ORIGINAL ARTICLE

Germline variants and phenotypic spectrum in a Canadian cohort of individuals with diffuse gastric cancer

M. Aronson MS,* C. Swallow MD,† A. Govindarajan MD,† K. Semotiuk MS,* Z. Cohen MD,*
P. Kaurah PhD,‡ L. Velsher MD,§ I. Ambus MS,§ K. Buckley MS,|| C. Forster-Gibson MD PhD,#
W.S. Meschino MD,** A. Blumenthal MD,†† R.H. Kim MD PhD,‡‡ and S. Brar MD†

ABSTRACT

Background *CDH1* pathogenic variants (PVs) cause most cases of inherited diffuse gastric cancer (DGC), but have low detection rates and vary geographically. In the present study, we examined hereditary causes of DGC in patients in Ontario.

Methods *CDH1* testing through single-site or multi-gene panels was conducted for patients with DGC meeting the 2015 International Gastric Cancer Linkage Consortium (IGCLC) criteria, or with isolated DGC at less than 50 years of age, or with a strong family history of cancer identified at the Zane Cohen Centre (ZCC). All *CDH1*-positive patients at ZCC, regardless of cancer history, were summarized.

Results In 15 of 85 patients with DGC (17.6%), a PV or likely PV was identified through *CDH1* single-site ($n = 43$) or multi-gene panel ($n = 42$) testing. The detection rate was 9.4% overall (8 of 85) and 11% using IGCLC criteria (7 of 65). No *CDH1* PVs were identified in patients with isolated DGC at less than 40 years of age, but 1 PV was identified in a patient with isolated DGC at less than 50 years of age. Multi-gene panels identified 9 PVs (21.4%), including *CDH1, STK11, ATM, BRCA2, MLH1,* and *MSH2*. Review of 81 *CDH1* carriers identified 10% with DGC (median age: 48 years; range: 38–59 years); 41% were unaffected (median age: 53 years; range: 26–89 years). Observed malignancies other than DGC or lobular breast cancer (LBC) included colorectal, gynecologic, kidney or bladder, prostate, testicular, and ductal breast cancers. Lobular-breast cancer was seen only in 3 families.

Conclusions In Ontario, the detection rate of *CDH1* PVs in patients with DGC was low: no PVs were identified in patients with isolated DGC at less than 40 years of age, and 1 was identified in a patient with isolated DGC at less than 50 years of age. Isolated LBC with no DGC was observed in *CDH1*-positive families, as were pathology-confirmed non-DGC or non-LBC malignancies, which had not previously been reported. Given a phenotype that overlaps with other hereditary conditions, multi-gene panels are recommended for all patients with DGC at less than 50 years of age and for those meeting IGCLC criteria.

Key Words Hereditary diffuse gastric cancer; HDGC; early-onset disease; breast cancer, lobular

INTRODUCTION

Gastric cancer arises in 1 of 3500 Canadians yearly[1]. Hereditary gastric cancer is rare: 10% of individuals with gastric cancer have a positive family history, but only 1%–3% are found to have a hereditary condition[2]. The most common hereditary condition associated with diffuse-type gastric cancer (DGC) is hereditary diffuse gastric cancer syndrome (HDGC), which is caused by a heterozygous germline pathogenic or likely pathogenic variant (PV) in the *CDH1* gene. The syndrome is associated with a lifetime risk for DGC of 70% in men and 56% for women, and a risk for lobular breast cancer (LBC) in women of 42%[3]. The penetrance of DGC declines when clinical *CDH1* families are not ascertained based on multiple cases of DGC, with the cumulative risk up to age 80 for gastric cancer being 42% for men and 33% for

women, and for LBC being 55%[4]. Signet-ring-cell colorectal cancer[5] has been associated with *CDH1* variants, as have cleft lip and palate[6,7]. The incidences of intestinal-type gastric cancer and ductal breast cancer are not described to be higher in HDGC[8].

In 2015, genetic testing criteria for *CDH1* were updated by the International Gastric Cancer Linkage Consortium (IGCLC)[9] to include affected individuals in families with

- 2 gastric cancers at any age, 1 confirmed as DGC; or
- DGC diagnosed at less than 40 years of age (DGC<40), regardless of family history); or
- personal or family history of DGC and LBC (1 diagnosed at less than 50 years of age).

Those criteria were revised from earlier versions outlined in 1999 and 2010 so as to broaden the eligibility of families with DGC. As a result, the detection of *CDH1* PVs declined to approximately 10%–20% from 25%–30% based on the original criteria[8,10]. To date, more than 150 *CDH1* PVs have been reported in HDGC[11].

Although *CDH1* is the most common cause of hereditary DGC, other hereditary conditions have been associated with DGC, including PVs in *CTNNA1* and *BRCA2;* Lynch syndrome caused by mismatch repair genes; and Li–Fraumeni syndrome and polyposis conditions such as familial adenomatous polyposis, Peutz–Jeghers syndrome, and juvenile polyposis[3,12]. Still, causes in most families suspected of having a hereditary DGC syndrome remain unidentified.

Because the prevalence of *CDH1* variants can vary geographically, studying the detection rate in various populations is important. The aim of the present study was to determine the frequency of *CDH1* and other germline PVs in individuals with DGC who underwent genetic testing in Ontario and to summarize the phenotype of a large cohort of *CDH1*-positive carriers from the only Canadian clinical gastrointestinal cancer registry.

METHODS

Individuals with gastric cancer referred for genetic consultation at the Zane Cohen Centre (ZCC) for Digestive Diseases at the Sinai Health System, Toronto, Ontario, between 1997 and 2018 were identified from a prospectively maintained database. The Familial Gastrointestinal Cancer Registry at the ZCC is the only active hereditary gastrointestinal cancer registry in Canada[13]. Individuals with a pathology confirmation of DGC were offered genetic testing if they met ZCC eligibility criteria: DGC diagnosed at less than 50 years of age (DGC<50), family history meeting IGCLC criteria, or family history suggestive of other hereditary cancer syndromes. Before 2016, genetic testing included *CDH1* sequencing and deletion or duplication testing by multiplex ligation-dependent probe amplification and by single-site testing for other hereditary conditions based on family history. Families tested after 1 January 2016 were offered multi-gene panels processed using next-generation sequencing. Those panels included these genes at minimum: *CDH1, CTNNA1, APC, MUTYH,* mismatch repair genes, *STK11, BMPR1A, SMAD4, BRCA1/2,* and *PALB2*. Attempts at re-contact with individuals tested before

2016 were made to offer updated testing. All individuals who accepted underwent *CDH1* screening through either single-site or multi-gene testing. For all individuals tested, a 3-generation family history was obtained.

The Familial Gastrointestinal Cancer Registry prospectively follows individuals identified with *CDH1* PVs. Biennial follow-up includes results of *Helicobacter pylori* testing, development of malignancy, prophylactic surgery, and oncologic resection. The present study was approved by the Mount Sinai Hospital institutional ethics review board.

RESULTS

Genetic Testing for Individuals with DGC
In the ZCC database, 208 individuals diagnosed with gastric cancer were identified, of whom 124 had pathology-confirmed DGC. Of those 124 patients, 119 met genetic testing criteria, but 34 did not consent to genetic testing or died before testing could occur. Genetic testing was performed for 85 individuals from 80 families when they met either the IGCLC criteria ($n = 65$), the ZCC criteria of isolated DGC diagnosed at 40–49 years of age ($n = 14$), or DGC diagnosed at 50 or more years of age with family history suggestive of a hereditary condition ($n = 6$). In 43 individuals, testing consisted of single-site *CDH1* sequencing and multiplex ligation-dependent probe amplification; in 42 individuals, it consisted of a multi-gene panel.

Ethnicity was available for 83 of the 85 patients, with 48% reporting European ancestry ($n = 40$), 24% Asian ancestry ($n = 20$), and 9% Canadian ancestry ($n = 8$), including First Nations, French Canadian, and Newfoundlander. The remaining 19% of families were ethnically Middle Eastern ($n = 5$), African ($n = 5$), Ashkenazi Jewish ($n = 4$), and Latino ($n = 1$).

The 15 PVs or likely PVs identified in 85 individuals with DGC meeting eligibility criteria (17.6%) included 8 *CDH1* PVs or likely PVs, and 7 PVs leading to other hereditary conditions. Of the 8 *CDH1* variants identified in this cohort (9.4%), 6 were found through single-site testing, and 2 (1 PV, 1 likely PV), through a multi-gene panel. The multi-gene panel testing (42 individuals) identified 9 PVs or likely PVs (21.4%), including *ATM* ($n = 1$), *CDH1* ($n = 2$), *BRCA2* ($n = 2$), *MLH1* ($n = 1$), *MSH2* ($n = 2$), and *STK11* ($n = 1$). Table I summarizes the demographics and variant results for the positive families. Table II summarizes genetic testing results for the entire cohort by age and eligibility criteria.

In the 65 individuals who met the IGCLC criteria, the positive *CDH1* diagnostic yield was 10.8% ($n = 7$). No mutations were identified in individuals meeting the criterion of isolated DGC<40 ($n = 18$), or in individuals with LBC and DGC ($n = 3$), or in those meeting the combined criteria of LBC and DGC<40 ($n = 1$).

Notably, 1 individual in the group 40–49 years of age who was diagnosed with isolated DGC did not meet the IGCLC criteria, but rather met the sole ZCC criterion of isolated DGC<50. Of the individuals meeting the IGCLC criteria, 4 were noted to also have other variants with hereditary implications, including *BRCA2, MLH1,* and *ATM.* Figure 1 outlines the IGCLC criteria, showing a breakdown of sole and group criteria met by the families and the resulting germline genetic results.

TABLE I Demographic and genetic information from 15 patients with mutation-positive diffuse gastric cancer (DGC)

ID	Ethnicity	Gene	Variant	Cancer diagnosis				DGC at PTG by age (years)	Other history of cancer	Unaffected CDH1 carriers		IGCLC criteria[a]	Reference[b]
				Proband		Known or obligate carriers				(n)	Age (years)		
				Type	Age (years)	Type	Age (years)						
1 (kin to 2)	Italian	CDH1	EX16del (novel)	DGC	49	Ductal BCa, ovarian, ovarian	49 74 72	27, 52, 59	Brainx2, ovarian	6	81, 66, 64, 62, 51, 29	None	
2 (kin to 1)	Italian	CDH1	EX16del (novel)	DGCx3, Testicular	47, 27, 37	Ductal BCa, ovarian, ovarian, DGC	49 74 72, 49					1	
3	German	CDH1	c.187C>T	DGC, Lobular BCa	57 50			29, 63	Prostate, Gastric NOS, Bladder, Ovarian, BCa NOS	0		1, 3	Gayther *et al.*, 1998[14]
4	Chinese	MLH1	c.1558+1G>A	DGCx2 CRC	77 77	CRC (multiple)						1	Viel *et al.*, 1998[15]
5	White	MSH2	c.1165C>T	DGC, CRC, Bladder	70 69 70	Endometrial	57		CRC (multiple)	NA		None	Mongiat-Artus *et al.*, 2006[16]
6	Ashkenazi Jewish	MSH2	c.1906G>C	DGC CRC Prostate	68 33, 67 77				Endometrial, CRCx2, Ureterx2, Prostate	NA		None	Goldberg *et al.*, 2010[17]
7	Japanese	CDH1	c.1212delC	DGC, DGC	44 59	Lobular BCa, Lobular BCa, CRC, Prostate, Lobular BCa, Lobular BCa	51 51 57 57 86 42	29, 53, 55, 56, 57	CRC, Gastric NOSx3, Bladder	1	72	1, 3	Yamada *et al.*, 2011[18]
8	British	STK11	c.910C>T	DGC, Lobular BCax2	67 64					NA		None	Resta *et al.*, 2013[19]
9	British	CDH1	c.1901C>T	DGC	38				Gastric NOSx2	1	72 (with PTG at 63)	1, 2	Suriano *et al.*, 2013[20]
10	French Canadian	CDH1	c.2100delT					27, 27	Gastric NOS, DGC	2	53, 57 (with PTG at 50)	1	Hansford *et al.*, 2015[3]
11 (kin to 12)	Italian	CDH1	EX1_2del	DGC	42	Lobular BCa, DGC, Lobular BCa, LCIS, DCIS	47 58 51 57	52	BCax2, Gastric NOS	0		1, 2	Lowstuter *et al.*, 2017[21]

TABLE I Continued

ID	Ethnicity	Gene	Variant	Cancer diagnosis						Unaffected CDH1 carriers		IGCLC criteria[a]	Reference[b]
				Proband		Known or obligate carriers		DGC at PTG by age (years)	Other history of cancer	*(n)*	Age (years)		
				Type	Age (years)	Type	Age (years)						
12 (kin to 11)	Italian	CDH1	EX1_2del	DGC, Lobular BCa	58	Lobular BCa, DGC, LCIS	47 42 51	52	BCax2, Gastric NOS, DCIS	0		1, 2	Lowstuter et al., 2017[21]
13	Italian	BRCA2	c.6591_6592delTG	DGC	41	Ductal BCa	47		Gastric NOSx2, Lobular BCa, Ductal BCa	NA		1, 3	Dominguez-Valentin et al., 2018[22]
14	Ashkenazi Jewish	ATM	c.5932G>T	DGC	57				Bladder, CRC, Gastric NOS			1	Estiar and Mehdipour, 2018[23]
15	Chinese	BRCA2	c.7480C>T	DGC	19	Gastric cancer	36		Gastric NOSx4	NA		1, 2	Kwon et al., 2019[24]

[a] (1) Diagnosis of 2 gastric cancers not otherwise specified, with 1 confirmed DGC at any age; (2) diagnosis of DGC at less than 40 years of age; (3) diagnosis of DGC and lobular breast cancer, 1 diagnosis at less than 50 years of age.

[b] If variant was previously reported in other families.

PTG = prophylactic total gastrectomy; IGCLC = International Gastric Cancer Linkage Consortium; BCa = breast cancer; NOS = not otherwise specified; CRC = colorectal cancer; NA = not applicable.

TABLE II Detection of *CDH1* and other genes in all patients with diffuse gastric cancer (DGC) by age and International Gastric Cancer Linkage Consortium (IGCLC) criteria

Age at DGC diagnosis	IGCLC criteria			CDH1 detection			Other genes		
	(A) 2 Gastric cancers NOS, with 1 being DGC	(B) DGC at <40 years	(C) Lobular BCa and DGC, 1 at <50 years	Tested *(n)*	Pathogenic variants *(n)*	Detection rate (%)	Tested *(n)*	Pathogenic variants *(n)*	Detection rate (%)
<40 Years		√ Only	—	17	0	0	10	0	0
	√	√	±√	9	1	11	4	1 (BRCA2)	25
40–49 Years	—	—	—	14	1	7	8	0	0
	—	At least 1 √	—	15	4	27	5	1 (BRCA2)	20
≥50 Years	—	—	—	5	0	0	4	3 (2 MSH2, 1 STK11)	80
	—	At least 1 √	—	25	2	8	11	2 (1 MLH1, 1 ATM)	44
TOTAL				85	8		42[a]	7	

[a] Of 85 individuals, 42 underwent multigene testing.

NOS = not otherwise specified.
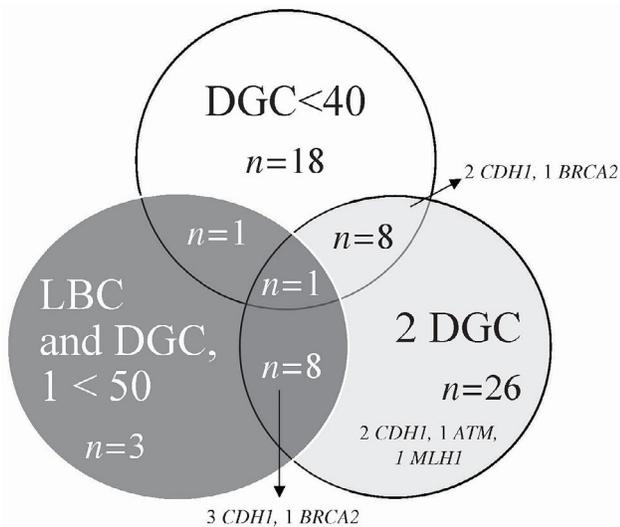
FIGURE 1   Patients with diffuse gastric cancer (DGC) meeting International Gastric Cancer Linkage Consortium criteria (*n* = 65). LBC = lobular breast cancer.

H. pylori in Patients with Very-Early-Onset DGC

Because no hereditary conditions were identified in individuals with isolated DGC<40, information about *H. pylori* infection was obtained to assess additional known risk factors. Infection information was available for 20 of 26 individuals with DGC<40. A history of *H. pylori* infection was present in 13 (65%), and all but 1 had been diagnosed within a year of their DGC. One individual had been treated 14 years before development of their malignancy.

Cancer Information for Patients with HDGC

In the ZCC database, 81 individuals with *CDH1* PVs (*n* = 77) or likely PVs (*n* = 4) from 18 HDGC families were identified. Of the *CDH1* variants, 8 were first identified in our cohort of patients with DGC (summarized in Table I). An additional 12 families were identified through patients in the ZCC database with non-DGC malignancies. Of the 81 individuals with *CDH1* mutations, 33 carriers had no cancer reported (40.7%; median age: 53.5 years; range: 26–89 years), including 4 individuals who had no microscopic cancer identified after prophylactic total gastrectomy. Invasive cancer was reported in 30 individuals with 36 malignancies (colorectal cancer, *n* = 3; kidney or bladder cancer, *n* = 2; gynecologic cancers, *n* = 3; prostate cancer, *n* = 2; testicular cancer, *n* = 2; ductal breast cancer, *n* = 2; lobular breast cancer, *n* = 14; and DGC, *n* = 8). In 6 individuals with invasive breast cancer, microscopic signet-ring-cell carcinoma was also found after prophylactic total gastrectomy. Overall, 26 individuals had microscopic signet-ring-cell carcinoma identified: 8 through endoscopic screening under the Cambridge protocol, and 18 from a prophylactic total gastrectomy specimen. Table III summarizes those cancer sites and patient ages.

The IGCLC criteria were met by 14 of 18 known HDGC families (78%). Of those 14 families, 12 met more than 1 criterion; the remaining 2 families met the sole criterion of 2 cases of DGC at any age. Of the 4 families that did not meet any IGCLC criteria, 1 met the ZCC criteria of a DGC<50, and the

other 3 had at least 1 case of LBC, with no history of DGC in the family. None of the families met the criteria of DGC<40 as the sole criterion. Table IV presents demographic and genetic information for the remaining *CDH1*-positive families.

DISCUSSION

In the present study, we report the results of genetic testing for hereditary causes of DGC in a large Canadian cohort. In our series of 85 individuals with DGC meeting the ZCC criteria of isolated DGC diagnosed at 40–49 years of age or meeting the IGCLC criteria, or with DGC diagnosed at 50 years of age or older and a family history suggestive of a hereditary cancer syndrome, we identified 15 (17.6%) with PVs or likely PVs, with more than half the variants (*n* = 8) being found in *CDH1* (9.4%). Overall, we identified 65 individuals who met the 2015 IGCLC eligibility criteria, in whom the *CDH1* mutation detection rate was 10.8% (*n* = 7). We found 1 HDGC carrier missed by the IGCLC criteria who was diagnosed with DGC at 49 years of age (no other DGC or LBC had been reported in the family at the time of diagnosis).

Our *CDH1* mutation detection rate of 10.8% in Ontario patients who met the 2015 IGCLC criteria is slightly lower than rates reported in other countries with populations having a low incidence of gastric malignancies. In 1999, criteria for *CDH1* genetic testing were published. Families were included if they had 2 cases of pathologically confirmed DGC in 1st- or 2nd-degree relatives, 1 DGC<50, or 3 or more pathology-confirmed DGC cases in 1st- or 2nd-degree relatives regardless of age[25]. Those criteria were developed based on the understanding of *CDH1* developed from highly penetrant families in New Zealand, leading to an ascertainment bias[28]. Using those original criteria, the *CDH1* detection rate was reported to be 25%–50%[5,29]. In 2010, the criteria were revised based on the acknowledged difficulty

TABLE III   Cancers in 62 patients with hereditary diffuse gastric cancer

Site	Pts [*n* (%)]	Age at Dx (years)	
		Median	Range
Microscopic SRCC	26 (32.1)	41.0	19–63
DGC, invasive[a]	8 (9.9)	48.0	38–59
Lobular BCa or LCIS[a,b]	14 (28.6)	50.0	41–86
Ductal BCa or DCIS[b]	2 (4.1)	48.0	47–49
Bladder	1 (1.2)	65	
Kidney	1 (1.2)	71	
Colorectal	3 (3.7)	57.0	46–77
Endometrial[b]	1 (2.0)		
Ovarian[b]	2 (4.1)	73.0	72–74
Prostate[b]	2 (6.2)	68.5	57–80
Testicular[b]	2 (6.2)	32.0	27–37

[a]   In 1 patient, a *likely* pathogenic variant was detected.
[b]   Percentage calculated based on sex-specificity.
Pts = patients; Dx = diagnosis; SRCC = signet-ring-cell carcinoma; DGC = diffuse gastric cancer; BCa = breast cancer; LCIS = lobular carcinoma *in situ;* DCIS = ductal carcinoma *in situ.*

TABLE IV Remaining 12 families in the registry who were *CDH1*-positive

ID	Ethnicity	Variant	Cancer diagnosis			Other history of cancer	Unaffected *CDH1* carriers		IGCLC criteria[a]	Reference[b]
			Known or obligate carriers		DGC at PTG by age (years)		*(n)*	Age (years)		
			Type	Age (years)						
16	Iranian	c.2029C>T (novel, likely pathogenic)	Lobular BCa	46		BCa NOS, Prostate, Leukemia, Kidney			None	
17	Pakistani	c.1792C>T			58	Gastic NOSx2, BCa NOS		40	1, 2[c]	Gayther et al., 1998[14]
18	White	c.1792C>T				BCa NOS, Gastric NOSx2, Lobular BCa, Urethral		34	1, 2[c]	Caldas et al., 1999[25]
19	Italian	c.832+1G>A	Lobular BCa, BCa NOS	41 50		Esophageal, BCa NOSx2	3	35,47, 69	None	Oliveira et al., 2002[26]
20	Ashkenazi Jewish	c.2398delC						29	1, 2[c]	Oliveira et al., 2002[26]
21	White	c.2398delC	CRC	77		Gastric NOSx3, BCa NOS, Esophageal		56	1, 2[c]	Oliveira et al., 2002[26]
22	White	c.2398delC				BCa NOSx4, Gastric NOS		63	1, 2[c]	Oliveira et al., 2002[26]
23	Canadian (NL)	c.2398delC			28, 29, 48	Unknown primary, BCa NOS		64 (with PTG at 54)	1, 2[c]	Brooks-Wilson et al., 2004[5]
24	Jordanian	c.1137G>A	Lobular BCa	50		Unknown primary, Gastric NOS, Leukemia, GEJx2		42	3	Oliveira et al., 2006[27]
25	Canadian (NL)	c.1189A>T	Lobular BCa	48	28				None	Hansford et al., 2015[3]
26	White	c.2100delT	Gastric NOS, CRC, Lobular BCa, Lobular BCa, BCa, Bladder, Kidney, Prostate	35 46 54 50s 65 71 80	19, 25, 28, 32, 50, 52, 52	DGC, CRC, BCa NOSx4, Gastric NOS		53, 53, 62, 88	1, 2, 3	Hansford et al., 2015[3]
27	British	c.1064dupT				DGC, Gastric NOS		40	1, 2	Hansford et al., 2015[3]

a   (1) Diagnosis of 2 gastric cancers not otherwise specified, with 1 confirmed DGC at any age; (2) diagnosis of DGC and lobular breast cancer, 1 diagnosis at less than 50 years of age; (3) diagnosis of DGC at less than 40 years of age.

b   If variant was previously reported in other families.

c   Even though the gastric cancer pathology unavailable, families were known to have multiple cases of gastric cancer and were known to have published founder mutations with DGC confirmed. The IGCLC criteria were based on the relevant references.

DGC = diffuse gastric cancer; PTG = prophylactic total gastrectomy; IGCLC = International Gastric Cancer Linkage Consortium; BCa = breast cancer; NOS = not otherwise specified; GEJ = gastroesophageal junction; CRC = colorectal.

in obtaining pathology confirmation for multiple cases of DGC in the family and included 3 cases of DGC in a family, 1 confirmed by pathology; 2 cases of DGC, 1 confirmed by pathology and being DGC<50; a case of DGC<40 regardless of family history; or individuals with a personal or family history of DGC and LBC, 1 case diagnosed at less than 50 years of age[30]. In 2015, the first two criteria were collapsed to include 2 cases of DGC at any age, 1 pathology-confirmed to be DGC. The remaining criteria of isolated DGC<40 or of DGC and LBC, 1 diagnosed at less than 50 years of age, remained the same[9]. Given the slight difference between the 2010 and 2015 criteria, an evaluation considering both found comparable sensitivity, with decreased specificity for the 2015 version[8]. Benusiglio *et al.*[8] found *CDH1* mutations in 19% of probands in France meeting the criteria (41 of 216), and van der Post *et al.*[10] reported a mutation rate of 14% for individuals in the Netherlands who met testing criteria (16 of 118). The highest positive *CDH1* detection rates have occurred in areas with known founder mutations, such as Newfoundland and New Zealand[29,31]. To date, a few small series are investigating *CDH1* mutations in populations with high incidences of gastric cancer, reporting relatively low positive yields of 7% (Japanese population) and 8% (Korean population)[18,32].

We examined each criterion from the 2015 IGCLC recommendations individually and then in combination to determine the most effective criteria. The highest yield of *CDH1*-positive detection using a single criterion was 2 cases of DGC in a family, where 2 of 26 tested positive for *CDH1* mutation (7.7%). Using criteria in combination, the highest yield occurred in families with multiple cases of DGC with or without LBC and with early age of onset. In our cohort, 5 of 16 individuals (31%) from families with either 2 cases of DGC (1 being DGC<40) or 2 cases of DGC and LBC (1 being diagnosed at less than 50 years of age) were found to have pathogenic *CDH1* variants. The combination of family history and early age of onset was shown to be most effective considering the positive rates in the original 1999 criteria. That finding was also supported when comparing the 2010 with the 2015 criteria, where a high *CDH1* detection rate of 39%–42% was observed in individuals having family history of DGC with or without LBC and with a young age of onset. The detection rate dropped to 28% once the age restriction was removed[8].

The least successful single criterion in our cohort was the sole criterion for DGC<40. No mutation was identified in those patients. More than half our mutation-positive index cases (5 of 8) were diagnosed with DGC between ages of 40 and 49 years. In our larger cohort of *CDH1* carriers, the median age of diagnosis with invasive DGC was 48 years. That age of onset is older than the median age of 40 years cited in previous reports[33], but it might be reflective of a clinical population in a low-incidence country that lacks some of the early ascertainment biases, and it is more comparable with similar studies reporting an age of DGC onset of 46.7 years[33]. Sporadic gastric cancer rarely affects young people, and only 5% of cases are diagnosed at less than 40 years of age[34]. The underlying cause of isolated DGC<40 is not well-understood, and geographic differences might be a factor affecting the mutation rate in young patients with DGC. Hakkaart *et al.*[31] reported that, in New Zealand, 67%

of individuals from the Maori population diagnosed with DGC at less than 45 years of age had a *CDH1* PV. The authors hypothesized a survival advantage of *CDH1* mutation, with carriers having an innate resistance to the foodborne pathogen *Listeria monocytogenes,* which has been linked to gastroenteritis, meningitis, and miscarriage[31]. Other studies have reported a low yield of mutations in isolated DGC<40[5,20], with the exception of Benusiglio *et al.*[8], who reported a 15% positive pick-up rate of *CDH1* PVs in patients with isolated early-onset DGC. Although the criterion of isolated DGC<40 was not successful at identifying *CDH1* carriers in our patients, the ZCC criteria of isolated DGC<50 was able to identify a carrier who would have been missed by the IGCLC criteria. Given certain of our observations (the lack of mutations in individuals with DGC<40, the small increase in the number of patients with DGC diagnosed between 40 and 49 years of age, and evidence of an older onset of DGC in the HDGC cohort), t is worth considering whether the age limit for the criterion of DGC<40 should be increased to 50 years of age.

H. pylori has long been seen as the most significant risk factor associated with intestinal-type gastric cancer. It has increasingly has been associated with DGC[35], and recent studies in Italian[36], Japanese[37], Indian[38], and Korean[39–41] populations suggest that it may contribute to early-onset DGC, especially in individuals with current infections. In our series, *H. pylori* was identified in 13 of 20 individuals with DGC<40 (65%). The estimated rate of *H. pylori* seropositivity in the Ontario population is less than 25%[42], and although causation cannot be determined, that rate would seem to support the role of *H. pylori* infection, possibly in conjunction with a host-related factor, as leading to early-onset DGC not explained by known disease susceptibility genes.

Positive family history of DGC or of DGC and LBC was an important factor in the *CDH1* detection rate, with 14 of 18 *CDH1*-positive families in our series meeting those IGCLC criteria. The exceptions were 3 families having a history of LBC only, and 1 family with a case of DGC diagnosed at 49 years of age and no other history of DGC or LBC. Notably, although the latter family did not have the hallmark cancers of HDGC, known carriers in the family had been diagnosed with brain, testicular, ovarian, endometrial, and ductal breast cancers at younger ages. We also reported the lack of a personal history of cancer in 41% of *CDH1* carriers (n = 33; median age: 53.5 years; range: 26–89 years), which might contribute to a lack of family history when kin are still young. Other challenges in fulfilling the testing criteria include limited knowledge of cancer diagnoses, lack of pathology confirmation, possible inaccurate diagnoses (gastroesophageal junction or esophageal cancer), and new diagnoses that might arise after the index case is first assessed.

Pathogenic *CDH1* variants were identified in 4 families presenting with LBC only. After 3-generation pedigrees were obtained, only 1 case of DGC was identified. Traditionally, families with LBC in the absence of DGC have not been considered at high risk for *CDH1* mutations, but incidental mutations have been identified in such families with the increased use of multi-gene panels[8,43–46]. Xicola *et al.*[47] recently reported that 36% of 113 HDGC families had cases of breast cancer in the absence of a family history of

gastric cancer, noting that full pedigrees were available on 38 families. Whether the genotype–phenotype correlation from variants identified in LBC-only families is associated with a reduced risk of DGC is unknown. However, caution is advised because an extensive family history might reveal DGC, and online databases might report DGC associated with those particular variants in other families. Several studies describe LBC-only families that are found to have signet-ring-cell carcinoma on endoscopy or examination of the resected specimen after prophylactic total gastrectomy[21,48]. Published case reports might also provide information leading an evolved understanding. For example, one of our LBC-only families having the variant c.832+1G>A had no DGC history. However, that particular variant has been reported in a family with DGC diagnosed at 40 years of age and with 3 relatives affected by gastric cancer at ages 36, 48, and 50 years[5].

Several other inherited syndromes have been associated with DGC, including Lynch syndrome, Peutz–Jeghers syndrome, juvenile polyposis, Li–Fraumeni syndrome, and familial adenomatous polyposis. Hansford *et al.*[3] identified PVs in *CTNNA1* and *BRCA2,* and likely or possible PVs in *MSR1, PRSS1,* and in DGC families who were tested using a 55-gene comprehensive panel. Indeed, we identified 7 families with other hereditary cancer predisposition genes (Peutz–Jeghers syndrome, *n* = 1; Lynch syndrome, *n* = 3; *BRCA2, n* = 2; and *ATM, n* = 1), 4 of whom met IGCLC criteria. The families with the *ATM* and *MLH1* mutations met IGCLC criteria, as did both families with pathogenic *BRCA2* variants, 1 member of which was a 19-year-old with DGC who had 5 close relatives with early-onset gastric cancer (no breast cancer reported). Three families did not meet IGCLC criteria, but were tested based on family histories suggestive of hereditary conditions, namely Lynch syndrome and Peutz–Jeghers syndrome. The individual with the pathogenic *STK11* mutation presented with 2 primary LBCs and DGC; however, the malignancies were diagnosed after 50 years of age, thereby not meeting the IGCLC criteria because of the age limitation. Although the patient underwent genetic testing because of mucopigmentation, the case highlights an overlapping of the phenotype with various hereditary syndromes and supports the need for multi-gene panel testing in at-risk families.

Our study has important limitations that warrant mention. Although this study is the first of hereditary DGC in Ontario, it is limited by the small number of patients with DGC. Another limitation was that only single-site *CDH1* testing was performed for members in the earlier part of the cohort, and although attempts were made to retest individuals with larger multi-gene panels, many whom we tried to contact were deceased.

CONCLUSIONS

In individuals meeting IGCLC criteria, we found detection rates for *CDH1* PVs that were lower than previously reported, and no *CDH1* PVs in individuals with DGC<40 as the sole eligibility criterion. We added to the literature observing LBC in the absence of DGC within *CDH1*-positive families despite obtaining 3-generation pedigrees, supporting the need to better integrate that phenotype into the *CDH1* diagnostic criteria. We also reported on pathology-confirmed malignancies in *CDH1* carriers not previously associated with HDGC patients. In *CDH1* carriers, DGC was observed at a slightly older age of onset, prompting our suggestion that age as the sole criterion in DGC be raised to 50 years from 40 years. Multi-gene panel testing is the recommended testing method, because several syndromes—such as Peutz–Jeghers syndrome—have been linked to both DGC and LBC and could have overlapping phenotypes. Despite comprehensive testing, most patients with high-risk DGC have no identifiable hereditary cause for their cancer. More research is needed to elucidate novel genes and risk factors for DGC.

AUTHOR AFFILIATIONS
*Sinai Health System, Zane Cohen Centre for Digestive Diseases, and †Sinai Health System, Department of Surgery, University of Toronto, Toronto, ON; ‡BC Cancer, Vancouver, BC; §North York General Hospital, Toronto, ‖Grand River Hospital, Kitchener, #Trillium Health Partners, Mississauga, **Porcupine Health Unit, Timmins, ††Lakeridge Health, Oshawa, and ‡‡Sinai Health System, Department of Medicine, Division of Medical Oncology, University of Toronto, Toronto, ON.

REFERENCES
1. Canadian Cancer Society's Advisory Committee on Cancer Statistics. *Canadian Cancer Statistics 2017.* Toronto, ON: Canadian Cancer Society; 2017.
2. Setia N, Clark JW, Duda DG, *et al.* Familial gastric cancers. *Oncologist* 2015;20:1365–77.
3. Hansford S, Kaurah P, Li-Chang H, *et al.* Hereditary diffuse gastric cancer syndrome: *CDH1* mutations and beyond. *JAMA Oncol* 2015;1:23–32.
4. Roberts ME, Ranola JMO, Marshall ML, *et al.* Comparison of *CDH1* penetrance estimates in clinically ascertained families vs families ascertained for multiple gastric cancers. *JAMA Oncol* 2019;:[Epub ahead of print].
5. Brooks-Wilson AR, Kaurah P, Suriano G, *et al.* Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. *J Med Genet* 2004;41:508–17.
6. Benusiglio PR, Caron O, Consolino E, *et al.* Cleft lip, cleft palate, hereditary diffuse gastric cancer and germline mutations in *CDH1. Int J Cancer* 2013;132:2470.
7. Obermair F, Rammer M, Burghofer J, *et al.* Cleft lip/palate and hereditary diffuse gastric cancer: report of a family harboring a *CDH1* c.687+1G>A germline mutation and review of the literature. *Fam Cancer* 2019;18:253–60.
8. Benusiglio PR, Colas C, Rouleau E, *et al.* Hereditary diffuse gastric cancer syndrome: improved performances of the 2015 testing criteria for the identification of probands with a *CDH1* germline mutation. *J Med Genet* 2015;52:563–5.
9. van der Post RS, Vogelaar IP, Carneiro F, *et al.* Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline *CDH1* mutation carriers. *J Med Genet* 2015;52:361–74.
10. van der Post RS, Vogelaar IP, Manders P, *et al.* Accuracy of hereditary diffuse gastric cancer testing criteria and outcomes in patients with a germline mutation in *CDH1. Gastroenterology* 2015;149:897–906.e19.
11. Figueiredo J, Melo S, Carneiro P, *et al.* Clinical spectrum and pleiotropic nature of *CDH1* germline mutations. *J Med Genet* 2019;56:199–208.

12. van der Post RS, Carneiro F. Emerging concepts in gastric neoplasia: heritable gastric cancers and polyposis disorders. *Surg Pathol Clin* 2017;10:931–45.

13. Madlensky L, Berk TC, Bapat BV, *et al.* A preventive registry for hereditary nonpolyposis colorectal cancer. *Can J Oncol* 1995;5:355–60.

14. Gayther SA, Gorringe KL, Ramus SJ, *et al.* Identification of germ-line E-cadherin mutations in gastric cancer families of European origin. *Cancer Res* 1998;58:4086–9.

15. Viel A, Genuardi M, Lucci-Cordisco E, *et al.* Hereditary non-polyposis colorectal cancer: an approach to the selection of candidates to genetic testing based on clinical and molecular characteristics. *Community Genet* 1998;1:229–36.

16. Mongiat-Artus P, Miquel C, Flejou JF, *et al.* Spectrum of molecular alterations in colorectal, upper urinary tract, endocervical, and renal carcinomas arising in a patient with hereditary non-polyposis colorectal cancer. *Virchows Arch* 2006;449:238–43.

17. Goldberg Y, Porat RM, Kedar I, *et al.* An Ashkenazi founder mutation in the *MSH6* gene leading to hnpcc. *Fam Cancer* 2010;9:141–50.

18. Yamada H, Shinmura K, Ito H, *et al.* Germline alterations in the *CDH1* gene in familial gastric cancer in the Japanese population. *Cancer Sci* 2011;102:1782–8.

19. Resta N, Pierannunzio D, Lenato GM, *et al.* Cancer risk associated with *STK11/LKB1* germline mutations in Peutz–Jeghers syndrome patients: results of an Italian multicenter study. *Dig Liver Dis* 2013;45:606–11.

20. Suriano G, Oliveira C, Ferreira P, *et al.* Identification of *CDH1* germline missense mutations associated with functional inactivation of the E-cadherin protein in young gastric cancer probands. *Hum Mol Genet* 2003;12:575–82.

21. Lowstuter K, Espenschied CR, Sturgeon D, *et al.* Unexpected *CDH1* mutations identified on multigene panels pose clinical management challenges. *JCO Precis Oncol* 2017;1:1–12.

22. Dominguez-Valentin M, Evans DGR, Nakken S, *et al.* Genetic variants of prospectively demonstrated phenocopies in *BRCA1/2* kindreds. *Hered Cancer Clin Pract* 2018;16:4.

23. Estiar MA, Mehdipour P. *ATM* in breast and brain tumors: a comprehensive review. *Cancer Biol Med.* 2018;15:210–27.

24. Kwon BS, Byun JM, Lee HJ, *et al.* Clinical and genetic characteristics of *BRCA1/2* mutation in Korean ovarian cancer patients: a multicenter study and literature review. *Cancer Res Treat* 2019;51:941–50.

25. Caldas C, Carneiro F, Lynch HT, *et al.* Familial gastric cancer: overview and guidelines for management. *J Med Genet* 1999;36:873–80.

26. Oliveira C, Bordin MC, Grehan N, *et al.* Screening E-cadherin in gastric cancer families reveals germline mutations only in hereditary diffuse gastric cancer kindred. *Hum Mutat* 2002;19:510–17.

27. Oliveira C, Seruca R, Carneiro F. Genetics, pathology, and clinics of familial gastric cancer. *Int J Surg Pathol* 2006;14:21–33.

28. Guilford P, Hopkins J, Harraway J, *et al.* E-Cadherin germline mutations in familial gastric cancer. *Nature* 1998;392:402–5.

29. Kaurah P, MacMillan A, Boyd N, *et al.* Founder and recurrent *CDH1* mutations in families with hereditary diffuse gastric cancer. *JAMA* 2007;297:2360–72.

30. Fitzgerald RC, Hardwick R, Huntsman D, *et al.* on behalf of the International Gastric Cancer Linkage Consortium. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. *J Med Genet* 2010;47:436–44.

31. Hakkaart C, Ellison-Loschmann L, Day R, *et al.* Germline *CDH1* mutations are a significant contributor to the high frequency of early-onset diffuse gastric cancer cases in New Zealand Maori. *Fam Cancer* 2019;18:83–90.

32. Kim S, Chung JW, Jeong TD, *et al.* Searching for E-cadherin gene mutations in early onset diffuse gastric cancer and hereditary diffuse gastric cancer in Korean patients. *Fam Cancer* 2013;12:503–7.

33. Pharoah PD, Guilford P, Caldas C on behalf of the International Gastric Cancer Linkage Consortium. Incidence of gastric cancer and breast cancer in *CDH1* (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. *Gastroenterology* 2001;121:1348–53.

34. Giryes A, Oweira H, Mannhart M, Decker M, Abdel-Rahman O. Exploring the differences between early-onset gastric cancer and traditional-onset gastric cancer. *J Gastrointest Oncol* 2018;9:1157–63.

35. Solcia E, Fiocca R, Luinetti O, *et al.* Intestinal and diffuse gastric cancers arise in a different background of *Helicobacter pylori* gastritis through different gene involvement. *Am J Surg Pathol* 1996;20(suppl 1):S8–22.

36. Rugge M, Busatto G, Cassaro M, *et al.* Patients younger than 40 years with gastric carcinoma: *Helicobacter pylori* genotype and associated gastritis phenotype. *Cancer* 1999;85:2506–11.

37. Koshida Y, Koizumi W, Sasabe M, Katoh Y, Okayasu I. Association of *Helicobacter pylori*–dependent gastritis with gastric carcinomas in young Japanese patients: histopathological comparison of diffuse and intestinal type cancer cases. *Histopathology* 2000;37:124–30.

38. Jindal Y, Singh A, Kumar R, *et al.* Expression of alpha methylacyl CoA racemase (amacr) in gastric adenocarcinoma and its correlation with *Helicobacter pylori* infection. *J Clin Diagn Res* 2016;10:EC10–12.

39. Ansari S, Gantuya B, Tuan VP, Yamaoka Y. Diffuse gastric cancer: a summary of analogous contributing factors for its molecular pathogenicity. *Int J Mol Sci* 2018;19:pii:E2424.

40. Puculek M, Machlowska J, Wierzbicki R, Baj J, Maciejewski R, Sitarz R. *Helicobacter pylori* associated factors in the development of gastric cancer with special reference to the early-onset subtype. *Oncotarget* 2018;9:31146–62.

41. Kwak HW, Choi IJ, Cho SJ, *et al.* Characteristics of gastric cancer according to *Helicobacter pylori* infection status. *J Gastroenterol Hepatol* 2014;29:1671–7.

42. Naja F, Kreiger N, Sullivan T. *Helicobacter pylori* infection in Ontario: prevalence and risk factors. *Can J Gastroenterol* 2007;21:501–6.

43. Xie ZM, Li LS, Laquet C, *et al.* Germline mutations of the E-cadherin gene in families with inherited invasive lobular breast carcinoma but no diffuse gastric cancer. *Cancer* 2011;117:3112–17.

44. Petridis C, Shinomiya I, Kohut K, *et al.* Germline *CDH1* mutations in bilateral lobular carcinoma *in situ*. *Br J Cancer* 2014;110:1053–7.

45. Corso G, De Scalzi A, Feroce I, Veronesi P, Bonanni B, Galimberti V. Clinical criteria revision for hereditary lobular breast cancer associated with E-cadherin germline mutations. *Per Med* 2018;15:153–5.

46. Huynh JM, Laukaitis CM. Panel testing reveals nonsense and missense *CDH1* mutations in families without hereditary diffuse gastric cancer. *Mol Genet Genomic Med* 2016;4:232–6.

47. Xicola RM, Li S, Rodriguez N, *et al.* Clinical features and cancer risk in families with pathogenic *CDH1* variants irrespective of clinical criteria. *J Med Genet* 2019;56:838–43.

48. Jacobs MF, Dust H, Koeppe E, *et al.* Outcomes of endoscopic surveillance in individuals with genetic predisposition to hereditary diffuse gastric cancer. *Gastroenterology* 2019;157:87–96.