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Expression and Localization of Huntingtin–Associated Protein 1 (HAP1) in the Human Digestive System

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Abstract

Background—Huntingtin-associated protein 1 (HAP1) is a neuronal protein that is predominantly expressed in neurons in the brain. HAP1 is critical for maintenance of neuronal survival as well as regulation of food intake and body weight in animals. In addition to the critical role of HAP1 in the central nervous system, HAP1 is also found in endocrine cells, raising an interesting issue of whether HAP1 is expressed in the digestive system.

Aims—To examine the expression and localization of HAP1 in the human gastrointestinal tract and to compare the differences of the HAP1 expression between benign and malignant tissues in the digestive system.

Methods—We used Western blot and immunohistochemistry to examine the expression and distribution of HAP1 in the human gastrointestinal tract tissues.

Results—We observed that the presence of HAP1-positive cells in the gastrointestinal tract was not uniform with immunohistochemistry staining. Western blot revealed that only one isoform (75KD) HAP1 was present in the human gastrointestinal system. Interestingly, the expression of HAP1 was higher in the stomach than other regions of the gastrointestinal tract and was at the lowest level in the intestine. We also found that HAP1 was unlikely altered in benign gastric polyps, but was downregulated in pancreatic cancer.

Conclusions—This is the first study showing the differential expression and location of HAP1 in the human digestive system. These findings suggested that HAP1 may have cell-type-dependent function in the gastrointestinal tract and may serve as a diagnostic marker for pancreatic cancer.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval

The study was approved by the Institutional Review Board at Emory University. The objectives of the study were explained to each patient, and written informed consent was obtained prior to enrollment, in accordance with the guidelines of the Declaration of Helsinki. The ethics approval number is IRB00084524.

Keywords

Huntingtin-associated protein 1; Digestive system; Pancreatic cancer; Feeding

Introduction

Huntingtin-associated protein 1 (HAP1) is a neuronal protein that interacts with huntingtin, a protein that can cause Huntington's disease (HD). HD is a neurodegenerative disorder caused by the polyglutamine expansion (> 37 units) in the N-terminal region of huntingtin. The expanded polyglutamine expansion causes HAP1 to bind more tightly to huntingtin, suggesting a potential involvement of HAP1 dysfunction in HD pathology [1].

Unlike huntingtin that is ubiquitously expressed, HAP1 is enriched in the brain. HAP1 is a cytoplasm protein that associates with microtubules and many types of membranous organelles, including mitochondria, endoplasmic reticulum, tubulovesicles, endosomal and lysosomal organelles, and synaptic vesicles [2]. In rat brain, HAP1 consists of two isoforms (HAP1-A, 75KD and HAP1-B, 85KD) that differ in their C-terminal sequences [3]. The expression level of HAP1 in the rodent brains varies, as HAP1 is enriched in the hypothalamus, amygdala, and brain stem [2, 4]. HAP1 in the hypothalamus may play a critical role in regulation of food intake and body weight. The strong evidence for the function of HAP1 in regulating food intake is that HAP1 knockout mice showed reduced food intake and postnatal death, a phenotype that is probably due to the degeneration of hypothalamic neurons in the absence of HAP1 [5].

Several studies suggest that HAP1 participates in vesicular trafficking, endocytosis of membrane receptors [5–9]. Consistent with the endocytosis function of HAP1, HAP1 is also found in endocrine cells such as pancreatic cells [2, 4, 10–12]. Although previous studies have shown that HAP1 is present in other systems such as stomach and duodenum in mice [4, 10, 11], it remains unclear whether HAP1 is also expressed in the human digestive system. Given the expression of different isoforms of HAP1 in rodents and humans [1], identification of HAP1 in the human digestive system may help us to determine the involvement of HAP1 in digestive diseases. In the present study, we carried out immunohistochemistry and Western blot to examine the expression and distribution of HAP1 in the human digestive system. We examined the expression of HAP1 in the normal tissues of the human digestive tract and some benign and malignant tissues. Our findings show that HAP1 is differentially expressed in the examined tissues and suggest that HAP1 also plays a role in endocrine cells in the human digestive tract.

Materials and Methods

Tissue Preparation

The gastrointestinal tract biopsy samples were collected from patients, aged 30–75 with normal liver and renal function and no digestive diseases during esophagogastroduodenoscopy (EGD) and colonoscopy examination. Biopsy samples were obtained using standard biopsy forceps. Some of them were immediately placed into 4%

paraformaldehyde to fix for 2 h, embedded in paraffin, and sectioned at 5 μm, and thaw-mounted onto gelatin-coated slides. The others were immediately placed into dry ice and then kept at − 80 °C refrigerator for Western blotting. The pancreatic samples were collected from immediately surgically resected pancreatic tissue in the operation room and then placed into 4% paraformaldehyde or dry ice.

Western Blot Analysis

The samples were homogenized in phosphate buffer saline (PBS) containing cocktail protease inhibitors (1 μg/ml pepstatin A, 1 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml leupeptin). Protein samples (60 μg) were denatured in SDS sample buffer at 100 °C for 5 min before loading onto a 10% SDS-PAGE. Proteins transferred to nitrocellulose membrane were blocked in 5% nonfat dry milk in PBS for 1 h and then incubated with the purified rabbit anti-hHAP1 antibody (1:1000, EM39 [1]) and mouse anti-actin antibody (1:200,000, Ab6276, USA) overnight at 4 °C. Following the incubation, the membrane was washed and incubated with secondary horse radish peroxidase (HRP)-conjugated donkey anti-rabbit and donkey anti-mouse antibodies (1:10,000, Jackson ImmunoResearch, USA) in 5% nonfat milk for 1 h at room temperature. Blots were visualized using SuperSignal ECL (Thermo, USA).

Immunohistochemistry

After being rinsed in PBS to reduce endogenous peroxidase activity and to prevent nonspecific antibody binding, tissue sections were treated in 10% hydrogen peroxide for 30 min and 2% normal goat serum (NGS) with 3% bull serum albumin (BSA) in PBS containing 0.2% Triton X-100 for 1 h for blocking. Sections were then incubated with a purified rabbit antibody to human HAP1 (1:1000, EM39) at 4 °C for 40–45 h, followed by incubation of biotinylated goat anti-rabbit IgG (1:200, Vector Laboratories, USA) at room temperature for 1 h and avidin–biotin complex (Vectastain Elite ABC Reagent, USA) at room temperature for 1 h. Primary and secondary antibodies were diluted with PBS containing 0.2% Triton X-100 and 2% NGS with 3% BSA. The tissue sections were rinsed in PBS between incubations. Finally, the HAP1-immunoreactive tissues were visualized by incubation with 3,3′-Diaminobenzidine tablets (DAB, Sigma, USA) for 2–5 min. Sections were then lightly counterstained with hematoxylin for less than 1 min, dehydrated, cleared with xylenes, coverslipped, and examined under light microscopy.

Statistical Analysis

Data were analyzed using SPSS 24.0 for Mac (SPSS Inc., Chicago, IL, USA) and Prism 7.0 for Macintosh (GraphPad Software, San Diego, CA, USA). All values were expressed as the mean ± SD. The differences between groups were analyzed using two-tail Student's t test and ANOVA. P values of less than 0.05 were considered statistically significant ($^{*}P < 0.05$).

Results

Patients Information

A total of 15 patients (11 females, four males) who accepted endoscopic examination or surgery between March 2017 and October 2017 and met the inclusion criteria were enrolled.

Demographic and pathology data are presented in Table 1. The gastric polyps were all benign lesions while pancreatic cancers were all adenocarcinoma.

Expression of HAP1 in the Human Gastrointestinal Tract

We performed Western blot analysis of biopsies in different parts of the human gastrointestinal tract, including the esophagus, the proximal and distal stomach, the proximal and distal small intestine, the proximal and distal colon, and the rectum. The blot was cut to stripes that were immuno-probed with antibodies to HAP1 and β-actin so that both proteins in the same samples were detected at the same time. Unlike the rodent HAP1 that consists of two isoforms (HAP1-A and HAP1-B) [3], only one HAP1 band (about 75KD) had been identified in the human samples, which had the molecular weight similar to that of rat HAP1-A. Interestingly, the expression of HAP1 was higher in the stomach than other parts of the gastrointestinal tract and is at the lowest level in the small intestine, which was confirmed by quantifying the ratio of HAP1 to β-actin (Fig. 1).

Localization of HAP1 in the Human Gastrointestinal Tract

Light microscopic immunohistochemistry with antihuman Hap1 antibody revealed that HAP1 is mainly expressed in the cytoplasm in the digestive system. There were regional distribution differences of HAP1-positive cells in the mucosa of human gastrointestinal tract. In the esophagus, which contains several scattered esophageal glands, moderate to strong HAP1 immunoreactivity was exclusively concentrated in the mucosa glands (Fig. 2a). Strong HAP1 immunoreactivity was found in the stomach in which many HAP1-immunopositive cells are densely distributed in the gastric glands (Fig. 2b). We biopsied duodenum as the representative of the proximal small intestine, and we found weak HAP1 immunoreactivity with scattered distribution of HAP1-immunoreactive cells in the villi and the intestine glands (Fig. 2c). In colon, weak to moderate HAP1 immunoreactivity was found with diffuse HAP1 staining in the colon glands (Fig. 2d).

Comparison of HAP1 in Normal Stomach Tissues and Stomach Polyps

We biopsied normal gastric mucosa and gastric polyps in each patient to examine the expression of HAP1. The pathology confirmed all polyps were benign, including fundic gland polyp, chronic inflammation, and hyperplasia. Western blot analysis (a) and densitometric analysis (b) showed there was no difference between normal stomach tissue and stomach polyp ($t = -0.165$, $P > 0.05$) (Fig. 3).

Comparison of HAP1 in Pancreatitis, Normal Pancreas, and Pancreatic Cancer Tissues

We collected normal pancreatic tissues from the same patient who had pancreatic cancer to compare the expression of HAP1. For pancreatitis, we only had one patient and used three different parts of the pancreatitis tissues. Immunohistochemical staining of HAP1-positive cells in pancreatitis and normal pancreas tissue showed that HAP1-positive cells are scattered in the pancreatic islet cells (Fig. 4a). They were distributed throughout the islets and localized in the cytoplasm. However, there was absence of HAP1 staining in pancreatic cancer tissues (Fig. 4a). Western blot analysis showed the similar HAP1 levels in both pancreatitis and normal pancreas tissues and non-detectable HAP1 level in pancreatic cancer

(Fig. 4c). Quantitative analysis of the relative levels of HAP1 by measuring its ratio to β-actin confirmed that HAP1 was markedly reduced or absent in the examined pancreatic cancer tissues (Fig. 4d).

Discussion

Earlier studies showed that HAP1 is a brain-specific protein that is widely expressed in the rodent brains [2, 3]. The neuronal function of HAP1 may be mediated by its regulating intracellular trafficking, recycling, and stabilization of receptors [13]. Indeed, decreasing HAP1 reduces the number of secreted vesicles and inhibits vesicles exocytosis [13]. These studies suggested that HAP1 may also be involved in hormone release in the endocrine cells that largely rely on receptor endocytosis and vesicle exocytosis. The important role of HAP1 in feeding behavior may be related to its endocytic regulation of neurotrophic factor receptors in the hypothalamus [5, 14]. It was yet unclear whether HAP1 also has peripheral function in the digestive system, as HAP1 is also found in endocytic cells in the digestive tract [10, 11, 15]. For example, HAP1 protein was found in singly dispersed cells of the mucosal layer of the stomach and duodenum of mice [10]. Enter-oendocrine cells were dispersed throughout the epithelia lining of the intestinal wall, and it is presumed that the HAP1-positive cells were enteroendocrine cells [15]. In the pancreas, HAP1 was selectively expressed in β-cells that release insulin [11]. A recent study showed that HAP1 was important for insulin release from β-cells, and when HAP1 expression was reduced, glucose-mediated insulin release was inhibited [13].

Because the expression of HAP1 isoforms in rodents and humans is not identical, it is important to investigate the expression of HAP1 in the human digestive tract in order to explore the potential role of HAP1 in digestive diseases. Our immunohistochemical study showed for the first time that HAP1 was present in the mucosa of the entire human gastrointestinal tract with regional expression differences. The expression of HAP1 was the highest in stomach and lowest in the small intestine. This may be associated with different digestive functions, as stomach plays an important role in secretion of gastric acid, enzymes, and many hormones, which was consistent with the idea that HAP1 is involved in the secretion of hormones.

Although we did not observe any significance difference in HAP1 expression between normal gastric mucosa and gastric polyps, we found that HAP1 is drastically decreased in pancreatic cancer tissues. These findings suggested that the expression of HAP1 may have a specific role in the pathogenesis of pancreatic cancer. Although more studies are required for understanding the significance of this marked reduction in pancreatic cancer tissues, several hypotheses can be offered on the basis of HAP1's function and previous findings. The intracellular trafficking function HAP1 is also likely involved in cell proliferation and apoptosis [5, 9]. Previous study showed that EGFR is highly expressed in a number of solid tumors and its expression correlates with tumor progression, resistance to chemotherapy, and a poor prognosis [16]. Overexpression of HAP1 prevents the trafficking of internalized EGFR from early endosomes to lysosomes, resulting in the suppression of EGFR degradation [5]. Some studies also demonstrated the relation of HAP1 expression with

tumorigenesis in some types of cells, as HAP1 is downregulated in breast tumor tissues and overexpression of HAP1 reduced in vitro cell growth in breast cancer cell lines [17].

In summary, our findings demonstrated for the first time the differential expression of HAP1 in the human gastrointestinal tract and HAP1 downregulation in the pancreatic cancer tissues. These findings suggested that HAP1 may have cell-type-dependent function in the human gastrointestinal tract and its expression may provide a diagnostic marker for pancreatic cancer.

Acknowledgments

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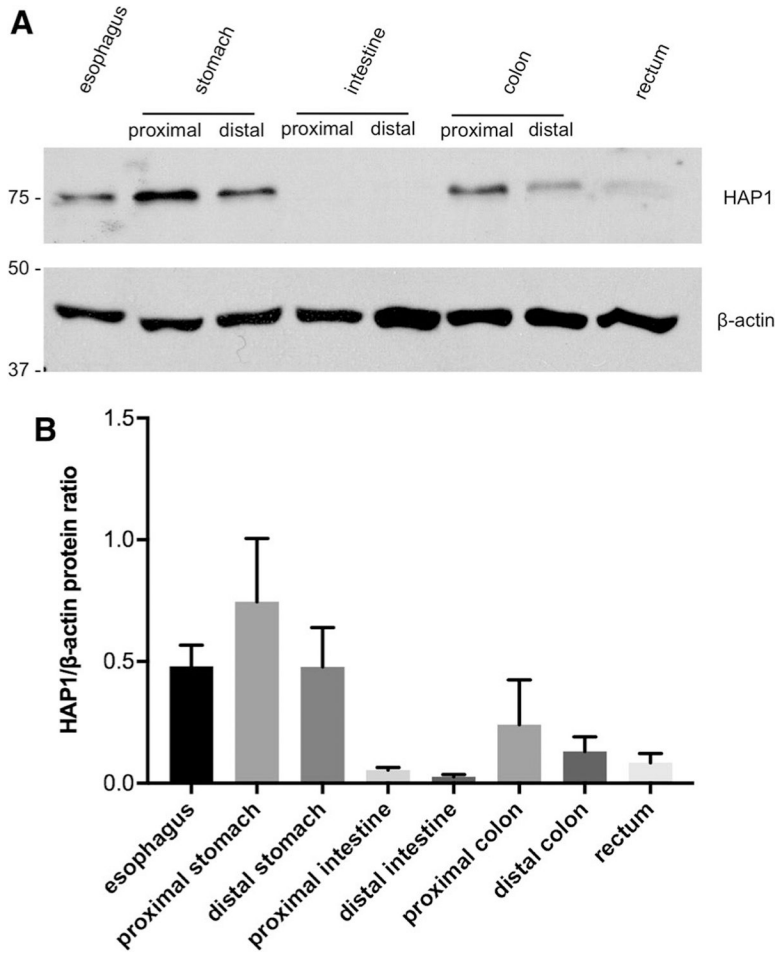

Fig. 1.

Western blots of huntingtin-associated protein 1 (HAP1) in different parts of the human gastrointestinal tract. **a** The blot was cut to stripes that were immunoprobed with antibodies to HAP1 and β-actin. Gastrointestinal tract protein extract (60 μg/lane) showed prominent immunoreactive bands at 75 kD for HAP1 and 42 kD for β-actin. Note that the expression of HAP1 was the highest in stomach and lowest in small intestine. **b** Densitometric analysis of the ratio of HAP1 to β-actin in each lane on the Western blots. Each bar represents mean ± SD ($n = 3$)

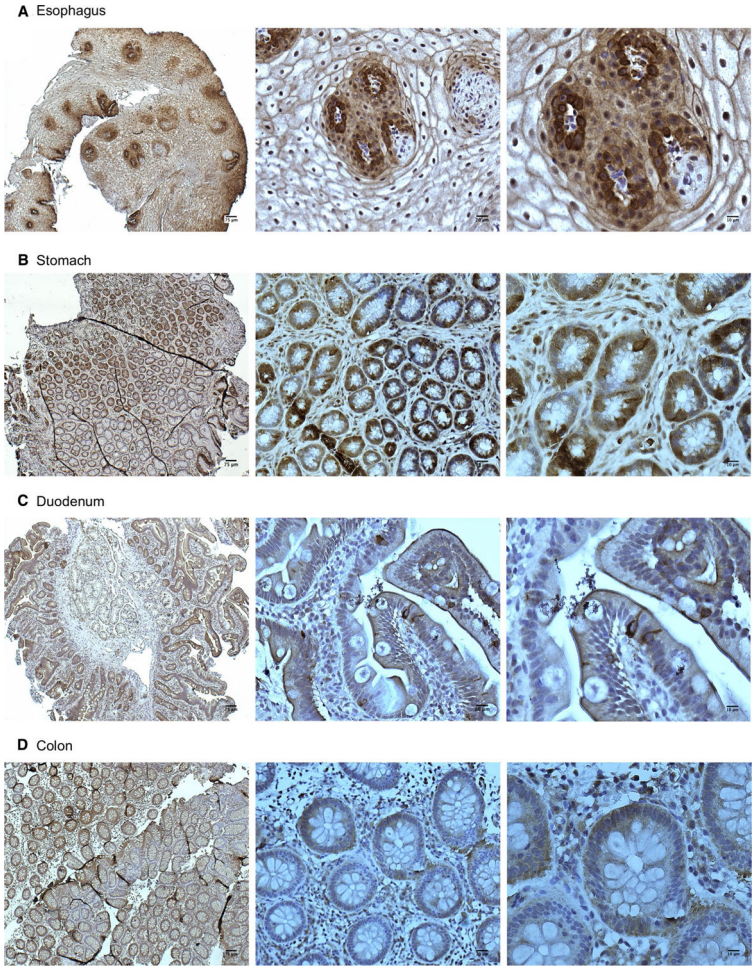

Fig. 2.

Immunohistochemical staining for HAP1 in the human gastrointestinal tract. HAP1 was expressed in the cytoplasm. **a** Esophagus: There are few glands in the mucosa of esophagus. HAP1-positive cells were concentrated in these glands. **b** Stomach: Immunopositive cells were densely distributed in the gastric glands. **c** Small intestine showed scattered distribution of HAP1-immunoreactive cells in the villi and the intestine glands. **d** Colon showed weak and diffuse HAP1 staining in the colon glands. Amplification: left panel (× 5), middle panel (× 20) and right panel (× 40)

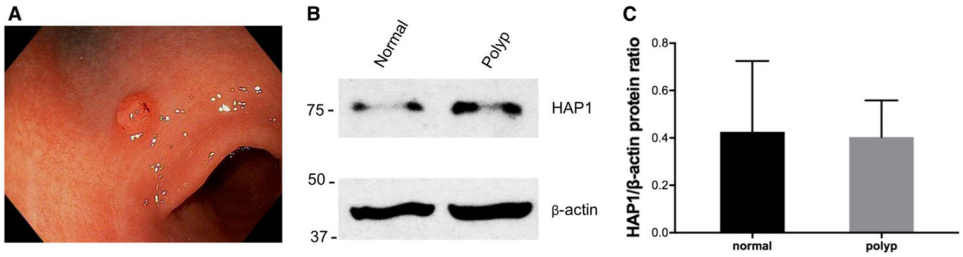

Fig. 3.

Expression of HAP1 in normal gastric mucosa and gastric polyps. **a** Endoscopic view of a gastric polyp. **b** Protein levels of normal gastric mucosa and gastric polyps were analyzed by Western blotting. β-actin served as a loading control. **c** Densitometric analysis of the expression of HAP1 showed no difference between normal gastric mucosa and stomach polyps. Each bar represents mean ± SD of values ($n = 3$, $P > 0.05$)

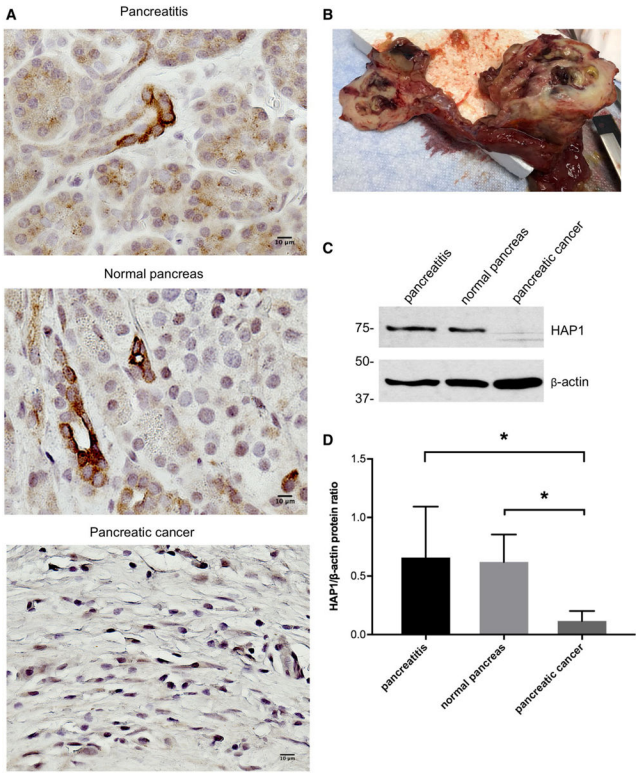

Fig. 4.

Comparison of HAP1 expression in pancreatitis, normal pancreas, and pancreatic cancer tissues. **a** Immunohistochemical staining of HAP1-positive cells in pancreatitis, normal pancreas, and pancreatic cancer tissue. Scale bar: 10 μm. **b** The head of the pancreas had been resected with Whipple procedure, and the pathology showed adenocarcinoma. **c** The expression of HAP1 in pancreatitis, normal pancreas, and pancreatic cancer tissue was detected by Western blotting. β-actin was a loading control. **d** Densitometric analysis showed the expression of HAP1 in pancreatitis, normal pancreas, and pancreatic cancer tissue ($n = 3$, $^*P < 0.05$)

Table 1

Demographic and pathology data of 15 patients

Patient no.	Sex	Age	Weight (kg)	Height (m)	BMI (kg/m^2)	Samples	Pathology
1	Female	71	59.5	1.68	21.1	Stomach, duodenum	N/A
2	Female	67	75.9	1.72	26.2	Stomach, duodenum	N/A
3	Female	53	63.5	1.55	26.5	Esophagus, stomach, duodenum	N/A
4	Male	69	74.8	1.78	23.6	Esophagus, stomach, duodenum	N/A
5	Female	51	67.1	1.70	23.2	Ileum, colon, rectum	N/A
6	Male	62	95.3	1.83	28.5	Esophagus, stomach, intestine, colon, rectum	N/A
7	Female	66	68.0	1.73	22.8	Ileum, colon, rectum	N/A
8	Female	32	59.0	1.57	23.8	Esophagus, stomach	N/A
9	Female	53	92.5	1.63	34.8	Pancreas	Fibrosis and chronic inflammation
10	Male	65	61.0	1.75	19.9	Pancreas	Adenocarcinoma
11	Female	89	57.0	1.65	20.9	Pancreas	Adenocarcinoma
12	Male	58	67.0	1.68	23.7	Pancreas	Adenocarcinoma
13	Female	69	78.0	1.60	30.5	Gastric polyp	Fundic gland polyp
14	Female	79	62.2	1.60	24.3	Gastric polyp	Chronic inflammation
15	Female	79	60.3	1.60	23.6	Gastric polyp	Hyperplasia