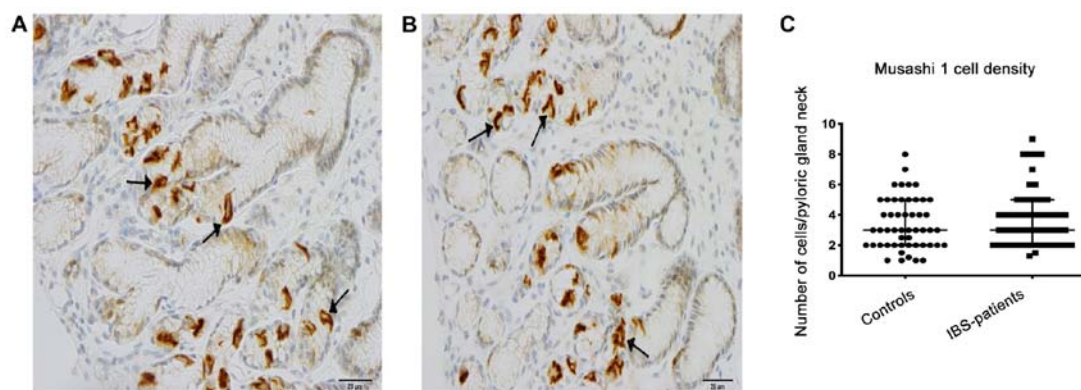

Figure 4. Musashi-1-positive cells (arrows) are present in the necks of the pyloric glands of the antrum. Musashi-1-positive cells in (A) a patient with irritable bowel syndrome and (B) in a healthy control. Densities of Musashi-1-positive cells in the antrum of patients with IBS and healthy controls (C) Scale, 20 μm. IBS, irritable bowel syndrome.

somatostatin-expressing cells are present in both the corpus and the antrum, ghrelin-positive cells are present only in the oxyntic mucosa of the corpus, while gastrin-positive cells are exclusive to the pyloric glands of the antrum (28,30). Several attempts have been made to identify a specific marker for gastric stem cells (33). Musashi-1, which is considered a marker for intestinal stem cells and their early progeny, is reportedly expressed by cells in the corpus and antrum of the stomach (35,36).

The present study found that Musashi-1-expressing cells were mostly present at the necks of the gastric glands of the corpus and of the pyloric glands of the antrum, which is consistent with previous observations (35,36). The density of Musashi-1-positive cells in both the corpus and antrum did not differ between patients with IBS and healthy controls. These observations may explain why, in a previous study, the total density of endocrine cells detected using chromogranin A in the corpus and antrum was similar in patients with IBS and healthy controls (29). In addition, the densities of ghrelin-, serotonin- and somatostatin-expressing cells in the oxyntic mucosa of the corpus did not differ between patients with IBS and healthy controls in a previous study (28). However, the densities of gastrin-positive cells were higher and somatostatin-positive cells were lower in the antrum of patients with IBS, compared with healthy controls. These differences could not be explained by

abnormalities in stem cells like those previously observed in the small and large intestines of patients with IBS, where density of Musashi-1-positive cells were lower than that of healthy controls (2,5,37).

The mechanisms underlying the changes in the densities of antral gastrin- and somatostatin-positive cells in patients with IBS remain unclear. These changes could represent a response to the changes in the intestinal chromogranin A, serotonin, secretin, cholecystokinin, gastric inhibitory peptide, somatostatin, peptide YY and enteroglucagon (oxyntomodulin) cells seen in these patients (2,5,7-15). The increase in the number of gastrin-expressing cells and the reduction in the number of somatostatin-expressing cells could result from activation of gastrin synthesis and inhibition of somatostatin synthesis, a phenomenon that has been observed in gastrointestinal endocrine cells (38,39). However, the present study was limited to measuring changes in densities of gastrin-, somatostatin- and Musashi-1-expressing cells. The expression or secretion of these active substances were not evaluated.

In conclusion, the changes in the density of antral gastrin- and somatostatin-expressing cells in patients with IBS cannot be explained by a low density of stem cells; thus as the gut endocrine cells integrate and interact with each other, the changes in gastrin-and somatostatin-expressing cells in patients with IBS may be caused by the direct changes in the intestinal endocrine cells.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

MES designed the study, obtained the funding, recruited the patients, performed gastroscopies, collected, analyzed and interpreted the data, and drafted the manuscript. TH and JGH contributed to the design of the study, and to the analysis and interpretation of the data, and critically revised the manuscript for important intellectual content. All authors read and approved the final the manuscript.

Ethics approval and consent to participate

The present study was approved by The Regional Committee for Medical and Health Research Ethics West, Bergen, Norway (approval no. 2017/1197/REK Vest). Oral and written consent was obtained from all subjects.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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