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Outcomes of screening gastroscopy in first-degree relatives of patients fulfilling hereditary diffuse gastric cancer criteria

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Background and Aims: The aim of this study was to determine the yield of endoscopic screening in first-degree relatives (FDRs) of *CDH1*-negative hereditary diffuse-type gastric cancer (HDGC) patients.

Methods: In this retrospective observational cohort study, in 2 expert centers in the Netherlands data were collected on FDRs from families fulfilling the international HDGC criteria that underwent endoscopic screening. Extensive inspection of the stomach was performed by gastroscopy, taking random and/or targeted stomach biopsy specimens to identify diffuse-type gastric cancer.

Results: Between 2004 and 2016, 90 persons (40% men; mean age, 48 years) from 40 families were offered endoscopic screening. The mean number of endoscopies per person was 3. The mean follow-up time was 46 months and mean endoscopic interval 20 months. Signet ring cell carcinoma foci restricted to the mucosa (pT1a) were identified in 4 persons (4%) from 1 family, which afterward was diagnosed with a germline *CTNNA1* mutation. Advanced poorly cohesive gastric carcinoma was diagnosed in 1 person from another family. Intestinal metaplasia was diagnosed in 38 persons (42%) and low-grade dysplasia in 4 persons (4%). Additionally, in 40 persons (44%) scar tissue was observed in the gastric mucosa, which can hinder the endoscopic detection of small white lesions typical for HDGC.

Conclusions: Endoscopic screening in HDGC families without a pathogenic *CDH1* mutation may be reasonable, as we detected signet ring cell carcinomas in 6% of persons screened. However, the criteria and frequency of screening may have to be reconsidered. (Gastrointest Endosc 2018;87:397-404.)

Comparable with other Western countries, the incidence of noncardia gastric cancer has markedly declined in the Netherlands during the last decades.^{1,2} In particular, the incidence of intestinal-type gastric cancer has decreased, but the incidence of diffuse-type gastric cancer (DGC) seems to be increasing.³⁻⁶ Younger gastric cancer patients especially are more often diagnosed with DGC.⁷⁻⁹

Intestinal-type gastric cancer can largely be attributed to *Helicobacter pylori* infections in a genetically susceptible host according to the Correa sequence,¹⁰ but the pathogenesis of DGC is less well understood. It has been suggested that *H pylori* plays a role in the development of DGC as well.^{11,12} In DGC genetic variations may play an important role. However, recent exome sequencing

Abbreviations: DGC, diffuse-type gastric cancer; FDR, first-degree relative; HDGC, hereditary diffuse-type gastric cancer; SRCC, signet ring cell carcinoma.

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studies in families that fulfill criteria of hereditary DGC (HDGC) failed to identify monogenic causes in most families.^{13,14} Mainly pathogenic *CDH1* mutations, causing a dysfunction in the adhesion molecule E-cadherin, are found in a select group of HDGC families.^{14,15}

Individuals with heterozygous *CDH1* mutations have a high cumulative risk of up to 70% to develop DGC.¹⁴ Because DGC develops from small mucosal foci of signet ring cells with a diffuse scattered growth pattern, it can be difficult to detect DGC by endoscopy, even in advanced stages. Therefore, prophylactic gastrectomy is offered to individuals with confirmed pathogenic *CDH1* mutations. Depending on personal circumstances and preferences, some patients opt for annual endoscopic surveillance according to the Cambridge protocol.¹⁶ Surveillance studies have shown that small, pale, nonelevated lesions can represent early-stage DGC, histologically showing signet ring cells within the lamina propria.^{16,17} However, these lesions can be difficult to detect as becomes apparent from the observation that even in experienced hands, endoscopy often reveals no abnormalities or only a few pale lesions, whereas resection specimens show tens to hundreds of small signet ring cell carcinomas (SRCCs).¹⁸⁻²¹

More than 70% of HDGC probably cannot be attributed to a germline *CDH1* mutation.^{14,22,23} The risk of developing DGC for these families is unknown but is presumably lower than reported in *CDH1* families. Little is known about the outcomes of screening in first-degree relatives (FDRs) of patients who fulfill HDGC criteria but tested negative for pathogenic *CDH1* gene mutations. Although evidence is lacking, the HDGC consensus guideline suggests annual endoscopic screening for FDRs of patients fulfilling HDGC criteria but without a detectable genetic defect.¹⁶ In the current study we describe the outcomes of endoscopies in FDRs from HDGC families without a pathogenic germline *CDH1* mutation. The primary endpoint was the endoscopic yield of microscopic early-stage DGC.

METHODS

Study population

This study, conducted by the Netherlands Cancer Institute/Antoni van Leeuwenhoek (NKI/AVL) and Radboud University Medical Center, is a retrospective observational cohort study. All clinicopathologic data were collected from medical records and anonymously stored in a combined database from persons who underwent endoscopic gastric screening between March 2004 and September 2016. Persons older than age 18 years who were FDRs of an affected person in a family fulfilling the updated HDGC criteria of 2015 were included (Table 1).¹⁶

Families were only included if genetic counseling had been performed. Family pedigrees of at least 3 generations

were analyzed for the number of affected family members with gastric cancer and other malignancies. Germline *CDH1* mutation analysis was performed in the affected patient with gastric cancer (index patient). When the DNA of an affected patient was not available, 1 or more relatives were tested to exclude a pathogenic germline *CDH1* mutation. Testing for other gene mutations, including *TP53*, mismatch repair genes, *PTEN*, *BRCA1/2*, *STK11*, *SMAD4*, and *BMP1a*, was performed when the family met the criteria for testing of that specific cancer syndrome according to the national and international guidelines.^{16,24,25} The original histopathologic reports from patients with gastric cancer were reviewed to confirm the diagnosis of DGC.

Information of persons was collected on age at endoscopy, number of endoscopies, prior malignancies, prior infection with *H pylori*, endoscopic abnormalities, endoscopic signs of scar formation, histopathologic findings of gastritis, *H pylori* infection, atrophy, intestinal metaplasia, dysplasia, and carcinoma. All persons from both centers were informed about the current screening guideline, the lack of evidence for endoscopic screening, and its procedural related risk. All persons gave their informed consent.

Endoscopic screening protocol

At NKI/AVL an endoscopic screening program for persons at risk for the development of DGC started in 2004. At the Radboud University Medical Center the endoscopic screening protocol started from 2010 onward. Endoscopy was performed with the patient under conscious sedation with midazolam or propofol. From November 2008 onward, after the International Gastric Cancer Linkage Consortium consensus meeting, gastroscopies were performed in line with the consensus protocol.²⁶ During at least 30 minutes the gastric mucosa was thoroughly inspected, searching for lesions that might be typical for early DGC. A biopsy specimen was taken from each aberrant mucosal lesion. Additionally, multiple random specimens were taken from each of the following anatomic regions of the stomach: antrum, transitional zone, body, fundus, and cardia. Although there was no strict screening protocol before 2008, all gastroscopies were performed with thorough inspection, often with both targeted and random biopsy specimens from the gastric mucosa.

The endoscopic screening at NKI/AVL and Radboud University Medical Center was performed or supervised by 1 of 3 gastroenterologists (A.C., J.v.D., T.M.B.) with experience in HDGC. At NKI/AVL a white-light high-definition endoscope with narrow-band imaging (GIF-H180/GIF-H190; Olympus, Tokyo, Japan) was used from 2008. At the Radboud University Medical Center the Pentax 3.2 EG29-i10 with iscan modality (Pentax, Tokyo, Japan) was used from 2014. Before that period Olympus 160-series endoscopes (Olympus, Tokyo, Japan) were used at both centers. Although the protocol does not describe screening with narrow-band imaging, it was used extensively at our

TABLE 1. Baseline characteristics of 90 persons in 40 families who underwent gastroduodenoscopy

Characteristic	Value
Men	36 (40)
Women	54 (60)
Total number of endoscopies	285
Mean interval between endoscopies, mo (SD; range)*	20 (17; 7-129)
Mean follow-up time, mo (SD; range)*	46 (40; 9-146)
Mean number of endoscopies per person (SD; range)	3 (3; 1-12)
Mean number of biopsy samples (range)†	27 (0-52)
Mean number of random biopsy samples (range)‡	25 (3-46)
Mean number of targeted biopsy samples (range)§	5 (1-24)
Endoscopies with ≥ 25 biopsy samples of 5 anatomic areas	157 (55)
Mean age during the first endoscopy, y (SD; range)	48 (13; 22-72)
HDGC criteria 2010, ²⁶ no. of persons (no. of families)	59 (30)
≥ 3 first- or second-degree family members with a diagnosis of DGC independent of age	7 (4)
≥ 2 cases of gastric cancer in first- or second-degree relatives, 1 confirmed DGC before age 50 y	26 (13)
Individual with DGC diagnosed before age 40 y	25 (12)
Combination of DGC and LBC in first- or second-degree relatives, 1 diagnosed before age 50 y	1 (1)
Not fulfilling HDGC criteria 2010	31 (10)
HDGC criteria 2015, ¹⁶ no. of persons (no. of families)	90 (40)
≥ 2 cases of gastric cancer in first- or second-degree relatives, 1 confirmed DGC independent of age	64 (27)
Individual with DGC diagnosed before age 40 y	25 (12)
Combination of DGC and LBC in first- or second-degree relatives, 1 diagnosed before age 50 y	1 (1)

Values are number of cases with percents in parentheses unless otherwise indicated.

SD, Standard deviation; HDGC, hereditary diffuse gastric cancer; DGC, diffuse gastric cancer; LBC, lobular breast cancer.

*Included are 69 persons (21 persons had only 1 endoscopy and no follow-up).

†Included are all endoscopies (in 2 no biopsy specimens were taken).

‡Included are 270 endoscopies (in 15 no random biopsy specimens were taken).

§Included are 188 endoscopies (in 97 no targeted biopsy specimens were taken).

centers because it facilitates visualization of the gastric pit structure and the typical white lesions that may represent signet ring cell foci.

Persons were included if they underwent at least 1 screening gastroscopy with more than 10 random biopsy samples from at least 4 anatomic regions. Endoscopies were included if the goal of the gastroscopy was screening. Endoscopies for evaluation of complaints or interval endoscopies for follow-up of dysplasia or cancer were noted but excluded from the analysis.

Histologic sampling and examination of biopsy specimens from the gastric mucosa

The primary outcome measure was the endoscopic yield of microscopic early-stage DGC (including mucosal signet ring cell foci). Other lesions associated with (especially intestinal-type) gastric cancer, such as atrophic gastritis, intestinal metaplasia, and dysplasia, are considered secondary findings because the relationship of these lesions and DGC is not clear. Experienced pathologists analyzed all gastric biopsy specimens. Biopsy specimens were examined using standard stains: hematoxylin and eosin and periodic acid Schiff-diastase (mucin-stain).

Additional (immuno-)histochemical stains were used in case of gastritis for the detection of *H pylori* or to detect or confirm another specific diagnosis.

RESULTS

Baseline characteristics

Between March 2004 and September 2016, 90 persons from 40 families fulfilling the 2015 HDGC criteria underwent endoscopic screening of the stomach. The baseline characteristics, including number of persons and family per HDGC criterion, are shown in Table 1 and Supplementary Table 1 (available online at www.giejournal.org). In total, 285 endoscopies were performed during the 12.5 years of the study, 52 before November 2008. Forty percent of the persons were men. The mean age (during the first endoscopy of each person) was 48 years (standard deviation, 12.9; range, 22-72). One to 18 family members were screened per family. In 75 persons (83%) at least 25 random biopsy samples of 5 gastric regions were taken during at least 1 endoscopy; in the other 15 persons more than 10

TABLE 2. Biopsy samples with diffuse gastric cancer, dysplasia, and intestinal metaplasia

	Families	Persons	Endoscopies	Biopsy samples	Random biopsy samples	Targeted biopsy samples
Total	40 (100)	90 (100)	285 (100)	7751 (100)	6825 (100)	926 (100)
Diffuse gastric cancer	2 (5)	5 (6)	7 (2)	27 (.3)	7 (.1)	20 (2)
Dysplasia	3 (8)	4 (4)	4 (1)	5 (.06)	2 (.03)	3 (.3)
Intestinal metaplasia	21 (53)	38 (42)	84 (29)	302 (4)	212 (3)	90 (10)

Values in parentheses are percents.

random biopsy samples were taken of at least 4 anatomic regions. Twenty-one persons underwent only 1 endoscopy, 30 underwent 2 endoscopies, and 39 persons had 3 or more endoscopies. The mean endoscopic interval for persons receiving more than 1 endoscopy was 20 months, and mean follow-up time was 46 months. None of the persons had a known medical history of gastric dysplasia or malignancy. Germline pathogenic *CDH1* mutation was excluded in all families, in 32 families by testing the DGC (index) patient and in 8 families by testing 1 or more family members of the index patient because no genetic material was available from the index patient.

Yield of the screening program

DGC was detected in 5 persons, and all cases were stage I (Table 2). In 1 person a highly suspicious tumor in the gastric body was detected during endoscopy (Table 3), whereas in the other persons the finding of DGC was found in random biopsy specimens. Well-defined small pale lesions that can represent small signet ring cell foci, as described in *CDH1* mutation carriers, were not found. The most advanced tumor was detected in a woman of 51 years (H190 endoscope, Olympus, Tokyo, Japan). She was first screened at age 44, and Barrett's mucosa with high-grade dysplasia was detected in the distal esophagus. After ablative treatment of the Barrett's esophagus, followed by 7 years without endoscopic screening, a nonsymptomatic tumor of 2 cm was found in the greater curvature of the body of the stomach, which was confirmed DGC histologically (Fig. 1A and B). After treatment with neoadjuvant chemotherapy, the resection specimen showed a diffuse carcinoma in the body of the stomach of 7 cm, growing into the peritoneal surface with omental metastases (ypT4aN0M1 DGC). Four siblings were previously diagnosed with advanced DGC (diffuse-type histology was reviewed and confirmed). Germline *CTNNA1* mutation was excluded in 2 siblings with DGC using Sanger sequencing.

In 4 other persons mucosal foci (pT1a) of SRCC were diagnosed (Fig. 1C and D). The first case was a 75-year-old man. SRCC was found in the fundus with random biopsy sampling without visible lesions (H180 endoscope) in 2010. Three earlier (2004-2008) and 5 later (2011-2015) gastroscopies with H180 and H190 endoscopes with biopsy sampling did not reveal abnormalities in this patient. In the second case, a 42-year-old man, random

biopsy specimens from the cardia showed SRCC foci in 2005 and 2006 (a second and third screening). No visible lesions were seen (GIF Q160 scope, Olympus, Tokyo, Japan). No endoscopic or histologic abnormalities were found in 2 following gastroscopies. In 2010, again SRCC was found in the cardia without endoscopic visible lesions (H180 endoscope). Gastrectomy was performed afterward. Multiple T1a SRCC foci were found in the resection specimen. Two other men aged 40 and 41 years, respectively, underwent their first gastroscopy in 2010 (H180 endoscope). No endoscopic lesions were found, but random biopsy specimens revealed SRCC foci, in the fundus in 1 and in fundus and cardia in the second. In both men gastrectomy was performed, and multiple T1a SRCC foci were found in both gastrectomy specimens.

These 4 people with SRCC foci belonged to 1 family (in which a total of 18 members were screened). The people in this family were included because of the first HDGC criterion (families with 2 or more patients with gastric cancer at any age, 1 confirmed DGC). In an additional family member of this specific family there was, next to intestinal metaplasia, a single atypical gland suspicious for in situ SRCC. However, more extensive abnormalities could not be confirmed in 6 follow-up endoscopies during the next 5 years. The findings in this family led to exome sequencing, which revealed a mutation in the *CTNNA1* gene, encoding the alpha-E-catenin molecule.²⁷ In the other 38 families no other gastric malignancies were detected.

Low-grade dysplasia was diagnosed in 4 persons (4%) (Fig. 1E and F), of whom 2 belonged to the same family in which a pathogenic *CTNNA1* mutation was detected. In the first person (man, 63 years) low-grade dysplasia was found in a random biopsy specimen in the antrum (2005, GIF Q160 scope). In the second person (woman, 64 years) there was a small elevation in the fundus, which was diagnosed histologically as a tubulovillous adenoma with low-grade dysplasia (2014, H180 endoscope). Later analysis showed the man was a carrier of the *CTNNA1* mutation, whereas the woman was not. In a third person (man, 47 years) the transitional zone showed a patchy aspect endoscopically, which histologically was diagnosed as extensive intestinal metaplasia and low-grade dysplasia within a background of chronic inflammation (2014, EG29-i10 endoscope, Pentax, Tokyo, Japan). In the fourth person (man, 62 years) erosive lesions were found in the antrum, which was microscopically diagnosed as extensive

TABLE 3. Outcomes of endoscopies per anatomic location

	Diagnosis	Pylorus	Antrum	Transitional zone	Body	Fundus	Cardia	Total no.
Random	Diffuse gastric cancer	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	4 (2)	7 (4)
	Dysplasia	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2)
	Intestinal metaplasia	4 (4)	65 (26)	23 (10)	19 (6)	3 (3)	14 (9)	70 (29)
	Total	2 (2)	265 (90)	263 (90)	267 (90)	206 (87)	256 (88)	270 (90)
Targeted	Diffuse gastric cancer	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)
	Dysplasia	0 (0)	0 (0)	2 (1)	0 (0)	1 (1)	0 (0)	2 (2)
	Intestinal metaplasia	10 (9)	27 (17)	14 (8)	13 (7)	3 (3)	7 (4)	38 (25)
	Total	26 (19)	79 (47)	75 (48)	93 (49)	40 (29)	25 (18)	188 (82)

Values are number of endoscopies with number of persons in parentheses.

intestinal metaplasia with low-grade dysplasia (2013, EG29-i10 endoscope). In the last person low-grade dysplasia was diagnosed again in biopsy samples taken in a second endoscopy after 3 months. Follow-up endoscopies in the following 3 years did not show dysplasia. In the other 3 persons, low-grade dysplasia was detected only once and was not diagnosed during follow-up endoscopies and biopsy sampling (follow-up 1.5, 3, and 10 years).

Intestinal metaplasia was diagnosed in 84 of 285 endoscopies performed (29%) (Fig. 1G and H). The biopsy specimens with intestinal metaplasia belonged to 38 persons (42%) with a mean age at diagnosis of 52 years (standard deviation, 12.5; range, 30-76). Intestinal metaplasia was isolated to the distal region (antrum, pylorus, and transitional zone) of the stomach in 63%. Intestinal metaplasia was found in 29% multifocal, in 3% proximal (cardia), and in 5% in the gastric fundus and/or body. Nonspecific chronic inflammation was the most common abnormality of the gastric mucosa in all persons (60 persons, 67%). Twenty-two persons had signs of active gastritis (24%) with histologically *H pylori* inflammation in 7 cases. An additional 10 persons had *H pylori* inflammation before screening started, and 9 of these patients also developed intestinal metaplasia. Furthermore, subtle endoscopic scar formation of the gastric mucosa (Fig. 1I and J), identified during follow-up after the first endoscopy, was observed in 39 persons (43%) and in 1 person during the first endoscopy. Biopsy samples from 82 endoscopies (29%) did not show any abnormality (no inflammation, atrophy, intestinal metaplasia, scar formation, polyps, dysplasia or carcinoma). No serious adverse events were reported in medical charts after endoscopy.

DISCUSSION

During a period of 12.5 years, 90 persons from 40 HDGC families without germline *CDH1* mutations were endoscopically screened. In this endoscopic screening program attention is paid to early-stage DGC, namely mucosal SRCC in biopsy samples, which may be seen as small pale

lesions during endoscopy as was learned from surveillance in *CDH1* mutation carriers. SRCC foci (pT1a) were found in random biopsy samples of 4 persons from 1 family in which additional genetic analysis revealed a germline *CTNNA1* mutation in all 4.²⁷ In a patient from another family, advanced DGC was found during the screening program. In none of the other 38 families gastric cancer was detected.

Because of the very focal nature of small mucosal SRCCs, endoscopic detection is extremely difficult, as was demonstrated previously even in patients with a very high chance of having SRCC foci.^{28,29} Therefore, endoscopic surveillance is not accepted as a safe alternative for prophylactic gastrectomy in individuals with a proven pathogenic *CDH1* mutation. Our study highlights the diagnostic difficulties in individuals without a known germline mutation, and therefore unknown risk, when advice on prophylactic gastrectomy cannot be given. In some patients in this study after the first positive random biopsy sampling, follow-up biopsy samples showed negative results, to become positive again in subsequent endoscopies. No mucosal abnormalities were visualized in these patients during endoscopy. Possibly, the evolution of the quality of endoscopes, the increasing resolution, and the availability of narrow-band imaging increases the visualization of subtle lesions, as the authors of this article experienced in the surveillance of individuals with a germline *CDH1* mutation (unpublished data). However, in the current study we could not confirm this. One could argue that a limitation of this study is that because of the long time-frame with long follow-up, different quality of endoscopes have been used. However, subtle lesions could not be visualized in screening with both older- and newer-generation endoscopes, even in the knowledge of biopsy specimen-proven SRCC. This finding suggests that currently endoscopy alone without random biopsy sampling is not sensitive enough as a screening modality in our study population.

The HDGC criteria were principally set up to select families for genetic *CDH1* testing and were recently broadened to test more families for this devastating syndrome.¹⁶ It is

Figure 1. Diffuse (poorly cohesive) gastric cancer, (**A** and **B**), endoscopy and histology of (**C** and **D**) signet ring cell carcinoma (coincidental, no specific endoscopic findings), (**E** and **F**) low-grade dysplasia (coincidental, no specific endoscopic findings), (**G** and **H**) intestinal metaplasia, and (**I** and **J**) scar formation.

not clear whether these *CDHI* testing criteria make sense as endoscopic screening criteria as well, when families are proven *CDHI* negative. In only .3% of the 7732 biopsy samples in this study was invasive carcinoma found.

However, when we take into account the number of persons and number of families enrolled, the yield of cancer is considerable: 6% and 5%, respectively, especially when we bear in mind that in this

heterogeneous HDGC study group far less than 50% of persons screened are at increased gastric cancer risk.

Kim et al³⁰ developed an algorithm for gastroscopic screening in the United States in which they proposed screening of all individuals with an FDR with a diagnosis of gastric cancer. They recommended screening should begin 10 years before the age at diagnosis in the FDR or at age 50 (whatever comes first). It is a very topical matter of debate when a screening program is considered useful. Harris et al³¹ suggested the “balance approach” to evaluate the usefulness of screening. Screening should only be considered if there is evidence that the magnitude of health benefit outweighs the magnitude of harm, and this net benefit justifies the required use of resources. For well-established screening programs such as colorectal cancer screening, all these criteria are met. For HDGC screening, this balance is questionable.

The current study is the largest cohort study on individuals at risk for DGC described thus far. However, the numbers presented are still too small and follow-up is too short to calculate an accurate lifetime risk for FDRs of *CDH1*-negative DGC patients fulfilling HDGC criteria. Lim et al¹⁸ described screening in 7 persons fulfilling HDGC criteria without *CDH1* mutations and found SRCC foci in 2 of 7 persons (28%). For a more accurate risk calculation for the development of DGC in non-*CDH1* HDGC families, larger studies with long follow-up are needed. In addition to large cohort follow-up, our suggestion for future research is to also use existing large genealogy databases. Several Scandinavian countries, Australia,³² and the state of Utah in the United States³³ have genealogy databases that include large numbers of people. These databases could be linked to cancer registries and hospital data to identify families with HDGC and determine risk in family members.

Interestingly, biopsy specimens in this cohort revealed a much higher incidence of dysplasia (4%) and intestinal metaplasia (42%) than generally reported in the Netherlands. Both are associated with *H pylori* infection and believed to be risk factors in the development of gastric cancer, especially IGC.³⁴⁻³⁷ Den Hoed et al³⁸ investigated the prevalence of premalignant gastric lesions in 383 asymptomatic patients (without a family history of gastric cancer) by taking 2 biopsy specimens from the antrum and 2 from the body. Dysplasia was found in 1.4% of subjects. They found intestinal metaplasia in 7% of subjects, increasing to 13% in those aged 60 to 70 years.³⁸ *H pylori* infection was demonstrated in 22% of persons in their study and is in line with our results (19%). Our high frequency of dysplasia and intestinal metaplasia may be influenced by a more extensive inspection and biopsy sampling protocol; a 30-biopsy sampling protocol has, to our knowledge, not been described in a healthy control group of persons without a family history of gastric cancer. Another possibility is that gastric cancer in families without a

confirmed monogenic cause is a pathophysiologically different, more heterogenic, kind of cancer.

In the 285 endoscopies performed in this study, no serious adverse events occurred, and although gastroscopy is not without adverse event risks, it is a relatively safe procedure. We report scar formation of gastric mucosa in 40 participants (44%), appearing endoscopically as small pale lesions. As first described by Shaw et al¹⁷ in 2005, foci of SRCC can also appear as small pale lesions. This may mimic scars endoscopically; however, microscopically this does not pose a diagnostic dilemma. In our analysis some participants underwent up to 12 endoscopies with on average 25 random plus additional targeted biopsy samples per endoscopy. Except for 1, no scars were found at baseline endoscopies, suggesting that scars were mostly induced by prior gastric biopsy samples. Although the shape of the lesion often suggests it is a scar rather than an SRCC focus, these scars may hinder endoscopic screening. Also, it is unknown if taking biopsy specimens from the gastric mucosa and thereby inducing inflammatory responses with release of cytokines influences carcinogenic pathways in this population. On the other hand, 4 SRCCs in this study were found because of random biopsy sampling and not because of visible lesions, indicating that random biopsy sampling cannot be omitted in screening. Two of the 4 individuals with SRCC foci were diagnosed at the first screening endoscopy.

In summary, this study presents the largest cohort of gastroscopy screening of a well-defined group at risk of HDGC. In these FDRs from HDGC patients without a germline mutation in *CDH1*, endoscopic screening with extensive biopsy collection resulted in the identification of DGC in 6% of asymptomatic persons. Screening may therefore be justified in this population, although adjustments in the endoscopic interval and stricter inclusion criteria for the screening program could be considered.

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SUPPLEMENTARY TABLE 1. Characteristics of families undergoing gastroscopy

Family identification no.	Center: Radboud (1), NKI/AVL (2)	No. of family members undergoing gastroscopy	2010 HDGC criteria*	2015 HDGC criteria†	Diagnosis index that was counseled (age)
1	1	5	1	1	DGC (42)
2	1	1		1	DGC (56)
3	1	1	1	1	DGC (45)
4	1	4	1	1	DGC (23), IGC (57)
5	1	1	1	1	DGC (47)
6	1	1		1	DGC (67)
7	1	2	1	1	NA
8	1	1	1	1	NA
9	1	2	1	1	DGC (34)
10	1	3	3	2	NA
11	1	1	4	3	LBC (45)
12	1,2	4	2	1	DGC (71)
13	1	1	1	1	DGC (43)
14	1	1		1	DGC (50)
15	1	1		1	NA
16	1	1		1	GC (50)
17	1	3		1	DGC (64)
18	1	1	3	2	NA
19	1	1	3	2	DGC (36)
20	1	3	1	1	DGC (35)
21	2	3		1	DGC (76)
22	2	2	1	1	NA
23	2	1	2	1	DGC (46)
24	2	1	3	2	NA
25	2	1	2	1	DGC (52)
26	2	1	3	2	DGC (28)
27	2	1	2	1	DGC (65)
28	2	2	3	2	DGC (25)
29	2	1		1	DGC (54), CRC (55)
30	2	1	1	1	IGC (52)
31	2	6	3	2	MGC (32)
32	2	18		1	DGC (62)
33	2	4	3	2	DGC (37)
34	2	2	3	2	DGC (31)
35	2	1	1	1	DGC (31)
36	2	1	1	1	DGC (43)
37	2	3	1 and 4	1 and 3	LBC (55)
38	2	1	3	2	DGC (33)
39	2	1	3	2	DGC (33)
40	1	1		1	GC (48)

BC, Breast cancer; BO, bone cancer; BRAT, brain tumor; CRC, colorectal cancer; CUP, cancer of unknown primary; DBC, ductal breast cancer; DCIS, ductal carcinoma in situ; DGC, diffuse-type gastric cancer; ES, esophageal cancer; ES SCC, esophageal squamous cell carcinoma; FDR, first-degree relative; GC, gastric cancer; HDGC, hereditary diffuse-type gastric cancer; HL, Hodgkin lymphoma; HNC, head and neck cancer; IGC, intestinal-type gastric cancer; LBC, lobular breast cancer; LC, lung cancer; Leu, leukemia; MGC, mixed-type gastric cancer; MM, malignant melanoma; NA, not available; NET, neuroendocrine tumor; NHL, non-Hodgkin lymphoma; NKI/AVL, Netherlands Cancer Institute/Antoni van Leeuwenhoek; OC, ovarian cancer; PC, pancreatic cancer; PrCa, prostate cancer; RC, renal cancer; ThC, thyroid cancer; UGC, undifferentiated gastric cancer.

*2010 HDGC criteria: 1 = ≥ 2 GC, 1 DGC < 50 y; 2 = ≥ 3 DGC; 3 = 1 DGC < 40 y; 4 = DGC and LBC, 1 < 50 y.

†2015 HDGC criteria: 1 = ≥ 2 GC, 1 DGC; 2 = 1 DGC < 40 y; 3 = DGC and LBC, 1 < 50 y.

SUPPLEMENTARY TABLE 1. Continued

GC or BC in FDR: frequency (age)	GC or BC in second-degree relatives: frequency (age)	Other cancer diagnoses in FDR and second-degree relative: frequency (age)
GC: 1 (69); DBC: 1 (47)	GC: 1 (69)	DCIS: 1 (53)
GC: 1 (66)	GC: 3 (53, 69, 73)	CRC: 1 (66); BO: 2 (57, 80)
IGC: 1 (77)	GC: 1 (70)	RC: 1 (60)
BC: 1 (52)	GC: 1 (50)	LC: 1 (79); BO: 1 (23)
DGC: 1 (84); DBC: 1 (50)		
DGC: 1 (70); GC: 1 (78)		Thymoma: 1 (72)
DGC: 1 (34)	GC: 1 (56)	CRC: 1 (60)
DGC: 2 (31, 42)		
	GC: 2 (59, 65); DBC: 1 (53)	
DGC: 1 (34); DBC: 1 (43)	BC: 1 (40)	Leu: 1 (80)
DGC: 1 (53)		PC: 1 (70)
DGC: 2 (59, 65)		RC: 1 (64); CRC: 2 (63, 65)
DGC: 1 (58); LBC: 1 (43)	BC: 1 (49)	PrCa: 1 (82)
DGC: 1 (50); GC: 1 (48)		ThC: 1 (48)
DGC: 1 (54); GC: 1 (56)	MGC: 1 (82)	CRC: 1 (74)
DGC: 2 (67, 77); GC: 2 (53, 60); LBC: 1 (62)	GC: 2 (NA); BC: 2 (NA)	CRC: 1 (45); LC: 1 (59); HNC: 1 (55)
GC: 1 (52)	GC: 3 (62, 64, 82)	CRC: 4 (62, 74, 82, 83)
DGC: 1 (32)		CRC: 1 (72)
BC: 1 (52)		HL: 1 (18)
DGC: 1 (51); GC: 2 (22, 42)		Thymoma: 1 (37)
IGC: 1 (73); UGC: 1 (51); GC: 2 (51, 69)	GC: 5 (60, 68, 70, 70, 74)	CUP: 1 (79); CRC: 1 (39)
DGC: 2 (42, 67)		ES: 1 (70); CRC: 1 (49); CUP: 1 (NA); NHL: 1 (NA)
DGC: 3 (26, 45, 51)		CRC: 1 (45)
DGC: 1 (34); IGC: 1 (53)		ES SCC: 1 (53); CRC: 1 (80); LC: 1 (53)
DGC: 1 (58)	DGC: 1 (85); GC: 1 (75)	CUP: 1 (82); RC: 1 (63)
		BRAT: 1 (NA)
DGC: 2 (59,71)		CRC: 1 (NA)
		CRC: 1 (74), OC: 1 (57)
DGC: 1 (65); LBC: 1 (62); GC: 1 (45)	GC: 3 (48, 68, 79); BC: 1 (60)	LC: 1 (48)
DGC: 2 (49, 74)	IGC: 1 (72), GC: 1 (82)	
DGC: 1 (63); GC: 2 (54, 71)	GC: 4 (45, 51, 57, 72)	BRAT: 1 (49)
	DBC: 1 (50)	BRAT: 1 (NA)
	BC: 1 (44)	NET lung: 1 (35); MM: 1 (NA)
DGC: 1 (79)		PrCa: 1 (76); Leu: 1 (41)
GC: 1 (51)		
DGC: 2 (49, 66)	BC: 1 (52)	PC: 1 (73); CRC: 1 (71); LC: 1 (NA)
DGC: 1 (51); IGC: 1 (78); BC: 1 (50)		