Prospective cohort study assessing outcomes of patients from families fulfilling criteria for hereditary diffuse gastric cancer undergoing endoscopic surveillance

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Background: Prophylactic total gastrectomy is performed in hereditary diffuse gastric cancer (HDGC) patients carrying the *CDH1* mutation because endoscopic surveillance often fails to detect microscopic disease.

Objective: The aim of this study was to determine the natural history and outcomes of patients with HDGC undergoing endoscopy.

Design: Prospective, cohort observational study.

Settings: Tertiary referral center.

Patients: Patients fulfilling criteria for HDGC who opted to undergo endoscopy.

Intervention: Research surveillance program using high-resolution white-light endoscopy with autofluorescence and narrow-band imaging combined with targeted and multiple random biopsies assessed by an expert histopathologist for the presence of signet ring cell carcinoma.

Main Outcome Measurements: The primary endpoint was the endoscopic yield of microscopic signet ring cell carcinoma according to patient mutation status and subsequent decision to undergo surgery. The secondary endpoint was the additional yield of targeted biopsies compared with random biopsies.

Results: Between September 2007 and March 2013, 29 patients from 17 families underwent 70 surveillance endoscopies. Signet ring cell carcinoma foci were identified in 14 of 22 (63.6%) patients with confirmed *CDH1* germline mutations and 2 of 7 (28.6%) with no pathogenic mutation identified. Eleven of 16 (9 *CDH1*-positive) patients proceeded to gastrectomy in a median 5.7 months. Five patients delayed surgery. In 1 patient, advanced gastric cancer developed 40.2 months after the first endoscopic findings.

Limitations: No control group.

Conclusions: Careful white-light examination with targeted and random biopsies combined with detailed histopathology can identify early lesions and help to inform decision making with regard to gastrectomy. Autofluorescence and narrow-band imaging are of limited utility. Delaying gastrectomy in individuals with signet ring cell carcinoma foci carries a high risk and has to be weighed carefully. (Gastrointest Endosc 2014;80:78-87.)

(footnotes appear on last page of article)

Hereditary diffuse gastric cancer (HDGC) is rare and accounts for 1% to 3% of all gastric cancers. The diagnosis of HDGC is suspected when one of the following criteria is fulfilled[1]:

1. Two or more family members with a diagnosis of gastric cancer with at least 1 diffuse gastric cancer diagnosed before the age of 50 years.
2. Three or more family members with a diagnosis of diffuse-type gastric cancer independent of age.
3. Diffuse gastric cancer is diagnosed before the age of 40 years.
4. Family history of diffuse gastric cancer and lobular breast cancer with 1 family member receiving a diagnosis before the age of 50 years.

An estimated 25% to 50% of patients fulfilling the first 2 criteria will carry an inactivating *CDH1* germline mutation

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and diffuse gastric cancer will develop upon loss or inactivation of the second allele.[1-4] This increases to 47% for patients fulfilling the first criterion. More recently, a review of 322 patients with suspected HDGC according to these standards yielded 29.2% positivity for a *CDH1* mutation.[4] The penetrance of a *CDH1* mutation is high, with an 80% lifetime risk of multifocal diffuse gastric cancer presenting at a mean age of 40 years (range 20-72 years).[2,5] In a *CDH1* mutation carrier, prophylactic total gastrectomy is recommended before the development of symptoms because of the very poor prognosis in established disease.[6] Of patients undergoing prophylactic total gastrectomy, 87% will have positive histopathological findings in their specimen ranging from microscopic signet ring cell carcinoma (SRCC) to linitis plastica.[4]

The decision to undergo prophylactic total gastrectomy is complex. Mortality is low (<1%); however, adverse events occur, and there are long-term effects on quality of life (QOL).[7] In the largest prophylactic total gastrectomy study to date (Newfoundland, n = 23), 17% had a major adverse event (anastomotic leak, abdominal abscesses) with an overall adverse event rate of 48%.[8] In a recent series involving 16 asymptomatic patients, 13 underwent prophylactic gastrectomy with no adverse events and were discharged by 1 week.[6] A second series with 10 patients similarly showed a hospital stay of 6 to 7 days; however, there were 4 late adverse events with 1 pulmonary embolus, 2 small-bowel obstructions, and 1 anastomotic stricture.[9] The long-term consequences include significant weight loss, dumping syndrome, and malabsorption as well as the psychosocial impact of altered eating habits and reduced body mass index.[10,11] For many patients with a *CDH1* mutation, the decision about whether and when to undergo prophylactic gastrectomy is an agonizing one. Some may prefer to delay the operation for reasons including child bearing and family responsibilities, loss of earnings, and fear of the associated mortality and morbidity. For these patients, endoscopic surveillance is an attractive option, but it has not yet been possible to provide evidence concerning the safety and efficacy of this approach. On the contrary, there has been concern that endoscopic surveillance may not detect the microscopic foci of SRCC adequately and may falsely reassure patients and increase their risk of the development of advanced gastric cancer subsequently.[6,8,9]

For families in whom no *CDH1* mutation has been identified or in whom the mutation is of uncertain pathogenic significance (eg, missense), surveillance is generally the only option because most surgeons would not undertake the operation without some evidence that the patient is at significant risk of gastric cancer. There are also individuals who are waiting for genetic testing results, and endoscopy is reasonable to provide a baseline status until more information is available.

The endoscopic protocol suggested in the international guidelines were based on discussion between experts and some limited data to suggest that annual multiple biopsy specimens (>30) taken from the representative areas from all anatomic areas of the gastric mucosa are required.[1,12] In the Newfoundland series, surveillance endoscopy failed to detect any foci of gastric cancer in 21 of 23 patients, all of whom were subsequently proven to have early cancer at gastrectomy.[8] In another study using methylene blue and Congo red dye, 33 *CDH1* mutation carriers underwent 93 surveillance endoscopies over a 5-year period.[13] Fifty-six targeted biopsy specimens were taken in 18 patients, and of these, 23 lesions in 10 patients (56% patients) were positive for SRCC. Despite its promising results, further use of this technique was curtailed because of concerns over dye toxicity.

Thus, the aims of this prospective study were to determine the natural history and outcomes of patients from families fulfilling HDGC criteria undergoing surveillance in the following groups: (1) CDH1 positive patients preferring to delay gastrectomy, (2) CDH1 mutations status unknown or uncertain at start of endoscopic surveillance, and (3) patients from CDH1 negative families. The primary endpoint was the yield of SRCC foci by endoscopy and gastrectomy (in those patients proceeding to surgery) according to mutation status and to correlate these findings with patient outcome (disease staging, curative surgery, and survival). The secondary endpoint was the additional yield of targeted biopsies compared with random biopsies.

Take-home Message

- In patients from families with hereditary diffuse gastric cancer, particularly those without confirmed causative germline mutations, endoscopic trimodal imaging with targeted and random biopsies detected microscopic foci of signet ring cell carcinoma (SRCC), which helped to inform decisions about gastrectomy.
- Once a focus of SRCC is identified, patients should be counseled about undergoing surgery because, although the natural history is uncertain, delay carries a substantial risk of progression to incurable disease.

METHODS

Patients, data collection, and analysis

Patients in the Familial Gastric Cancer Registry held in Cambridge fulfilling criteria for HDGC were invited to participate in a research surveillance program using high-resolution white-light endoscopy with autofluorescence imaging (AFI) and narrow-band imaging (NBI) combined with targeted and multiple random biopsies assessed for the presence of SRCC. Patients are managed by a multidisciplinary team consisting of a medical geneticist, gastroenterologists, upper GI surgeons, and a specialist nurse. All patients are recommended to undergo prophylactic total gastrectomy when a deleterious *CDH1* mutation is

detected, and all are offered a baseline endoscopy before surgery. Those who prefer to defer surgery (because of patient choice or clinical recommendation as a result of physical or psychological comorbidity) are offered endoscopic surveillance. Patients in whom no *CDH1* is identified or those awaiting clarification of their genetic status (eg, owing to a lack of confirmed mutation in the family index case) are also enrolled in a surveillance program.

Ethical approval was obtained from the ethics research committee (MREC 97/5/32). A dedicated specialist nurse obtained informed consent from each patient and prospectively collected basic demographic information, family history, endoscopic findings, and pathology reports. A separate review of the clinical notes was conducted to assess the reason for surveillance and the impact of SRCC on the subsequent management plan.

Data are given as mean with standard deviation, median with interquartile range, and proportion where appropriate. The proportion of patients with detected foci of SRCC in each cohort and the subsequent intervention or delay in surgery are described. For those who underwent prophylactic total gastrectomy, disease staging and completeness of resection are reported. The yield of SRCC through targeted biopsies, random biopsies, or a combination is also reported. Follow-up is defined as the time from the first endoscopic examination (including previous endoscopic surveillance) to the most recent endoscopic examination. On a per-patient analysis, sensitivity and negative predictive value were calculated for each biopsy modality. Specificity, accuracy, and positive predictive value were also calculated for AFI and NBI targeted biopsies.

Endoscopic protocol

A detailed research endoscopy surveillance protocol incorporating high-resolution white-light endoscopy with AFI and NBI was set up in 2007 so that the procedures are conducted in a standardized fashion by the same endoscopists (K.R., M.d.P., R.C.F.) with 1 person performing the procedure and another recording any abnormalities. One of the endoscopists (K.R.) had significant previous expertise in AFI and NBI lesion recognition, and he reviewed the images as required. *N*-acetylcysteine as a mucolytic agent is first administered orally 30 minutes before the procedure. Patients are rolled from the right to the left to ensure full distribution of the mucolytic agent. All patients are offered conscious sedation with midazolam with supplemental fentanyl or pethidine as required. The examination is conducted with a high-resolution endoscope that provides 115× magnification and a maximal resolution of 7.9 μm (GIF-FQ240Z, ELURA CLV260SL, OEV261H; Olympus, Tokyo, Japan). The stomach is carefully insufflated, and at least 30 minutes are spent systematically examining various anatomic segments. Any abnormality is described and further assessed by AFI and NBI. AFI positivity is defined by an area of well-demarcated magenta on a background of normal green mucosa. NBI changes are classified as either normal or abnormal (described as irregular, suspicious, or distorted). All endoscopic findings are recorded in a custom reporting proforma. Any focal lesions identified undergo biopsy in addition to obtaining 24 random biopsies (4 each of the prepylorus, antrum, T zone, body, fundus, and cardia), and a biopsy specimen from each area is snap frozen for research. All pathological specimens are stained with periodic acid–Schiff with diastase and examined by a upper GI pathologist specialist (M.O.), with a second pathologist confirming any findings of SRCC cells including in situ lesions.

For patients previously undergoing endoscopy before 2007, these data are shown in the figures and are included in the follow-up length, but are not part of the analysis because they did not have the same standardized protocol.

RESULTS

Cohort demographic characteristics and *CDH1* status

Between September 2007 and March 2013, 29 patients from 17 families (Table 1) underwent 70 surveillance endoscopies.

Twenty-two patients (75.9%) were confirmed carriers of a *CDH1* mutation, either at the time of enrollment into the study or subsequently. The remaining 7 patients were entered into surveillance based on the clinical criteria for HDGC. Of these, 4 patients were from *CDH1* mutation–negative families, 1 had a possible variant with insufficient evidence of pathogenicity to warrant gastrectomy, and 2 were untested because there were no affected family members alive to provide samples for genetic testing. Overall, the yield of SRCC foci detected by endoscopy was 63.6% (14 of 22) in those with a *CDH1* mutation (either known at the time or confirmed subsequently) and 27.6% (2 of 7) in those with a negative or unconfirmed mutation status. The median follow-up was 20.5 months (interquartile range 11.0-62.0 months).

CDH1-positive patients

Fourteen patients had a known *CDH1* mutation from the start of enrollment in the study but decided to undergo surveillance endoscopy rather than proceed straight to prophylactic total gastrectomy (Figs. 1 and 2). Of these 14 patients, 9 had foci of SRCC detected during surveillance, 7 at the initial endoscopy, 1 at a second endoscopy (9 months), and 1 at a third endoscopy (22 months). Four patients with SRCC detected proceeded straight to prophylactic total gastrectomy with a further patient requiring treatment for his Barrett's esophagus before surgery. Of the remaining 4 patients, 1 had type 1 diabetes mellitus and despite advice to have gastrectomy was worried about the effect on his glycemic control and QOL with his

TABLE 1. Patient characteristics

	Overall	Known *CDH1* mutation	Subsequent *CDH1* mutation confirmed	Untested or *CDH1* negative
Sample size	29	14	8	7 (3 negative)
Median age, y (range)	36 (19 – 65)	29.5 (19 – 65)	40 (27- 59)	40 (20-55)
Sex, male:female	16:13	8:6	5:3	3:4
Follow-up, mo, median (IQR)	20.5 (11.0–62.0)	19.8 (9.0–52.2)		23.4 (11.8–71.4)
SRCC identified	16	14		2

IQR, Interquartile range; *SRCC*, signet ring cell carcinoma.

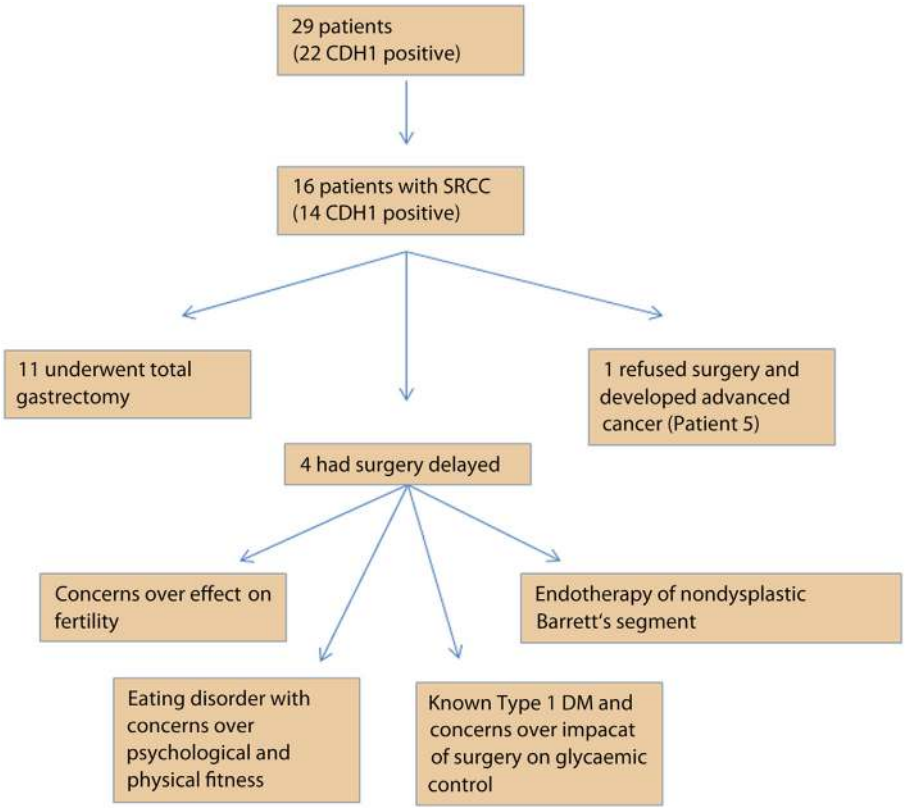

Figure 1. Flowchart of clinical management of patients with SRCC.

children, 1 patient wanted to delay surgery for as long as possible because of concerns over fertility, 1 had surgery delayed because of an eating disorder and concerns by the clinicians over psychological and physical fitness for prophylactic total gastrectomy, and 1 patient initially refused surgery in whom invasive disease subsequently developed before the patient agreed to a radical D2 gastrectomy. This last patient is discussed further in the following.

Five patients have not had signet ring cell foci detected and are not keen to pursue surgery despite advice to do so. So far, 3 of these patients have had 2 endoscopies, 1 patient has had 1 endoscopy, and 1 patient has had 4 endoscopies, all with multiple samples taken and no SRCC cells found. Median follow-up in these patients was 13.1 months (range 3.1–20.3 months).

CDH1 mutation status unknown at the start of endoscopic surveillance

Eight patients did not know their mutation status when they joined the study and thus opted for endoscopic screening for the identification of SRCC. In 5 of 8 of these

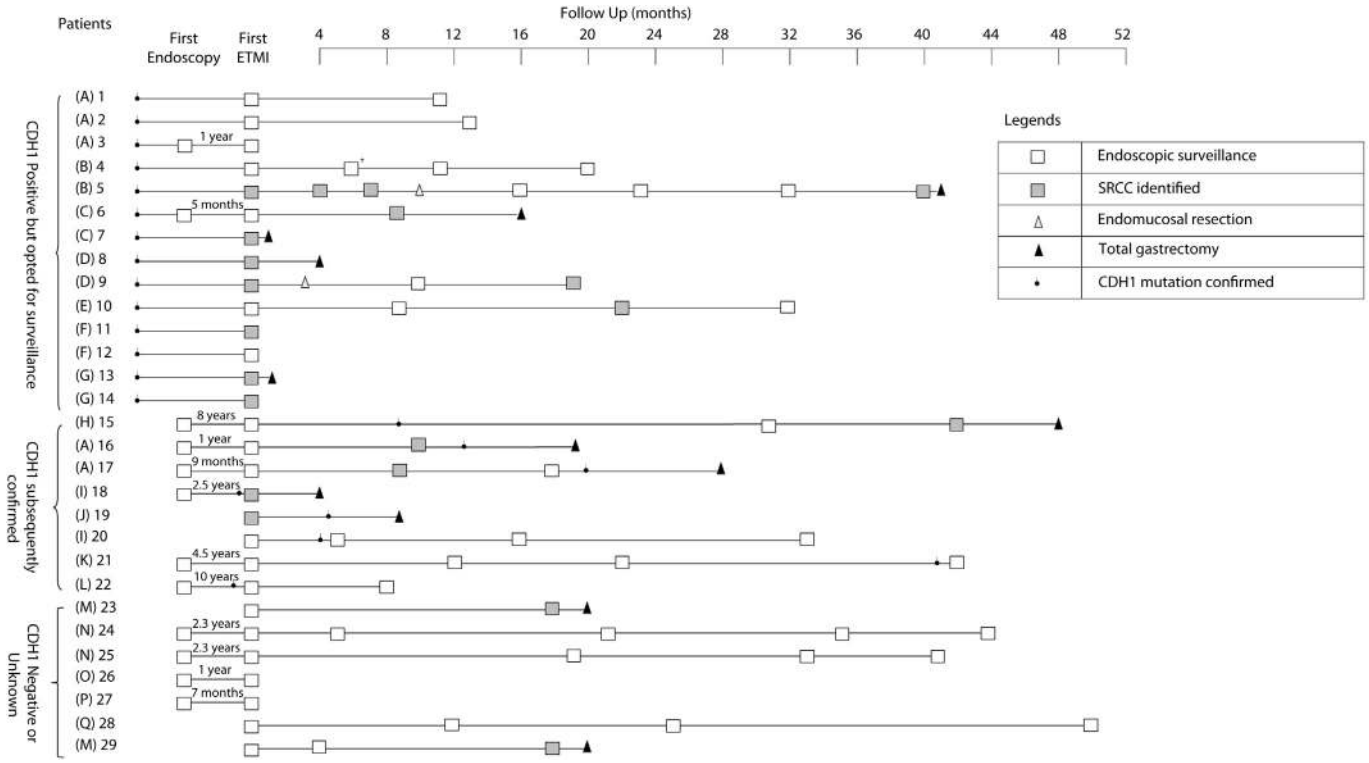

Figure 2. Endoscopic trimodal imaging surveillance endoscopy with biopsy result and follow-up.

patients, SRCC cells were identified either before (4 patients) or shortly after the mutation status was determined (1 patient). All of these patients went ahead with total gastrectomy at a median of 7.0 months after their positive endoscopy findings and a median of 5.0 months after their mutation was confirmed. Of the remaining 3 patients, 1 had a whole *CDH1* gene deletion and a personal history and family history of lobular breast cancer with 1 confirmed case of young-onset gastric cancer (although the histological type is unknown). She remains under surveillance in view of the lack of confirmed cases of diffuse gastric cancer and uncertainty about her risks for this disease given the uncommon mutation type and atypical family history. Two remaining patients later tested positive for *CDH1* but despite a 3-year and 10-year follow-up, respectively, no SRCC cells have ever been detected, and both remain unwilling to undergo prophylactic total gastrectomy for personal reasons.

CDH1-negative patients

Seven patients from families fulfilling the criteria for HDGC in whom no deleterious *CDH1* mutations were identified also took part in research endoscopy. Two patients were not tested for reasons described previously; 1 patient had a family member with a possible variant *CDH1* identified but she herself tested negative. Of the remaining 4 patients, 2 were siblings from a *CDH1*-negative family and in whom SRCC subsequently developed and underwent successful total gastrectomy. The *CDH1* mutation testing was performed by gene sequencing and multiplex ligation-dependent probe amplification.

Endoscopic findings

All 29 patients had a minimum of 24 random biopsy specimens taken as well as specimens reserved for future research purposes. Twenty-four patients had abnormal findings during endoscopic examination, from whom 77 targeted biopsy specimens were taken (Table 2). Four specimens were excluded because they were follow-up biopsy specimens taken from an EMR site. The average procedure time was 33.8 minutes (range 13-63 minutes).

Of the 73 targeted biopsies, 70 targets were identified with white light (57 pale, 5 nodular, 2 erosions, 6 polyps) and 3 with AFI. Ten of 73 lesions had an abnormal magnifying NBI pattern, 4 of which contained SRCC foci (Figs. 3 and 4). However, 3 were from the same lesion at repeat examinations in the same patient who deferred prophylactic total gastrectomy. A further 3 signet ring cell foci were seen on targeted biopsies but had a normal magnified NBI appearance. A total of 696 random biopsy specimens were taken in which 22 signet ring cell foci were identified (11 fundus, 1 body, 4 antrum, 3 transitional zone, 2 cardia, and 1 unspecified).

TABLE 2. Characteristics of targeted lesions

	Pale, no. (%)	Nodular, no. (%)	Erosions, no. (%)	Polyp, no. (%)	AFI positive, no. (%)	Total, no. (%)	SRCC
Fundus	3 (4.1)	1 (1.4)	0	1 (1.4)	0	5 (6.85)	0
Body	1 (1.4)	2 (2.7)	0	2 (2.7)	0	5 (6.85)	0
Antrum	20 (27.4)	1 (1.4)	1 (1.4)	0	2 (2.7)	24 (32.9)	2*
TZ	21 (28.8)	1 (1.4)	0	3 (4.1)	1 (1.4)	26 (35.6)	5†
Prepyloric	12 (16.4)	0	1 (1.4)	0	0	13 (17.8)	0
Total	57 (78.1)	5 (6.9)	2 (2.7)	6 (8.2)	3 (4.1)	73 (100.0)	
SRCC	5	0	1	1	0		7 (9.6)

AFI, Autofluorescence imaging; SRCC, signet ring cell carcinoma; *TZ*, transitional zone.
*One pale lesion and 1 erosion.
†Four pale lesions and 1 polyp.

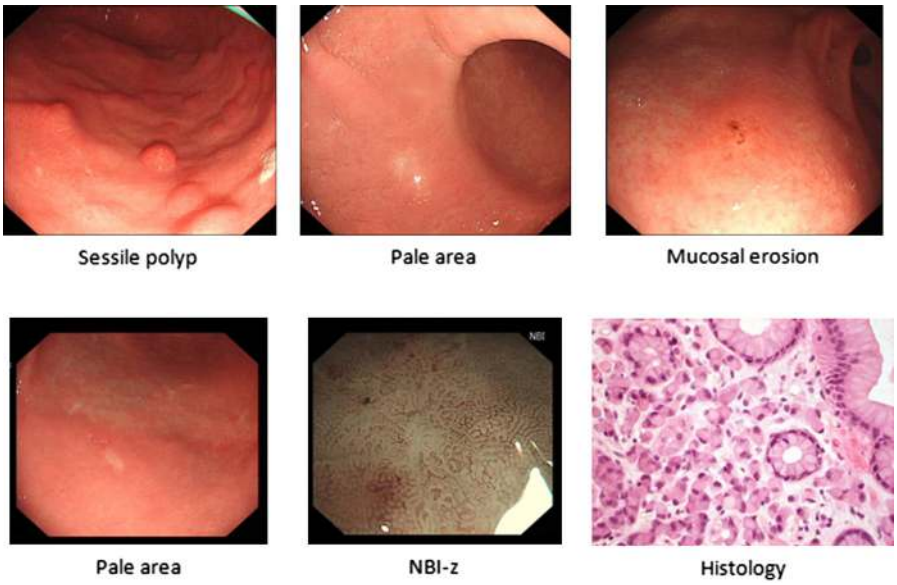

Figure 3. Examples of endoscopic lesions associated with foci of SRCC (top panels). Bottom panels represent a pale area (left) with corresponding NBI-Z pattern (middle) and histology showing a focus of SRCC (right).

On a per-patient basis, 11 of 16 patients with SRCC foci were detected by random biopsies only, 3 (18.8%) by targeted biopsies only, and 2 (12.5%) by both methods. The additional use of targeted biopsies together with random biopsies did identify a further 3 cases (18.8%); however, overall very few targeted biopsies corresponded to SRCC (7 of 73 [9.6%]) (Tables 2 and 3).

Gastrectomy findings and correlation with endoscopic SRCC foci

Twelve patients proceeded to total gastrectomy, of which 11 were conducted at our center. Ten of 11 cancers were limited to the mucosa (T1a), and surgery was deemed curative. In 9 patients with early disease, 7 patients had SRCC cells identified endoscopically in just 1 anatomic segment; however, when the surgical specimen was examined, 4 of them had SRCC in multiple areas. Nine of 10 patients had foci in the proximal stomach (cardia/fundus), with the remaining patient harboring SRCC cells in the body of the stomach (Table 4). All patients with biopsy-detected foci of signet ring cell were confirmed to have malignancy on the resected specimen.

Patient 5 needs special mention. He was a known *CDH1* carrier who underwent his first endoscopy in 2009 at the age of 65 years. A pale area with an irregular blood vessel pattern was noted in the transitional zone (TZ). Biopsy of this area showed a single focus of SRCC, and he was recommended to have a total gastrectomy but declined, preferring to delay gastrectomy to maximize his QOL. A follow-up endoscopy 3 months later confirmed 1 persistent SRCC focus in the targeted biopsy, and the remaining 27 (24 random, 3 targeted) samples were normal, but he still

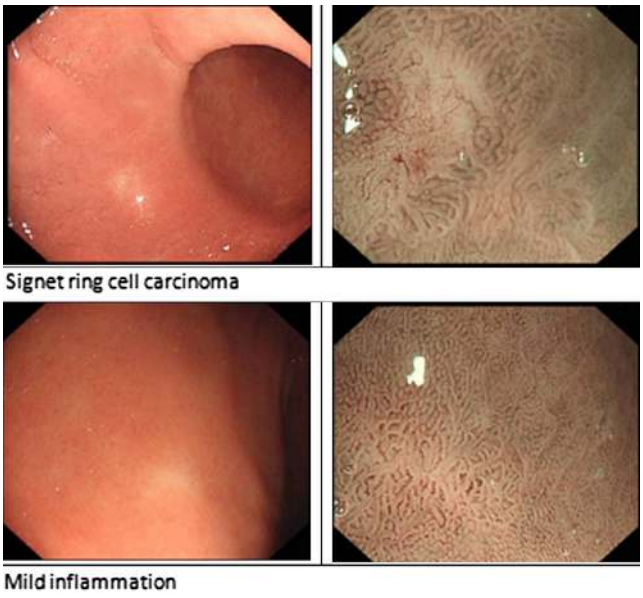

Figure 4. Endoscopic examples of pale areas with a focus of SRCC (top panels) and without evidence of SRCC (bottom panels). High resolution endoscopy pictures are shown in the left panels and NBI-Z patterns are shown in the right panels.

declined surgery. His third surveillance endoscopy 3 months later showed persistent SRCC cells in the same location with more than 90 random biopsies from other segments from around the whole gastric mucosa being negative. An EMR was therefore performed to provide information on the extent and depth of disease. The procedure was uncomplicated, and histological assessment showed complete excision of the malignant focus (free lateral and deep margins), as well as no invasion into the submucosa with a small number of tumor cells extending into the superficial muscularis mucosae (T1a). Over a period of a further 22 months and 3 further surveillance endoscopies with 96 biopsies including biopsies of the EMR site, no further SRCC cells were identified. Eight months later, biopsy specimens showed SRCC with poorly differentiated characteristics in the TZ. He proceeded to a radical D2 gastrectomy, which revealed widespread poorly differentiated adenocarcinoma with serosal invasion, 1 of 25 lymph nodes positive, and microscopic involvement of the distal resected margin (stage 4 disease, pT4a pTn1, pM0–R1 resection).

DISCUSSION

This is the largest prospective cohort study of endoscopy performed to date in patients from families fulfilling criteria for HDGC, and overall we found SRCC foci in 63.6% (14 of 22) of patients with *CDH1* mutations and 28.6% (2 of 7) of patients without an identified mutation. This is a higher yield than in previous studies, which often report a significant underestimate of SRCC cells obtained from samples taken from a macroscopically normal endoscopic examination compared with histopathological examination of gastrectomy specimens.[6,8] In addition to the 2 of 23 patients with foci detected at preoperative endoscopy in the Newfoundland series, Pandalai et al[9] and Chen et al,[6] respectively, reported 1 of 10 and 2 of 13 SRCC cells detected in these presurgical patients.

Here we show that it is possible to increase the yield of SRCC by adhering to a strict endoscopy protocol, and we demonstrate how this information can be used to guide the decision-making process in patients in whom the causative mutation is unknown or uncertain. In patients with known pathogenic *CDH1* mutations, even though the international guideline is clear concerning the recommendation of prophylactic total gastrectomy, the decision-making process is complex, and, in our experience, there is a group of individuals who are very reluctant to undergo surgery. The data from this cohort shed further light on the natural history of the disease and how this might help inform management of this patient group in the future.

For those patients in whom no *CDH1* mutation has been found, the identification of SRCC provides the phenotypic information required to know that the patient is at high risk of diffuse-type gastric cancer, presumably because they have inherited a mutation at an unidentified locus. This is important because *CDH1* mutations only account for approximately 30% of cases fulfilling the HDGC criteria and although a mutation in α-catenin has recently been identified in a large diffuse gastric cancer pedigree,[14] many families remain uncertain about the genetic cause in their case. With the advent of exome sequencing, it is hoped that further predisposing mutations to this syndrome will be identified, but in the meantime, endoscopy seems to be a reasonable option.

The majority of our patients in whom SRCC cells were identified had a pathogenic *CDH1* mutation (14 of 16). Once the mutation has been identified, all of these individuals are recommended to undergo prophylactic total gastrectomy and attend a multidisciplinary clinic to discuss this in detail. The patients have the opportunity to discuss the risks of the development of invasive cancer in mutation carriers (80% penetrance), the practicalities of surgery including the operative mortality (<2% in our center for gastrectomy for all causes and 0% in prophylactic total gastrectomy), morbidity, length of recovery, and long-term effects on QOL. Patients are offered the opportunity to talk to other patients who previously underwent surgery, and, where possible, the contact is with someone who has similar considerations (eg, work, family). The discussion aims to be realistic about the consequences of this surgery while giving patients the confidence to make the decision. In 3 of 16 (18.8% of patients), the SRCC cells were found before the genetic testing results, and in these individuals who were anxious to move ahead with some form of monitoring, this enabled them to make a prompt decision. Five of 16 of our patients (31.3%) with a known *CDH1* mutation and

TABLE 3. Sensitivity and specificity of biopsy sampling (per-patient analysis)

	Sensitivity, % (no.)	Specificity, % (no.)	Accuracy, % (no.)	PPV, % (no.)	NPV, % (no.)
Random biopsies	81.3% (13/16)	NA	NA	NA	81.3 (13/16)
WLE targeted	31.3% (5/16)	NA	NA	NA	54.2 (13/24)
AFI	0%	100.0 (13/13)	44.8 (13/29)	0	44.8 (13/29)
NBI-Z*	40.0% 2/5	79.0 (15/19)	70.8 (17/24)	33.3 (2/6)	83.3 (15/18)

PPV, Positive predictive value; *NPV,* negative predictive value; *NA, not applicable; WLE*, white-light endoscopy; *AFI*, autofluorescence imaging; *NBI-Z*, narrow-band imaging zoom.
*Patients who had targeted biopsy specimens taken.

TABLE 4. Signet ring cell carcinoma staging and gastrectomy specimen

Patient	Sampling method	Biopsy location, differentiation, and staging	Gastrectomy specimen	
			No. of foci	Location
5	Targeted	TZ and body. G3 intramucosal carcinoma*	Tumor involving submucosa, muscularis propria, and subserosa involving most of the fundus, body, and posterior antrum. Tumor involved 99/110 block	
6	Targeted	Antrum. G2 intramucosal carcinoma	11	Fundus and body
7	Both	Targeted: TZ. G1 intramucosal carcinoma Random: fundus and cardia. G1 intramucosal carcinoma	Multiple, >250 mm	Cardia, fundus, body, TZ, and antrum
8	Random	TZ. G1 intramucosal carcinoma	8	Cardia, fundus, body, and antrum
9	Targeted	TZ. G1 intramucosal carcinoma	Awaiting gastrectomy	
10	Random	Cardia. G1 intramucosal carcinoma	Awaiting gastrectomy	
11	Random	TZ. G1 intramucosal carcinoma	Awaiting gastrectomy	
13	Both	Targeted: antrum. G1 intramucosal carcinoma Random: fundus and cardia. G1 intramucosal carcinoma	182	Fundus, body, TZ and antrum
14	Random	Antrum and fundus. G1 intramucosal carcinoma	Awaiting gastrectomy	
15	Random	TZ. G1 intramucosal carcinoma	1	Body
16	Random	Unspecified. G1 in situ† carcinoma	1	Cardia
17	Random	Fundus. G1 in situ carcinoma	10	GEJ, fundus, body, and antrum
18	Random	Fundus. G1 intramucosal carcinoma	Gastrectomy elsewhere	
19	Random	Fundus. G1 intramucosal carcinoma	Multiple, >30 mm	Fundus
23	Random	Fundus. G1 intramucosal carcinoma	62	Cardia, fundus, and body
29	Random	Body. G1 in situ carcinoma	24	Fundus and body

TZ, transitional zone; *GEJ*, gastroesophageal junction; *G3*, poorly differentiated; *G2*, moderately differentiated; *G1*, well differentiated.
*Intraepithelial carcinoma without evidence of invasion into the lamina propria.
†Invasion into or beyond the lamina propria.

positive endoscopic findings delayed the decision to undergo prophylactic total gastrectomy. The reasons were highly personal and complex but included cultural and religious beliefs, issues about childbearing, loss of earnings, ability to do physical activities, QOL with young children, and effects on comorbid conditions such as type 1 diabetes. We found that providing plenty of time for discussion and building a relationship with individuals through endoscopy and clinic visits enabled many patients (12 of 16) to make a decision to proceed with surgery, particularly once SRCC was identified and on average 6.3 months after endoscopy was performed.

The danger of delaying surgery in *CDH1*-positive individuals with SRCC identified is highlighted by patient 5. This patient was the oldest in our cohort at 65 years of age and had SRCC identified 3.5 years before his gastrectomy. Although SRCC cells were identified, the extent of disease was not apparent because the endoscopic appearance was unremarkable (normal gastric distensibilty and intact mucosa) and the resection specimen was remarkable for the diffuse infiltration by poorly differentiated, small diffuse cells that started in the submucosal layer with remarkably few classic signet ring forms (99 of 110 blocks examined that formed the resection specimen lacked signet ring cells).

With regard to the type of endoscopic modality, we used careful inspection and multiple biopsy samplings of random areas coupled with biopsy of any abnormal lesions. Pale lesions were the most common focal abnormality seen, and these occurred anywhere. This is similar to the study by Shaw et al,[13] although chromoendoscopy was not required in our experience and Congo red is no longer approved for use because of concerns over toxicity. In Asia, methylene blue or indigo carmine are used extensively to characterize subtle mucosal irregularities.[15-17] However, the role of these agents in detecting microscopic foci of SRCC cells has not been examined, and because these lesions are often deep to the superficial mucosa, they may have more limited use in this context.

Interestingly, SRCC is commonly detected in the proximal stomach (9 of 16 on biopsy and 9 of 10 on gastrectomy) compared with previous reports of a predilection for the TZ.[18] In the New Zealand study, the 6 histological specimens showed cancer foci in the body and stomach in all 6 patients as well as the antrum in 4, suggesting possibly a more advanced stage.[18]

The use of high-resolution white-light endoscopies with random biopsies alone was sufficient to diagnose 13 of 16 cases of SRCC, but targeted biopsies identified a further 3 foci that were missed. All had subtle changes identifiable on white light. The use of NBI zoom is thought to be helpful because it highlights mucosal and vascular abnormalities, although sensitivity for SRCC was poor. Meticulous mucosal assessment with a high-definition endoscope should form the minimum standard.

In this study, AFI did not prove to aid in the detection of early foci of signet ring cell neoplasia; however, NBI was a useful imaging adjunct to delineate pale areas and, in conjunction with magnification, allowed careful assessment of vascular and mucosal patterns, with a high negative predictive value. The limited value of AFI is most likely related to the very early stage and small size of the lesions identified, which in some cases only involved a few clusters of SRCC cells. In our cohort, the average procedure time was longer than 30 minutes, and close liaison with an expert histopathologist is essential because of the focality and subtlety of these lesions. In the future, it will be interesting to see whether EUS or techniques such as optical coherence tomography and confocal endomicroscopy might increase the sensitivity for detecting disease occurring deep to the superficial mucosa.

There are limitations to this study. Because HDGC is rare, it would not have been feasible to have a control group. However, the observational nature of our study reflects a real-life clinical scenario for each individual, which thus influences the clinical course. The influence of mutation status and endoscopy on patient decision making was discussed, but this has not been formally evaluated and is the subject of an ongoing qualitative study.

In conclusion, we believe that endoscopy does have an important role to play in guiding the timing of total gastrectomy, particularly for those individuals in whom the mutation status is uncertain or unknown at that time and for those reluctant to proceed straight to prophylactic surgery. Careful white-light examinations with both random and targeted biopsies identified foci of SRCC and prompted patients to proceed safely to curative surgery except for 1 patient who refused. However, the risks of delayed surgery in mutation-positive individuals, especially when SRCC cells have been identified, should not be underestimated, and we believe that it is important for these data and experiences to be shared with the community of clinicians caring for patients with familial diffuse gastric cancer syndromes.

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Abbreviations: AFI, autofluorescence imaging; HDGC, hereditary diffuse gastric cancer; NBI, narrow-band imaging; QOL, quality of life; SRCC, signet ring cell carcinoma; TZ, transitional zone.

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