Cancer risk and genotype-phenotype correlation in Japanese patients with Cowden syndrome

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Abstract

Background Cowden syndrome (CS) is an autosomal-dominant hereditary disorder caused by a germline *PTEN* variant and characterized by multiple hamartomas and a high risk of cancers. However, no detailed data on CS in Asian patients nor genotype-phenotype correlation have been reported.

Methods We performed the first Japanese nationwide questionnaire survey on CS, and obtained questionnaire response data on 49 CS patients.

Results Patients included 26 females (median age 48 y). The incidence of breast, thyroid, endometrium, and colorectal cancer was 32.7%, 12.2%, 19.2% (among females), and 6.1% respectively. The incidence of any cancers was relatively high among all patients (46.9%, 23/49), and particularly female patients (73.1%, 19/26), compared with previous reports from Western countries. Gastrointestinal (GI) polyps were more frequently found throughout the GI tract compared with previous studies. *PTEN* variants were detected in 95.6% (22/23) of patients; 12 in the N-terminal region (11 in phosphatase domain) and 10 in the C-terminal (C2 domain) region. The incidence of cancer in the C2 domain group was significantly higher than in the N-terminal region (phosphatase) group. All female patients with C2 domain variant had breast cancer.

Conclusion Our data suggest that Japanese patients with CS, particularly female patients and patients with C2 domain variant may have a high risk of cancers.

Introduction

Cowden syndrome (CS) is an autosomal dominant inherited disease characterized by multiple hamartomas and a high risk of cancers including breast, thyroid, kidney, endometrial, and colorectal cancer (CRC) [1-3]. Currently the NCCN guideline for Cowden syndrome/PTEN hamartoma tumor syndrome (PHTS) [4], is widely used worldwide. In this guideline, the 8 major diagnostic criteria are composed of breast cancer, thyroid follicular cancer, endometrial cancer, gastrointestinal (GI) hamartomas, macrocephaly, mucocutaneous lesions, Lhermitte-Duclos disease (LDD), and penile freckling. Moreover, CS is associated with CRC and renal cancer, glycogenic acanthosis in the esophagus, autism spectrum disorder, intellectual disability, and vascular anomaly [4]. The most common cancer is reportedly breast cancer, followed by thyroid cancer, endometrial cancer, and CRC [2,3,5,6]. However, the incidence of these cancers varied to a large extent among studies. Moreover, all relevant studies on CS have been performed in Western countries, and no data from Asian patients with CS have been reported.

Several studies of hamartomatous polyps in the GI tract of CS patients have been reported to date [7-9]. However, most of the studies on GI polyps have been small-scale, and only 2 large-scale (n>50) studies on GI polyps in CS have been reported [8,9]. Although CRC is expected to develop from colorectal polyps, detailed analysis on the number of polyps and the histology in each region of GI tract in CS have not been reported. Moreover, some patients

with GI polyps have been reported to present with GI bleeding, but a detailed analysis of symptomatic GI polyps including small bowel polyps has not been performed.

The germline *PTEN* variant site has been found scattered over the entire *PTEN* gene in CS. However, the variant site is predominantly distributed in the N-terminal phosphatase domain of the *PTEN* gene [3,5,9]. On the other hand, somatic mutation of *PTEN* is found preferentially in the C-terminal region of the *PTEN* gene in various cancers [10-12]. In addition, no clear genotype-phenotype correlation has been found in CS, although several studies investigated the correlation between CS phenotypes and *PTEN* gene variant sites [3,9,13,14]. Moreover, all of these studies on the *PTEN* gene were performed in Western countries, and data on Asian patients with CS are lacking. Therefore, in this study, we performed the first nationwide survey of CS in Asia, and investigated incidence of cancer, pathological findings of tumors, and other clinical manifestations in various organs. We then analyzed germline *PTEN* gene variants and genotype-phenotype correlations in patients with CS.

METHODS

Questionnaire and patients

We performed a preliminary questionnaire survey of CS among 3337 gastroenterologists from all over Japan. We received responses from 539 gastroenterologists and found that CS

patients had been followed up in 49 hospitals. We then sent a detailed questionnaire to the physicians in those hospitals, and received responses from 35 hospitals describing 57 patients. We determined whether these patients met the diagnostic criteria of the NCCN guideline version 1. 2019 [4], or those of the International Cowden Consortium version 2000 [15], or had germline *PTEN* variant. We excluded 8 patients because of the non-fulfillment of those diagnostic criteria and analyzed 49 patients from 30 hospitals (Supplementary Fig.). The registration period was between January 2015 and December 2018. Patient information including major and minor diagnostic criteria and *PTEN* variant were investigated in questionnaire (Supplementary Information). This study was approved by the Institutional Review Board and Human Genome and Gene Analysis Research Ethics Review Board in Tokushima University and in each hospital, and informed consent was obtained from the participants, according to the rules of the hospitals. All methods were performed in accordance with the relevant guidelines and regulations.

Details for the germline *PTEN* gene variant and statistics are provided in Supplementary information.

RESULTS

Patient characteristics

The baseline characteristics of CS patients enrolled are provided in Table 1. Of the 49

patients, 26 (53.1%) were female. The median age of all patients was 48 years (range, 19-98); that of females and males was 49 years (range, 28-98) and 47 years (range, 19-76), respectively. Seventeen patients (34.7%) had a family history of CS; i.e., they had a first- or second-degree relative with CS. Of the 49 patients, a set of 2 patients were related to each other, as were another set of 3 patients. In total our cohort included 46 probands (families) and 49 patients. The germline *PTEN* gene variant was tested in 23 (46.9%) patients.

Clinical manifestations

We first evaluated the incidence of clinical manifestations including 8 major symptoms in patients with CS (Table 2). Overall, GI polyps (95.9%, 47/49), mucocutaneous lesions (87.8%, 43/49), thyroid disease (81.6%, 40/49), and breast disease (76.9%, 20/26, only female) were found to have incidence rates greater than 70% in patients with CS. Endometrial disease was found in 12 of 26 female patients (46.2% among females). The incidence of breast disease and thyroid disease was obviously higher in female than in male patients respectively, consistent with previous reports [5,13,16].

Among the various types of mucocutaneous lesions, acral keratosis, cutaneous facial papules, and papillomatous papules were most commonly identified (73.5%, 71.4%, and 57.1% of patients, respectively). Penile freckling was seen in only 1 male patient (4.3%). Mental disease was found in 14.3% (7/49) of patients and was more common among males

than females (6/23 vs 1/26). Macrocephaly, vascular anomaly and LDD were found in 38.8% (19/49), 28.6% (14/49), and 10.2% (5/49) of patients, respectively.

Malignant and benign lesions in major organs

The incidence of cancers in each organ is shown in Table 3. Breast cancer was the most frequent among all patients with CS (32.7%, 16/49). The incidence of breast cancer was obviously higher in females (61.5%, 16/26) than in male patients (0%, 0/23), consistent with previous reports [5,13,16]. Moreover, among all breast diseases, cancer was the most common histologic type (80.0%,16/20; Fig.1A). Of the patients with breast cancer, 5 patients (31.3%, 5/16) had bilateral breast cancers. The onset age of the youngest breast cancer patient was 23 years, and that patient had bilateral cancer. Information on the histology and immunogenicity of the invasive cancers examined is provided in Supplementary Table 2. The cancers showed various types of histology, but most cancers (8/9) were estrogen receptor- and progesterone receptor-positive. Among benign breast diseases, fibrocystic disease was the most common (15.4%, 4/26), and 25% of those patients had breast cancer. There was no significant difference in age between patients with and without breast cancer.

The incidence of thyroid cancer was 12.2% (6/49) (Table 3), and it was the second most common cancer in all CS patients. The incidence of thyroid cancer tended to be higher in female (19.2%, 5/26) than in male patients (4.3%, 1/23; p=0.14). The onset age of the

youngest thyroid cancer patient was 17 years. Among all thyroid diseases, the incidence of benign lesions was even higher than that of cancers in both males and females; 60.9% (14/23) and 76.9% (20/26), respectively (Fig.1B). Of these benign lesions, goiter was the most common among females (42.3%, 11/26) and males (30.4%, 7/23), followed by adenoma and Hashimoto's disease.

The incidence of endometrial cancer and benign diseases alone was 19.2% (5/26) and 26.9% (7/26), respectively (Fig.1C). One patient had both endometrial cancer and benign disease (myoma). Among all endometrial diseases, cancer was the most common, followed by myoma, polyps, and endometrial hyperplasia.

As for other organs, CRC, renal cancer, lung cancer, and ovarian cancer were found in 3 (6.1%), 2 (4.1%), 2 (4.1%) and 1 (3.8%) of patients, respectively.

The overall incidence of any cancers in female patients (73.1%, 19/26) was obviously higher than in male patients (17.4%, 4/23), consistent with previous reports [13,16]. The median age [interquartile range; IQR] of patients with any cancer was significantly higher than that of patients without cancer (54 [42-71] vs 47 [35.8-60]; p=0.026). Moreover, the median onset age of the first cancer among patients with multiple cancers tended to be lower than that in patients with single cancer; (40, [30.5-44] vs 58, [39-78]; p=0.08).

Incidence, number, and pathology of GI polyps

The incidence, number, and pathological findings of GI polyps are shown in Figure 2. Esophageal polyps were found in 85.1% (40/47) of patients (Fig.2A). Among 33 patients whose number of polyps was described in questionnaire, the majority of these patients (57.6%, 19/33) had more than 100 polyps. Histologically, the most common lesions in the esophageal polyps were glycogenic acanthoses (53.2%, 25/47) followed by hamartomas, papilloma, and lymphoid cells infiltration.

Gastric polyps were present in 91.7% (44/48) of patients (Fig.2B). Although a majority of these patients (60.0%, 24/40) had 1-50 polyps, 25% (10/40) had more than 100 polyps. Histologically, various types of polyps including hyperplastic polyp (27.1%, 13/48), hamartoma (22.9%, 11/48), fundic gland polyp (18.8%, 9/48), and inflammatory polyp (10.4%, 5/48) were found.

Duodenal polyps were observed in 70.2% (33/47) of patients, and most of these patients (69.7%, 23/33) had 1-50 duodenal polyps (Fig.2C). The most common polyp was hamartoma (21.3%, 10/47) followed by lymphoid follicle and inflammatory polyps.

Jejunal/ileal polyps were found in 94.7% (18/19) of patients, and most patients (55.6%, 10/18) had 1-50 jejunal/ileal polyps. Histologically they included hemangioma (15.8%, 3/18), lymphoid follicle (15.8%, 3/18), inflammatory polyp (10.5%, 2/19), hamartomatous polyps (5.3%, 1/19), and hyperplastic polyp (5.3%, 1/19). One patient had a large hamartomatous polyp (≥20 mm), which should be removed by endoscopic resection to prevent ileus. Another

patient with multiple hemangiomas presented an intermittent hematochezia.

Colorectal polyps were found in 97.7 % (43/44) of patients (Fig.2E). Most patients (73.0%, 27/37) had 1-50 of colorectal polyps, and only 5.4% (2/37) had more than 100 polyps. Various histological types of polyps were found; the most common were hamartoma (34.1%, 15/44), followed by adenoma (27.3%, 12/44), hyperplastic polyp (20.5%, 9/44), and inflammatory polyp (7/44, 15.9%). CRCs were found in 3 patients; 2 were male and 1 was female. Details of the female patient with cancer were unknown. Of the 2 male patients, 1 had a cancer in adenoma and the other had advanced sigmoid colon cancer. The latter was diagnosed as stage III colon cancer at age 39 years and underwent surgical operation. However, he relapsed soon after surgery, presenting with liver metastasis. Although he received systemic chemotherapy, he finally died from CRC at age 41 years. The former patient with CRC had 1 to 50 colorectal polyps. However, the latter patient had no polyps other than CRC.

GI lesion complications occurred in 20.0% of patients (6/30); All these patients presented with bleeding and 1 had abdominal pain.

Genotype-phenotype correlation

The germline *PTEN* variants were detected in 22/23 of patients (95.7%). The patient characteristics and variant site in all 22 patients are described in Supplementary Table 1. The

PTEN variants in 22 patients comprised 9 nonsense mutations (40.9%), 8 frameshift mutations (36.4%), 3 missense mutations (13.6%), and 2 splice site mutations (9.1%). Of the 22 variants, 15 were classified as pathogenic/likely pathogenic in ClinVar database and 4 were previously reported in CS. The remaining 3 (all frame shift variants) were classified as variant of uncertain significance but all the patients fulfilled the diagnostic criteria of CS.

To evaluate the distribution of variant sites and genotype-phenotype correlations, the *PTEN* gene was divided into the N-terminal region (including phosphatase domain) and C-terminal region (including C2 domain and PDZ) (Fig.3). Of these 22 patients, 12 had *PTEN* variants in the N-terminal region (11 in phosphatase domain) and 10 in the C-terminal region (C2 domain). Cancers were found in 33.3% (4/12) of the N-terminal region group (27.3% [3/11] in phosphatase domain) and in 80.0% (8/10) of the C2 domain group. The incidence of cancer in the C2 domain group (8/10) was significantly higher than in the N-terminal region (p=0.038) group (phosphatase domain group, p=0.023). Breast cancers were found in 100% (7/7) of females in the C2 domain group and 50.0% (4/8) of females in the N-terminal group (42.9% [3/7] phosphatase domain group). The incidence of breast cancer in the C2 domain group was marginally higher than in the N-terminal region group (p=0.051). No significant differences in the incidence of cancer among 4 variant types of nonsense mutations, frameshift mutations, missense mutations, and splice site mutations were observed.

Discussion

In the first Japanese nationwide multicenter study of CS, we have demonstrated that the incidence of cancers in CS patients was high (46.9%), particularly among female patients (73.1%), in comparison with previous studies from Western countries. We also found GI polyps at substantially higher rates in all regions of the digestive tract including the esophagus (85.1%), stomach (91.7%), duodenum (69.7%), jejunum/ileum (94.7%), and colorectum (97.7%) compared with previous reports. Additionally, the incidence of cancer was significantly higher in patients with germline *PTEN* gene variant in the C2 domain than in the other domains, suggesting a genotype-phenotype correlation in CS.

The overall incidence of any cancer in CS has been reported to be 31%~51%; 42%~61% in female and 17%~33% in male patients [9,13,16]. The incidence of any cancer in female patients (73.1%, 19/26) in this study was very high; it was higher than in previous reports, although the mean age was slightly higher in current study than previous studies (49 vs 32-44 years). Moreover, the incidence of multiple cancers in female patients was 46.2% (12/26) although the incidence of multiple cancers in female patients had not been previously reported. There is a possibility that the incidence of any cancer among female patients with CS may be higher in Japan than in Western countries. On the other hand, of the 23 male patients, only 4 (17.4%) had cancers in this study, which is slightly low but within the range of previous reports (17%~33%) [9,13,16]. Regarding the comparison between the genders,

because the diagnostic criteria for CS included breast cancer and thyroid cancer, the incidence of which is significantly higher in female than male patients, and endometrial cancer only for female, it may not be appropriate to make such a comparison. In addition, our cohort includes 49 patients from 46 probands (families). The number of probands among all the patients in this study is relatively higher compared with previous studies [9,13]. This may suggest that our results on cancer incidence are more reliable even though the cohort size was small.

The cumulative risk of breast cancer among female patients with CS at the age of 70 years is reportedly estimated to be 77-85% although the actual frequency of breast cancer overall regardless of age was reported to be 24%-45% [3,5,9,13,16]. Among female patients in this study, breast cancer was the most frequent cancer, followed by thyroid cancer and endometrial cancer, consistent with previous reports [13,16]. Notably, the incidence of breast cancer in our study was the highest among studies to date. Since the breast is a paired organ, it is necessary to pay attention to the opposite breast when a patient initially presents with breast cancer. Interestingly, somatic *PTEN* mutation has been reported to be associated with triple-negative breast cancers [17]. The breast cancers in our study showed various types of histology but most cancers were positive for estrogen and progesterone receptors. Further study with a larger cohort of patients is needed.

The incidence of colorectal polyps in our study (97.7%) was higher than in previous

studies (85.0%~92.5%) [8,9], whereas the incidence of CRC (6.1%) was roughly comparable with previous reports (2.2%~15.4%) [8,9,13,18]. It has been reported that CS patients with numerous colorectal polyps have a high risk of CRC [8]. In fact, a male patient with CRC in this study had multiple polyps (range 1~50). However, the other CRC patient had no other colorectal polyps. Moreover, when the CRC was found by colonoscopy, his CRC was already advanced (stage III), and he died ultimately due to CRC. These data suggest that even patients with a small number of colorectal polyps should undergo colonoscopy for surveillance of CRC. Only one other study to date has examined colorectal polyps and cancers in a large number of CS patients (n>40); Heald and associates found CRCs in 13.4% (9/67) of patients with CS by colonoscopy [8]. However, their study included many symptomatic patients, whereas most of our patients were non-symptomatic and underwent colonoscopy as surveillance for colorectal tumors. Thus, the high incidence of CRC (13.4%) in their study might be explained by the difference in the reason for performing colonoscopy.

Regarding gastric polyps, only 1 large-scale study (n>40) on CS patients has been reported to date. Bubien and associates reported the incidence of gastric polyps (73.3%, 44/60) but did not examine the histological types of the polyps [9]. In the present study, the incidence of gastric polyps (91.7%) was considerably higher than theirs, and we evaluated the incidence of each histological type of the polyps (Fig.2B). Similarly, the incidence of esophageal polyps and duodenal polyps was higher than in previous reports [8,9]. We also

showed various histological types of these polyps other than hamartomas. In addition, although there have been no studies investigating jejunal/ileal polyps in CS aside from a few cases [19,20], we found a very high incidence of jejunal/ileal polyps (94.7%). Notably, one patient had large hamartoma (>2 cm) and another patient showed hematochezia from multiple hemangiomas. These jejunal/ileal lesions, which should be treated, were found in 10.6% (2/19) in CS patients. Thus, the incidence of GI polyps in CS was higher in this study than in previous studies. This result may be explained by 2 hypotheses; 1) the incidence of GI polyps in Japanese (Asian) patients with CS is high, or 2) GI endoscopy is popular in Japan due to the universal insurance system.

The incidence of macrocephaly (38.8%) and penile freckling (4.3%) was lower in this study than in previous reports [5,9,21]. This could be due to the current questionnaire being answered by a gastroenterologist and not by a pediatrician or dermatologist.

It has been reported that the *PTEN* germline variant site is located predominantly in the phosphatase domain (N-terminal region) rather than the C2 domain (C-terminal region) [3,5,9,22]. In this study, however, it was found almost equally in the phosphatase domain (n=11) and C2 domain (n=10), suggesting that the frequency of the germline variant in the C2 domain may be higher among Japanese patients with CS than in those of Western countries. It has been well documented that germline variant sites in many hereditary diseases are geographically varied [23,24]. Moreover, we showed that the incidence of

cancer patients with C2 domain variant was significantly higher than in those with other domain variants. In addition, the incidence of breast cancer in patients with the C2 domain variant was marginally higher than in those with the other variant. Interestingly, Sun and associates generated a knock-in mouse with nonsense mutation in the *PTEN* C2 domain and demonstrated that this region played an essential role in suppression of tumorigenesis [25]. These may be related to the fact that incidence of cancer in our study was higher than in previous reports from Western countries. However, as a limitation of this study, the number of patients analyzed for *PTEN* gene was small, and therefore the results should await conformation in a large-scale study of CS patients.

The youngest patient who developed breast cancer was 23 years old. The NCCN guideline recommends to start periodic examination of the mammary gland at 25 years of age, or to start from 5~10 years earlier than the onset age of breast cancer patient in a family younger than 25 years. However, there were no female CS patients in her family. Therefore, caution should be exercised regarding the breast cancer even in a female patient younger than 25 years when she does not have a female family member with CS. The age of onset of CRC in 1 patient in this study was 39 years, which is older than the age at which colonoscopy surveillance is recommended (35 years). However, he had never undergone prior colonoscopy. Our results may support the guidelines for CRC surveillance, which recommend colonoscopy for CRC surveillance starting at age 35 years. In addition, in this

Japanese nationwide study of CS, cancer surveillance had been performed in most patients based on NCCN guideline; according to the guidelines exactly (11/27) or approximately according to the guideline (13/27).

In conclusion, the results of the present nationwide study suggests that Japanese/Asian patients with CS, particularly female patients and patients with the C2 domain variant in the *PTEN* gene, may have a high risk of cancers.

Author contributions

Satoshi Teramae and Tetsuji Takayama designed the research study. Naoki Muguruma, Koichi Okamoto, Kumiko Oseto, Ryutaro Nishikawa, Takayuki Tanoue, Keiji Hirata, Shunichi Yanai, Takayuki Matsumoto, Seiji Shimizu, Jun Miwa, Yu Sasaki, Kazuo Yashima, Hiroyuki Ohnuma, Yasushi Sato, Yoshitaka Kitayama, Yoshio Ohda, Atsushi Yamauchi, Yoji Sanomura, Kumiko Tanaka, Yoshiaki Kubo, and Hideki Ishikawa collected the data and Satoshi Teramae analyzed the data. Yoshimi Bando evaluated histology of breast cancers. Tomoko Sonoda performed statistical analysis. Tetsuji Takayama contributed to the final approval of the manuscript.

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Disclosure Statement

The authors have no conflict of interest.

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Figure legends

Fig. 1

Cancer and benign lesions in breast, thyroid, and endometrium in female and male patients with Cowden syndrome. A circle graph showing cancers and benign lesions, and a bar graph showing cancers and each benign lesion are presented. A. Breast. One patient had both fibrocystic disease and cancer. Another patient had fibrocystic disease and adenoma. One more patient had mastitis and papilloma. B. Thyroid. Two patients had goiter and Hashimoto disease, respectively. C. Endometrium. One patient had endometrial cancer and uterine myoma, and another patient had endometrial polyp and endometrial hyperplasia.

Fig. 2

Number and histology of gastrointestinal polyps in patients with Cowden syndrome. A circle graph of positivity, a bar chart of the number of polyps, and a bar graph of the number of each histology type. A. Esophageal polyp, B. Gastric polyp, C. Duodenal polyp, D. Jejunal/ileal polyp, E. Colorectal polyp.

Fig. 3

Distribution of germline *PTEN* gene variant site in patients with Cowden syndrome. The variant site is shown in the corresponding site of the PTEN protein. NP_000305 was used as

a reference sequence for the *PTEN* gene and its domain structure. Phosphatase domain is located in the N-terminal region at codons 24-181. C2 domain and PDZ-binding domain are located in the C-terminal region at codons 188-349 and codons 401-403, respectively. The PTEN protein is divided into N-terminal region and C-terminal region [26].

Supplementary Figure legend

Patient inclusion flowchart following questionnaire distribution.

Table 1. Baseline characteristics of patients with Cowden syndrome

Clinical characteristics	All patients	Female	Male
No. of patients	49	26 (53.1%)	23 (46.9%)
Age (y), median (range)	48(19-98)	49 (28-98)	47 (19-76)
Family history of CS [n, (%)]*	17 (34.7)	9 (34.6)	8 (34.8)
PTEN gene tested [n, (%)]	23 (46.9)	16 (61.5)	7 (30.4)

CS, Cowden syndrome.

*Family history was defined as having a first- or second-degree relative with Cowden syndrome.

Table 2. Clinical manifestations in patients with Cowden syndrome

	All patients (n=49)	Female (n=26)	Male (n=23)
	n (%)	n (%)	n (%)
Gastrointestinal (GI) polyps	47 (95.9)	24 (92.3)	23 (100.0)
GI hamartoma	22 (44.9)	11 (42.3)	11 (47.8)
Esophageal glycogen acanthosis	25 (51.0)	14 (53.8)	11 (47.8)
Mucocutaneous lesions	43 (87.8)	22 (84.6)	21 (91.3)
Acral keratosis	36 (73.5)	17 (65.4)	19 (82.6)
Cutaneous facial papules	35 (71.4)	17 (65.4)	18 (78.3)
Papillomatous papules	28 (57.1)	15 (57.7)	13 (56.5)
Trichlelemmomas	7 (14.3)	4 (15.4)	3 (13.0)
Penile freckling	- (-)	- (-)	1 (4.3)
Thyroid disease	40 (81.6)	25 (98.2)	15 (65.2)
Breast disease	20 (40.8)	20 (76.9)	0 (0.0)
Endometrial disease	-	12 (46.2)	-
Macrocephaly	19 (38.8)	8 (30.8)	11 (47.8)
Vascular anomaly	14 (28.6)	6 (23.1)	8 (34.8)
Mental disease*	7 (14.3)	1 (3.8)	6 (26.1)
Lhermitte-Duclos disease	5 (10.2)	2 (7.7)	3 (13.0)

*Mental disease includes intellectual disability (5), depression disorder (1), and unknown (1).

Table 3. Incidence of each cancer in patients with Cowden syndrome.

	All patients (n=49)	**Female (n=26)**	**Male (n=23)**
	n (%)	n (%)	n (%)
Breast caner	16 (32.7)	16 (61.5)	0 (0.0)
Bilateral breast cancers	5 (10.2)	5 (19.2)	0 (0.0)
Thyroid cancer	6 (12.2)	5 (19.2)	1 (4.3)
Endometrial cancer	- (-)	5 (19.2)	- (-)
Colorectal cancer	3 (6.1)	1 (3.8)	2 (8.7)
Renal cancer	2 (4.1)	2 (7.6)	0 (0.0)
Lung cancer	2 (4.1)	0 (0.0)	2 (8.7)
Ovarian cancer	-	1 (3.8)	-
Cancer of unknown primary*	1 (2.0)	1 (3.8)	0 (0.0)
Any cancer	23 (46.9)	19 (73.1)	4 (17.4)
Multiple cancers	13 (26.5)	12 (46.2)	1 (4.3)

*Pathologically squamous cell carcinoma.

Figure 1

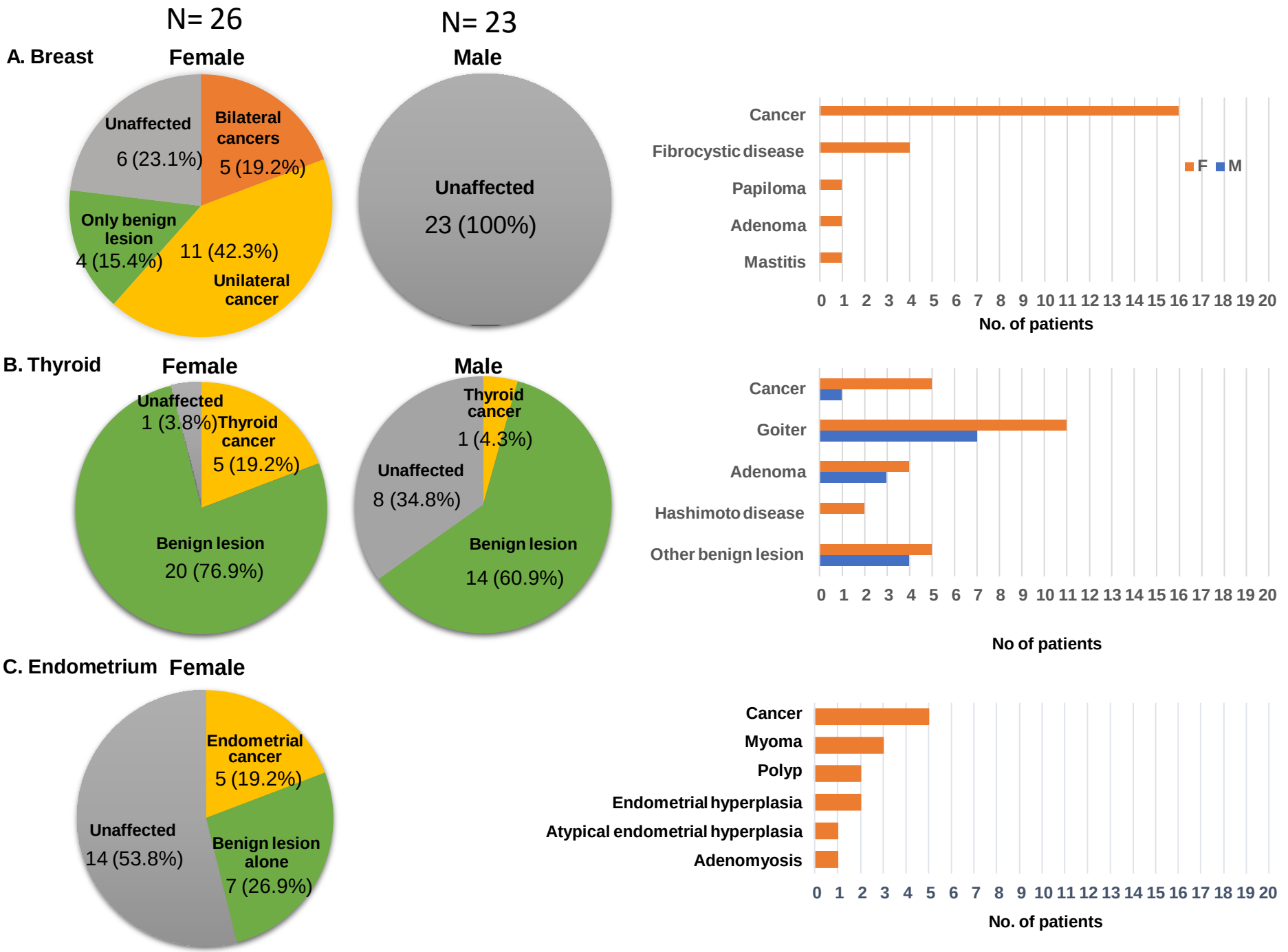

Figure 2

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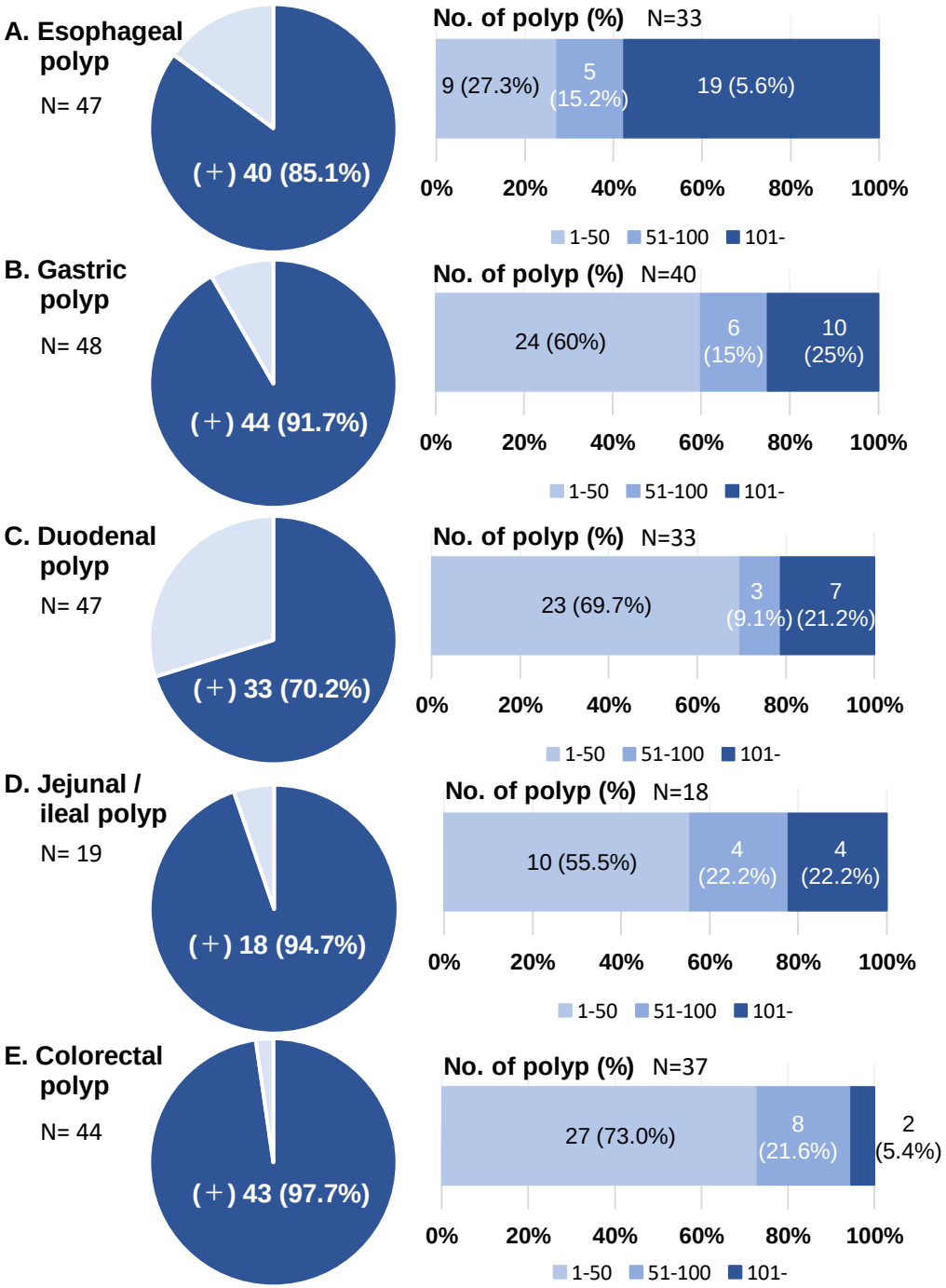

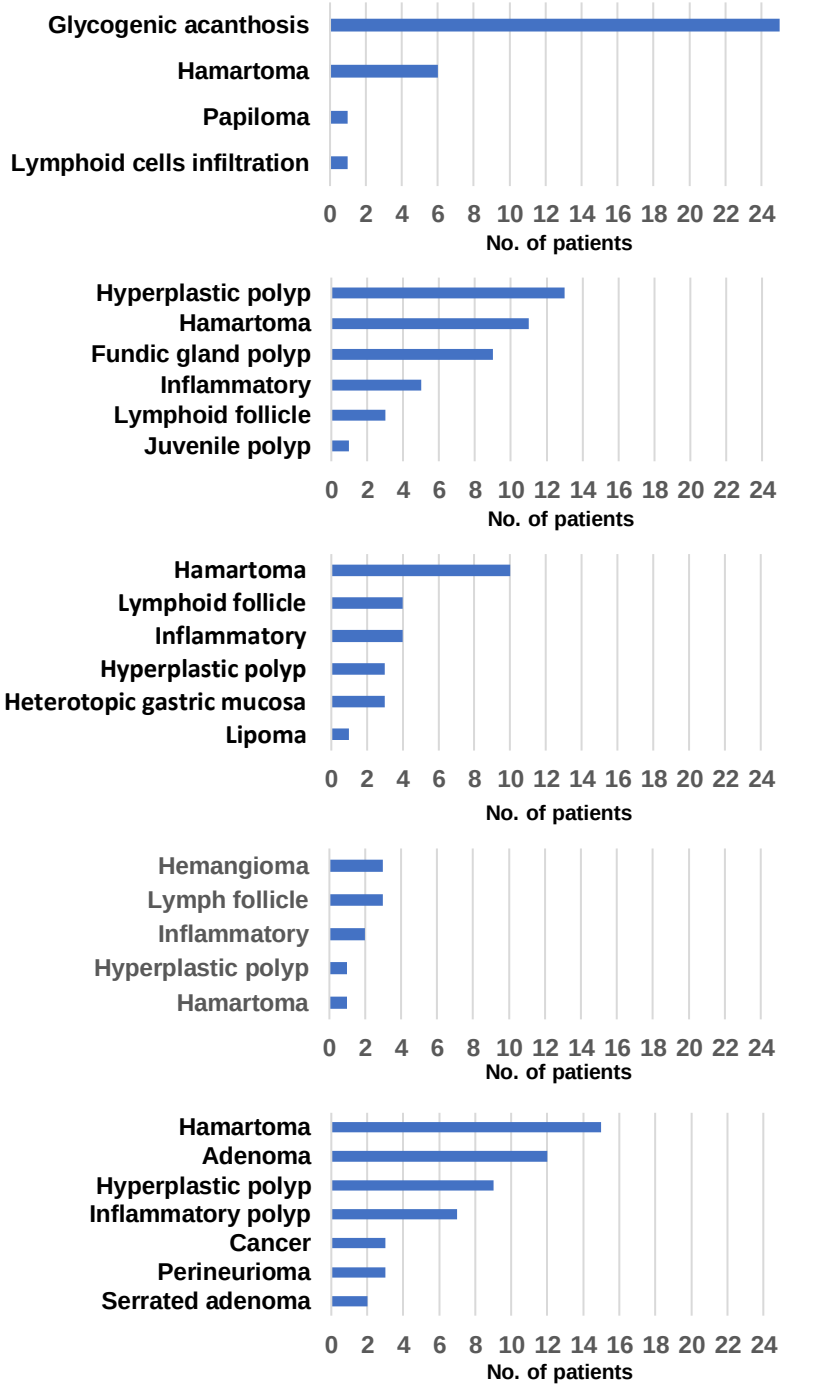

Figure 3