Original Investigation

Hereditary Diffuse Gastric Cancer Syndrome
CDH1 Mutations and Beyond

Samantha Hansford, MSc; Pardeep Kaurah, MSc; Hector Li-Chang, MD; Michelle Woo, PhD; Janine Senz, BSc; Hugo Pinheiro, PhD; Kasmintan A. Schrader, MBBS, PhD; David F. Schaeffer, MD; Karey Shumansky, MSc; George Zogopoulos, MD; Teresa Almeida Santos, MD, PhD; Isabel Claro, MD; Joana Carvalho, PhD; Cydney Nielsen, PhD; Sarah Padilla, BSc; Amy Lum, BSc; Aline Talhouk, PhD; Katie Baker-Lange, MSc; Sue Richardson, RGN; Ivy Lewis, BN, RN; Noralane M. Lindor, MD; Erin Pennell, RN; Andree MacMillan, MSc; Bridget Fernandez, MD; Gisella Keller, PhD; Henry Lynch, MD; Sohrab P. Shah, PhD; Parry Guilford, MSc, PhD; Steven Gallinger, MD; Giovanni Corso, MD, PhD; Franco Roviello, MD; Carlos Caldas, MD; Carla Oliveira, PhD; Paul D. P. Pharoah, PhD; David G. Huntsman, MD

IMPORTANCE E-cadherin (*CDH1*) is a cancer predisposition gene mutated in families meeting clinically defined hereditary diffuse gastric cancer (HDGC). Reliable estimates of cancer risk and spectrum in germline mutation carriers are essential for management. For families without *CDH1* mutations, genetic-based risk stratification has not been possible, resulting in limited clinical options.

OBJECTIVES To derive accurate estimates of gastric and breast cancer risks in *CDH1* mutation carriers and determine if germline mutations in other genes are associated with HDGC.

DESIGN, SETTING, AND PARTICIPANTS Testing for *CDH1* germline mutations was performed on 183 index cases meeting clinical criteria for HDGC. Penetrance was derived from 75 mutation-positive families from within this and other cohorts, comprising 3858 probands (353 with gastric cancer and 89 with breast cancer). Germline DNA from 144 HDGC probands lacking *CDH1* mutations was screened using multiplexed targeted sequencing for 55 cancer-associated genes.

MAIN OUTCOMES AND MEASURES Accurate estimates of gastric and breast cancer risks in *CDH1* mutation carriers and the relative contribution of other cancer predisposition genes in familial gastric cancers.

RESULTS Thirty-one distinct pathogenic *CDH1* mutations (14 novel) were identified in 34 of 183 index cases (19%). By the age of 80 years, the cumulative incidence of gastric cancer was 70% (95% CI, 59%-80%) for males and 56% (95% CI, 44%-69%) for females, and the risk of breast cancer for females was 42% (95% CI, 23%-68%). In *CDH1* mutation–negative index cases, candidate mutations were identified in 16 of 144 probands (11%), including mutations within genes of high and moderate penetrance: *CTNNA1*, *BRCA2*, *STK11*, *SDHB*, *PRSS1*, *ATM*, *MSR1*, and *PALB2*.

CONCLUSIONS AND RELEVANCE This is the largest reported series of *CDH1* mutation carriers, providing more precise estimates of age-associated risks of gastric and breast cancer that will improve counseling of unaffected carriers. In HDGC families lacking *CDH1* mutations, testing of *CTNNA1* and other tumor suppressor genes should be considered. Clinically defined HDGC families can harbor mutations in genes (ie, *BRCA2)* with different clinical ramifications from *CDH1*. Therefore, we propose that HDGC syndrome may be best defined by mutations in *CDH1* and closely related genes, rather than through clinical criteria that capture families with heterogeneous susceptibility profiles.

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Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: David G. Huntsman, MD, Centre for Translational and Applied Genomics, Department of Pathology and Laboratory Medicine, British Columbia Cancer Agency and University of British Columbia, Vancouver, BC V5Z 4E6, Canada (dhuntsma@bccancer.bc.ca).

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Gastric cancer (GC) is the third most common cause of cancer-related mortality worldwide.[1] The 2 major subtypes, diffuse GC (DGC) and intestinal-type GC, are distinguished by molecular, epidemiologic, and morphologic features.[2] Although GC is usually sporadic, familial aggregation occurs in approximately 10% of cases.[3,4] Clinically defined hereditary DGC (HDGC) (OMIM #137215) is characterized by early-onset, multigenerational DGC and lobular breast cancer. Clinical criteria for this entity was established by the International Gastric Cancer Linkage Consortium (IGCLC) (**Table 1**).[5,6] Approximately 40% of HDGC families have germline mutations in *CDH1* (E-cadherin) (Ensembl ENSG00000039068; OMIM *192090), and over 100 different pathogenic germline mutations are reported across multiple ethnicities (eTable 1 in the Supplement).

Current cumulative lifetime GC risk in *CDH1* mutation carriers are derived from a small number of families, 11 in one study and 4 families sharing a founder mutation in another, with predicted risks ranging from 40% to 67% and 63% to 83% in male and female carriers, respectively.[7,8] Female carriers also have risk of breast cancer (BC) between 39% and 52%,[7,8] with lobular BC being most characteristic. The first objective of this study is to derive reliable estimates of cancer risk for *CDH1* mutation carriers based on a collated analysis of cohorts, including previously published families,[7-12] mutation-positive families from this study, and previously unpublished families. The findings from this study will provide a reliable assessment of risk and represents the largest series of *CDH1* mutation carriers studied to date.

Our second objective was to catalogue a comprehensive list of all reported germline mutations in the literature to date. Lastly, we aimed to determine whether other cancer susceptibility genes contribute to HDGC in families meeting IGCLC criteria but lacking *CDH1* mutations. We used a multiplexed, next-generation sequencing approach to simultaneously interrogate a selected panel of genes implicated in upper gastrointestinal tract cancer or susceptibility syndromes across 144 *CDH1*-negative HDGC families (eFigure 1 in the Supplement).

At a Glance

- The cumulative risk for gastric cancer in *CDH1* mutation carriers is 70% by 80 years for men and 56% for women.
- Cumulative risk for breast cancer by 80 years is 42% for women with *CDH1* mutations.
- Families meeting clinical criteria for HDGC, but lacking *CDH1* mutations, may harbor mutations in genes associated with other cancer predisposition syndromes.
- Two families harbored pathogenic, truncating mutations in the *CTNNA1* (α-catenin) gene inferring a genocopy of *CDH1*.
- HDGC syndrome may be defined not only by mutations in *CDH1* but also by mutations in closely related genes.

Methods

Study Population

Institutional review board (IRB) approval was obtained for *CDH1* analysis at all study sites. All patients or next of kin from deceased individuals provided written informed consent. Pedigrees and available medical records were collected by genetic specialists at the referring centers and centrally reviewed (P.K. and D.G.H.). The samples from Portugal and Italy were collected under local IRB guidelines and sent to us without identifiers.

The present analysis is part of an ongoing study on risk assessment in HDGC families. A total of 183 new index cases meeting IGCLC 2010 clinical criteria for HDGC[5] were recruited between February 2006 and June 2013 to undergo *CDH1* testing. Also, 144 *CDH1* mutation-negative HDGC families, collected from different centers (British Columbia Cancer Agency, Vancouver, British Columbia, Canada; IPATIMUP-Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; and University of Siena, Siena, Italy) underwent multigene panel screening.

CDH1 Genetic Testing

Sequencing of the *CDH1* exons was performed on genomic DNA extracted from peripheral blood, saliva, or paraffin-embedded sections as previously described (eMethods 1 in the Supplement).[8,12] Samples with no significant mutations (point or small insertions or deletions) were tested for copy number variations using multiplex ligation-dependent probe amplification.

Catalogue of All Known *CDH1* Germline Mutations

To obtain a comprehensive list of reported germline truncating or missense *CDH1* mutations, a MEDLINE search for articles from 1998 to 2013 was conducted using the following search terms: *CDH1*, *E-cadherin*, *germline mutation*, *gastric cancer*, *hereditary*, *familial* and *diffuse gastric cancer*. The mutations were catalogued according to exon, location, and type (missense-pathogenic, missense-unclassified, nonsense, insertion, deletion, or splicing). Somatic mutations were excluded, as were mutations reported as silent.

Penetrance Analysis

Pedigree information was used to estimate the penetrance of *CDH1* mutations using the MENDEL program.[13] Families with *CDH1* missense mutations with unknown pathogenicity and families where no carrier test information was available were excluded from the analysis (eTable 1 in the Supplement). Penetrance analysis was thus performed on 17 of 34 eligible families identified with pathogenic mutations in this study, as well as on 58 additional families, of which some were previously reported[7-14] (eTable 2 in the Supplement). The model was parameterized in terms of log relative risk for GC and BC in mutation carriers compared with population risk, irrespective of ethnic origin (eMethods 2 in the Supplement). The number of families from different countries was too small to enable country-specific penetrance estimates.

Noncarriers of the deleterious mutation in each family were assumed to have probability of developing this disease as reported in the United Kingdom, thus assuming that the cancer incidence is the same for all families regardless of ethnicity. The relative risk of GC was estimated separately for male and female participants and allowed to vary with age using 6 age

Table 1. Summary of *CDH1* Germline Mutation Type Identified in Index Cases From the Study Population

Criteria[a]	Definition[a]	No. of Index Cases in Study Population	Mutations, No. (% of Total)			Total No. of Pathogenic Mutations (% of Total for Each Criteria)
			Truncating	Splice Site	Pathogenic Missense[b]	
1	Family with 2 or more cases of GC, with at least 1 DGC diagnosed before the age of 50 y	84	12 (14)	9 (11)	1 (1)	22 (26)
2	Three confirmed DGC cases in first- or second-degree relatives independent of age	1	0	0	0	0
3	Isolated individual diagnosed with DGC at age <40 y from a low incidence population	38	0	2 (5)	0	2 (5)
4	Personal or family history of both DGC and LBC, with 1 affected person aged <50 y at time of diagnosis	60	6 (10)	4 (7)	0	10 (17)
Total		**183**	**18** (10)	**15** (8)	**1** (1)	**34** (19)

Abbreviations: *CDH1*, E-cadherin; DGC, diffuse gastric cancer; GC, gastric cancer; LBC, lobular breast cancer.

[a] Criteria and definition based on the 2010 International Gastric Cancer Linkage Consortium guidelines (Fitzgerald et al[5]).

[b] Unclassified variants have been removed from this table.

groups between 10 and 79 years. The relative risk of BC for female participants was modeled to be constant with age. The *CDH1* mutant allele was assumed to be rare in the general population with a frequency of 0.001.

Gene Panel Testing

Multiplexed panel sequencing across 55 selected genes was performed on germline DNA from 144 HDGC probands without *CDH1* mutations. Genes were selected based on implication in upper gastrointestinal tract cancers or syndromes identified through literature review and unpublished data from concurrent projects (eTable 3 in the Supplement). When necessary, samples from additional family members and/or tissue sections were requested for downstream analysis. Multiplexed sequencing analysis (eMethods 3 in the Supplement) was performed using Illumina's TruSeq Custom Amplicon assay on the MiSeq platform (Illumina). Eight germline DNA samples with known *CDH1* mutations were included as controls to confirm reliability of the assay and analysis software. Individual sample data were sorted for candidate mutations (novel frameshift, nonsense or splice-site mutations, and rare (<1% population) missense variants with previously reported functional relevance), and validated by Sanger sequencing (eMethods 4 in the Supplement). Secondary analysis by a bioinformatician was also performed on 25 samples to predict reliability of on-instrument data analysis (eMethods 5 in the Supplement). Such mutations were considered pathogenic if occurring in a highly penetrant gene directly implicated in GC or a GC predisposition syndrome. Truncating variants in genes with previously known low to moderate penetrance in GC-related syndromes were called "likely pathogenic." Novel missense mutations predicted to be damaging from at least 2 *in silico* methods (SIFT, PROVEAN, and PolyPhen) were called "variants of unknown significance" (VUS), since pathogenicity of such mutations is not well supported (eTable 4 in the Supplement).

Results

Germline *CDH1* Mutations

Overall, 34 of 183 index cases (19%) who met current IGCLC criteria[5] were found to have germline pathogenic *CDH1*-mutations, and 4 of 183 index cases displayed *CDH1* VUS (Table 1). Thirty-one distinct mutations (14 novel and 17 previously reported) were found (Table 1 and eTable 5 in the Supplement). A higher frequency was observed in 22 of 84 index cases (26%) with 2 or more cases of GC with at least 1 DGC diagnosed before the age of 50 years. Previously reported mutations were seen in 10 of 34 index cases (29%) and included positions c.1137G, c.1792C, c.1565, and a large deletion encompassing exons 1 and 2. All mutations were heterozygous.

Catalogue of *CDH1* Germline Mutations to Date

The majority of studies reviewed were original reports. Two articles with tabulated reported mutations were used as cross-references.[14,15] A total of 155 *CDH1* mutations, of which 126 are pathogenic and 29 are unclassified variants, have been described to date (eTable 1 in the Supplement). Plots of the mutations according to exon location (**Figure 1**A) and known break points for deletions (Figure 1B) demonstrate that germline mutations in HDGC families are spread across the gene. Bona fide germline *CDH1* mutations have recently been reported in high-incidence GC populations (Chinese and Japanese ethnicities), whereas this phenomenon had been rare previously (eTable 1 in the Supplement). This could reflect more prevalent *CDH1* testing in these populations.

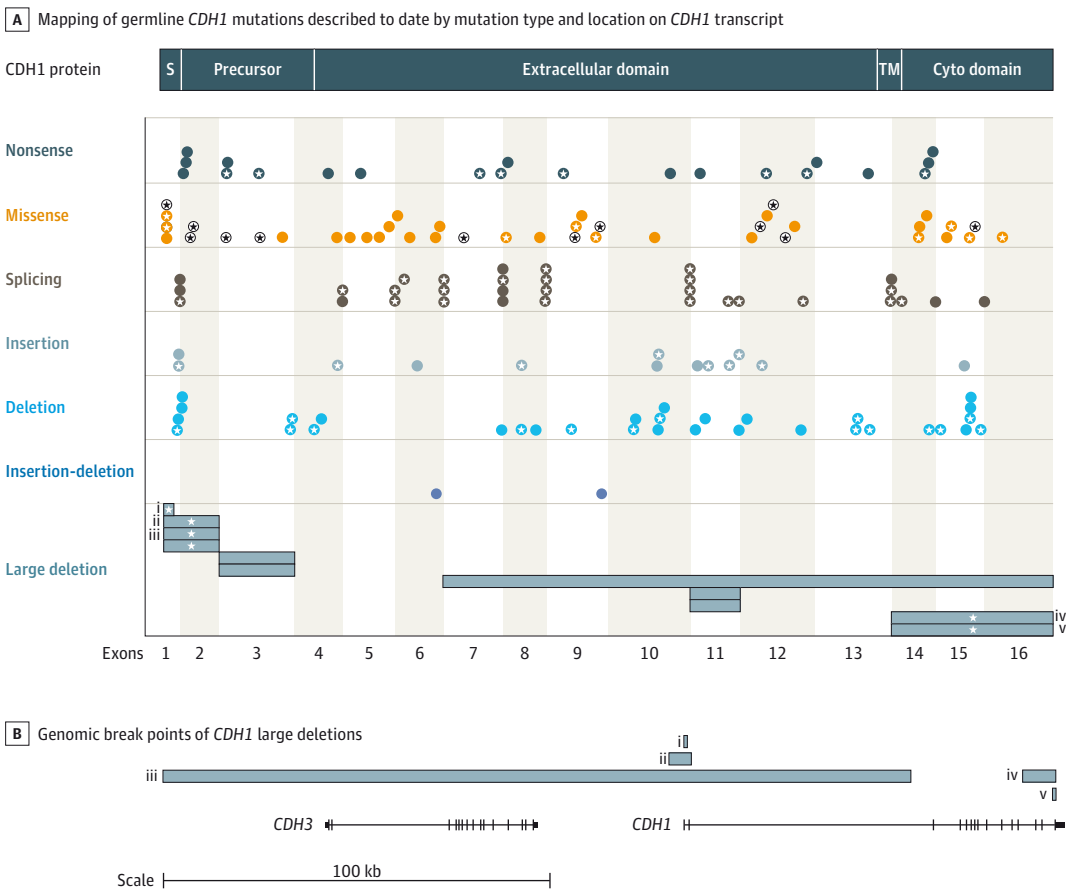
Penetrance Analysis

Cumulative risks of GC and BC are shown in **Figure 2** and given in eTable 6 in the Supplement. The cumulative incidence of GC by 80 years was 70% (95% CI, 59%-80%) for male participants and 56% (95% CI, 44%-69%) for female participants. The risk of BC for female participants was 42% (95% CI, 23%-68%) by 80 years.

Panel-Based Screening

Of the 144 probands, we identified potentially pathogenic variants in 16 cases (11.1%) (**Table 2** and eFigure 2 in the Supplement). Novel truncating mutations in *CTNNA1* (α-catenin) (N71fs and R129X) were found in 2 unrelated HDGC families. The germline *CTNNA1* mutation in family P25 was validated in the affected mother (**Figure 3**A). Immunohistochemical staining of tumors from both *CTNNA1* mutation-positive families

Figure 1. Mapping of Germline *CDH1* Mutations and Genomic Break Points



The panels provide a mapping between the large deletions in the transcript/protein view (A) and in the genome view (B). Lower case roman numerals i to v signify the large deletions with known genomic coordinates; and stars inside circles and bars, the mutations that our laboratory found in the present study. Cyto indicates cytoplasmic; S, signal peptide; and TM, transmembrane domain.

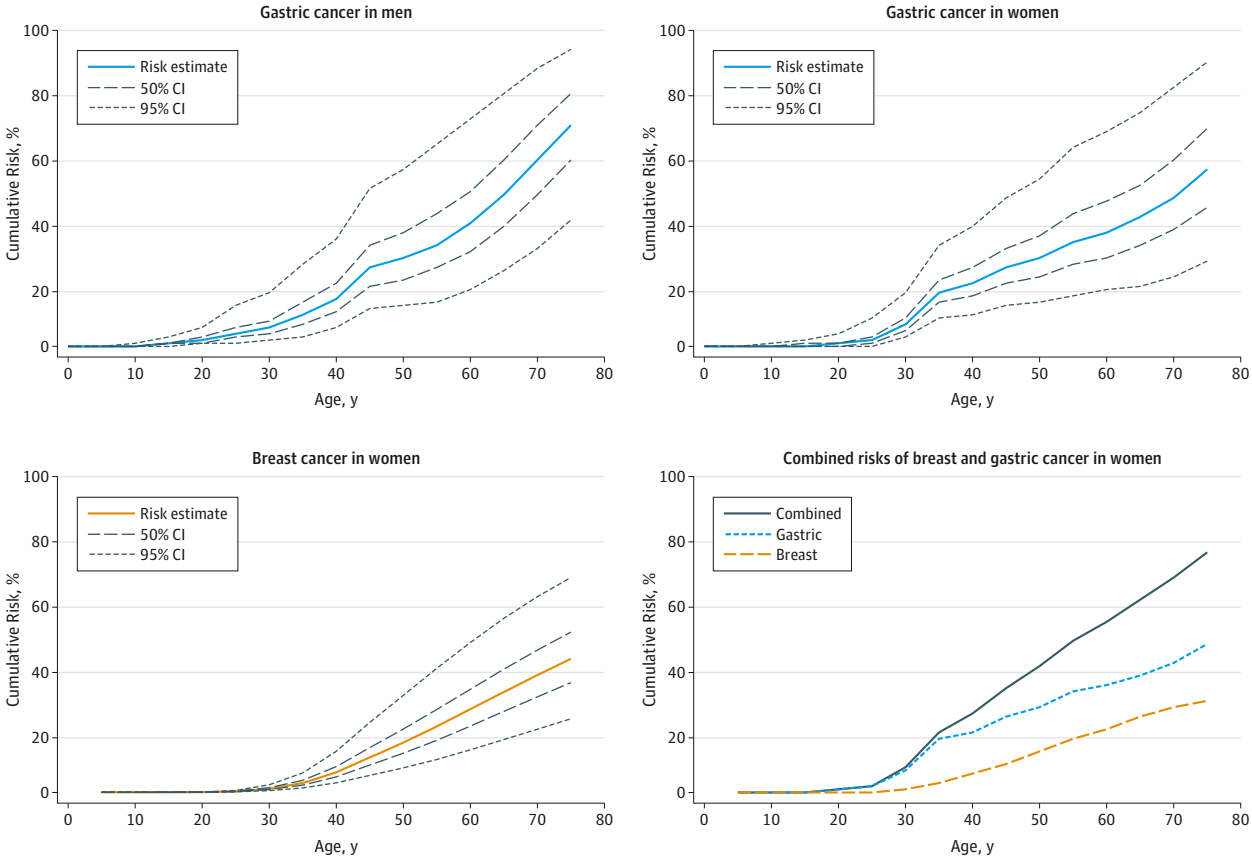
showed loss of α-catenin expression, suggesting the occurrence of a second hit event at the *CTNNA1* locus (eMethods 6 and eFigure 3 [panels E and F] in the Supplement), while E-cadherin expression was preserved (eFigure 3 [panels C and D] in the Supplement). In addition, a novel *BRCA2* truncating variant was identified in 1 proband (N1287fs) (Figure 3B and eFigure 4 in the Supplement), and a truncating mutation in an upper gastrointestinal tract-related gene *PRSS1* was found in another case (Q86X) (eFigure 4 in the Supplement). Heterozygous protein-truncating variants within genes of predicted low to moderate penetrance in upper gastrointestinal tract cancers were identified in *ATM* (E1267fs, Y2791fs, and R521fs) and *PALB2* (V398fs) (Table 2 and eFigure 4 in the Supplement). We also identified rare, pathogenic missense variants in *SDHB* (S163P) (n = 1) (Figure 3C), *STK11* (F354L) (n = 3) (Figure 3D), and *MSR1* (R293X) (n = 4), previously associated with Peutz-Jeghers syndrome, Cowden-like syndrome, and esophageal cancer, respectively (Table 2, Figure 3, and eFigure 4 in the Supplement).[16-19] The *MSR1* variant R293X is also associated with prostate cancer risk.[20] Limitations of sample availability prevented extensive germline screening of candidate variants across relatives and somatic mutation analysis of tumor DNA.

Discussion

Heterozygous germline *CDH1* mutations have been described in up to 40% of HDGC families.[8,11,21] We found only 34 mutations in 183 HDGC index cases (19%): 26% of mutation-positive index cases fulfilled IGCLC criteria 1 (2 cases of GC and at least 1 DGC occurring in an individual younger than 50 years), followed by index cases with both DGC and lobular BC (criteria 4) (Table 1). Compared with several previously published reports,[8,10,11,14,22] our results reveal less than half the expected numbers of HDGC families investigated have a germline *CDH1* germline mutation. This number could be a reflection of the varied ethnicities within our consecutive series; it is well known that the frequency is highly variable between countries with different incidences of GC, and prior ascertainment of kindred with the strongest family histories may have skewed past reports. This reduced number may be useful in managing patient expectations during the counseling process.

The 31 pathogenic, germline *CDH1* mutations and 4 VUS described herein and other previously reported mutations are distributed throughout the *CDH1* gene including splice-site se-

Figure 2. Cumulative Risk of Gastric and Breast Cancer for *CDH1* Mutation Carriers by Sex



quences (Figure 1). Approximately 27% (41 of 155) of total reported *CDH1* pathogenic mutations have been reported in multiple families (eTable 1 in the Supplement), suggesting that germline mutations can either arise from a common ancestor[8,12] or be the result of novel events at mutational hot spots.

The clinical utility of *CDH1* mutation identification in HDGC families lies in determining whether unaffected relatives are at risk for DGC and BC. At present, the only recommended GC risk reduction strategies are gastroscopy, with multiple random biopsies, or prophylactic total gastrectomy.[5] This represents a difficult choice between the 2 procedures because gastroscopy screening has repeatedly been shown to miss early DGC[9,11,23,24] and gastrectomy carries certain morbidity.[25-28] With no validated biomarkers available to assist in the timing of prophylactic total gastrectomy, accurate risk assessment is essential. A previous study of 11 families calculated that GC risks were 67% in men and 83% in women, with an additional risk of BC in women mutation carriers of 39% at the age of 80 years.[7] We have also previously demonstrated a GC risk of 40% for males and 63% for females, and BC risk of 52% in 4 families with the 2398delC mutation.[8] Owing to small sample sizes in these 2 studies, risk figures had to be interpreted with caution, highlighting the need for a more comprehensive analysis.

Our new penetrance estimates are more precise because of the larger cohort used. The families used to compile these data are from multicultural backgrounds, primarily from regions where GC is present at low incidences. Gastric cancer penetrance among *CDH1* mutation carriers is incomplete, and it is likely that modifiers of risk exist, such as environmental or other inherited genetic factors. These unknown modifiers are likely to cluster in multicase families. While clinically appropriate, the penetrance estimates presented herein may be higher than the average penetrance in truly unselected carriers.

Multiplexed panel-based sequencing enables in-depth sequencing of targeted regions of interest simultaneously across multiple samples. Applying this assay to unexplained HDGC, we identified novel and previously known potentially pathogenic germline mutations in genes associated with GC or GC risk syndromes.

Among the germline abnormalities uncovered in this cohort, *CTNNA1* mutations are most likely to mirror the genetic and functional significance of *CDH1* mutations. Like *CDH1*, *CTNNA1* is involved in intercellular adhesion and is a suspected tumor suppressor and susceptibility gene for DGC.[29] We identified 2 *CDH1*-negative HDGC families with novel *CTNNA1* germline truncating mutations (Figure 3 and Table 2). Loss of α-catenin expression with preservation of E-cadherin in tumor material from 2 probands was observed (eFigure 3 in the Supplement). This variant was also confirmed in an affected parent (family P25). Our data support that germline *CTNNA1* alterations cause HDGC on occasion and should be considered in screening of prospective families.[29] This also suggests that the

Table 2. Candidate Germline Variants From Multiplexed, Panel Sequencing of HDGC Families

ID (Race/ Ethnicity)	IGCLC Criteria[5]	Age at Diagnosis (Years)			Additional Family Hx	Gene (Chr)/ Position	AA Change[b]	Depth[f]	Mutation Type/ Pathogenicity
		Proband	Relatives With GC or BC	Family Hx of Other Cancers					
P16 (White)	2	DGC (58)	GC (72), GC MDA (51), GC (52), DGC (NA)	Uterine (NA), cervical (61), lung (71), bladder (69), EA (NA), thyroid (51), prostate (NA), PC (62), bone (9), CRC (61)	NR	*ATM* (11) c.3800AG>A	E1267fs	244x	Frameshift/ possibly pathogenic
P42 (Unknown)	4	BC (59)	IGC (71), GC (82), GC (53), GC (38), GC (42), GC (59), GC (NA), GC (73)	Ovarian (49), ovarian (74), head and neck (78), CRC (42), CRC (39), leukemia (NA), leukemia (55), CRC with GC metastases (57), PC (70)	NR	*ATM* (11) c.8369GATAC>G	Y2791fs	258x	Frameshift/ possibly pathogenic
P58 (English)	4	LBC (56)	GC (40s), GC (NA), BC and brain (70), bilateral BC (40s), BC (55), BC (69), LBC (49), BC (NA), BC (NA)	CRC (50s)	Blood clots, stroke	*ATM* (11) c.1560CAG>C	R521fs	2866x	Frameshift/ possibly pathogenic
P2 (East Indian)	1	DGC (64)	DGC (21), BC (50)	NR	NR	*BRCA2* (13) c.3862TAATA>T	N1287fs	205x	Frameshift/ pathogenic
P25 (White)	3	DGC (22)	GC (59), BC (70)	Brain (70), GEJ (82)	NR	*CTNNA1* (5) c.211A>AT	N71fs	245x	Frameshift/ pathogenic
P80 (Italian)	[a]	DGC (72)	DGC (52)	NR	NR	*CTNNA1* (5) c.385C>T	R129X	442x	Nonsense/ pathogenic
P90 (Portuguese)	3	DGC (22)	NR	NR	NR	*MSR1* (8) c.877C>T	R293X[c]	93x	Nonsense/ possibly pathogenic
P107 (White)	4	DGC (36) and uterine (25)	GC (69), GC (68), GC (64), BC (42), BC (54)	Uterine (51), lung (NA) ×2, pancreatic (85), bladder/ prostate (86), bladder (82), prostate (80), bladder (69), cervical (22), CRC	Gilbert syndrome	*MSR1* (8) c.877C>T	R293X[c]	488x	Nonsense/ possibly pathogenic
P110 (Unknown)	4	DGC (51)	BC (NA), GC (NA)	CRC (45), myeloma (NA)	NR	*MSR1* (8) c.877C>T	R293X[c]	432x	Nonsense/ possibly pathogenic
P61 (Unknown)	1	NA	GC (50s), GC and BC (78), GC (50), GC and liver (77), GC (87), GC (62), GC (47), BC (42)	Prostate (74), skin (NA), prostate (82)	NR	*MSR1* (8) c.877C>T	R293X[c]	94x	Nonsense/ possibly pathogenic
P124 (Portuguese)	4	DGC (45)	GC (38), BC (42), BC (45)	CRC (59), prostate (75)	NR	*PALB2* (16) c.1193AC>A	V398fs	1786x	Frameshift/ likely pathogenic
P123 (English)	1	DGC (42)	GC (45), GC (NA)	Lung (52)	Cirrhosis, emphysema	*PRSS1* (7) c.256C>T	Q86X	49x	Nonsense/ possibly pathogenic
P13 (Italian)	4	LBC (39)	GC (53), GC (44), BC and uterine (34), BC (NA)	Brain (NA)	DD	*SDHB* (1) c.487T>C	S163P[d]	248x	Missense/ likely pathogenic
P117 (Unknown)	1	DGC (45)	GC (45)	NR	NR	*STK11* (19) c.1062C>G	F354L[e]	616x	Missense/ likely pathogenic
P44 (White)	3	DGC (37)	GC (70), LBC (45), BC (33), BC (56)	GEJ (NA), CRC (70), prostate (NA)	NR	*STK11* (19) c.1062C>G	F354L[e]	681x	Missense/ likely pathogenic
P46 (Unknown)	3	DGC (22)	NR	Lung (NA)	NR	*STK11* (19) c.1062C>G	F354L[e]	281x	Missense/ likely pathogenic

Abbreviations: AA, amino acid; AOD, age of diagnosis; BC, breast cancer; Chr, chromosome; CRC, colorectal carcinoma; DD, developmental delay; DGC, diffuse gastric cancer; EA, endometrium adenocarcinoma; fs, frameshift; GC, gastric cancer; GEJ, gastroesophageal junction cancer; HDGC, hereditary diffuse gastric cancer; Hx, history; IGC, intestinal gastric cancer; IGCLC, International Gastric Cancer Linkage Consortium; MDA, moderately differentiated adenocarcinoma; NA, not available; NR, not reported; PC, pancreatic cancer.

[a] Family meets original criteria for HDGC from Caldas et al, 1999,[6] and IGCLC criteria.[5]

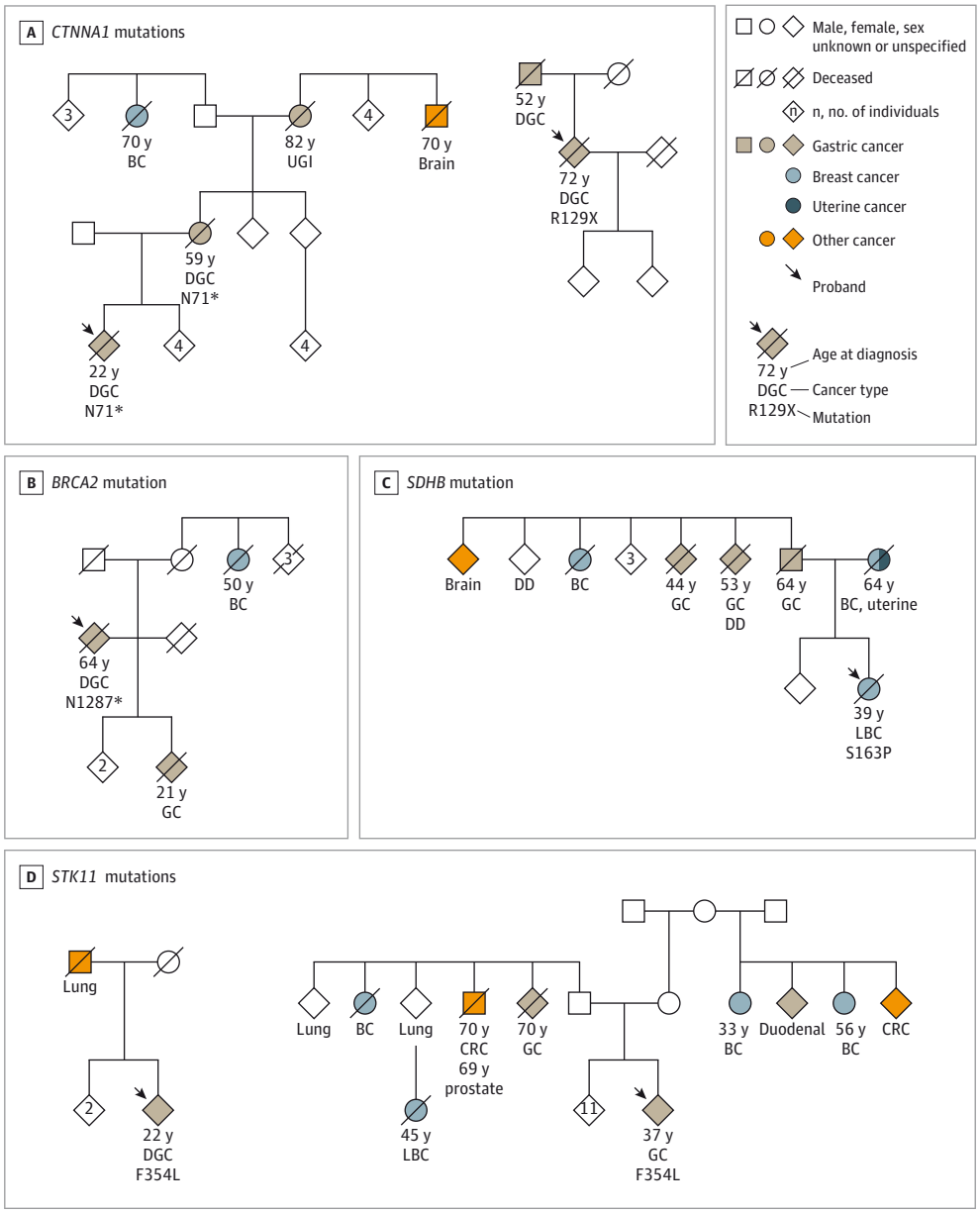
[b] Variants found at less than 1% frequency of the North American population: [c]rs41341748, [d]rs33927012, [e]rs59912467.

[f] Depth of sequencing coverage, across the nucleotide of interest; x indicates number of times covered.

pathogenicity underpinning GC susceptibility in *CDH1* mutation carriers is transmitted through a *CDH1/CTNNA-1* signaling axis. Identifying additional *CTNNA1* mutation-positive HDGC families will be required for penetrance analyses.

We also identified additional pathogenic germline involving genes associated with other cancer predisposition syndromes. Germline *BRCA2* mutations predispose carriers to hereditary breast and ovarian cancer,[30,31] with mutations

Figure 3. Familial Pedigrees of HDGC Families With Germline Pathogenic or Likely Pathogenic Mutations Identified Through Panel Sequencing



Extensive family pedigrees for familial intestinal gastric cancer families with pathogenic mutations were not available. Age in years is age at diagnosis. The asterisks indicate a translation termination or stop codon; BC, breast cancer; CRC, colorectal carcinoma; DD, developmental delay; DGC, diffuse gastric cancer; GC, gastric cancer; HDGC, hereditary diffuse gastric cancer; LBC, lobular breast cancer; and UGI, upper gastrointestinal tract cancer unspecified.

in exon 11 being associated with an elevated risk for other cancers, including GC.[32,33] In some *BRCA2*-positive families, there is an overrepresentation of GC.[33-36] The novel *BRCA2*-truncating variant (N1287fs) identified in a HDGC family (Table 2) in this study represents, to our knowledge, the first identification of a truncating *BRCA2* mutation in HDGC, further suggesting GC as a phenotypic-manifestation of *BRCA2* mutations. Gastric cancer families found to carry *BRCA2* mutations would likely benefit from preventive and therapeutic measures used for *BRCA2*-associated malignant conditions. Further work is necessary to uncover the GC risk associated with *BRCA2* variants.

Within several genes associated with gastrointestinal cancer predisposition syndromes, we identified rare missense mutations that have been previously reported as pathogenic. Three HDGC cases had a pathogenic missense variant (F354L) in the highly penetrant Peutz-Jeghers syndrome susceptibility gene, *STK11* (*LKB1*).[16,17] In vitro analyses of F354L have shown impaired activation of 5′ adenosine monophosphate–activated protein kinase (AMPK) pathways and disruption of cellular polarity.[16] Family P44 had this variant and presented with 2 cases of GC (DGC confirmed in one), as well as breast, colorectal, and duodenal cancers, suggestive of a broader cancer syndrome such as Peutz-Jeghers syndrome (Figure 3 and Table 2).

The proband of family P46 presented with DGC at age 22 years. It is unknown if members of either family had other features of Peutz-Jeghers syndrome such as hamartomatous gastrointestinal tract polyps or mucosal pigmentation. Similarly, a rare *SDHB* mutation (S163P), previously associated with the cancer risk disorder Cowden-like syndrome,[18] was identified in a HDGC family (family P13). Conflicting data exist in the literature to support pathogenicity of this variant; however, broader analysis revealed disease patterns suggestive of Cowden-like syndrome on both sides of the pedigree P13, with GC, BC, and developmental delay on the paternal side (Figure 3 and Table 2). The S163P mutation has been shown by in vitro analysis to increase activity of both AKT and MAPK (mitogen-activated protein kinase) pathways.[18] We also found 4 HDGC families with a rare truncating *MSR1* variant (R293X), a previously identified risk allele for esophageal and prostate cancers.[19,20] More evidence will be required before this mutation can be used for clinical risk stratification.[19,20,37,38] Frequencies of the 3 mutations described vary but are uncommon in North America.[39]

Truncating mutations in the low- to moderate-penetrance genes *ATM* and *PALB2* (Table 2 and eFigure 4 in the Supplement) were also identified through our gene panel. Conflicting risk estimates of *ATM* and *PALB2* variants have been reported in hereditary breast, ovarian, and pancreatic cancers.[40-44] However, a recent publication on the penetrance of *PALB2* mutation carriers shows that loss of function mutations are an important cause of hereditary BC, with a 33% to 58% increased risk for disease in mutation carriers by 70 years.[45] A heterozygous truncating variant (Q86X) was also identified in gene *PRSS1*. Such mutations are associated with high, incomplete penetrance for hereditary pancreatitis.[46,47] A recent report of all documented germline *PRSS1* variants highlights that truncating variants are rare but pathogenic.[48] This family presented with extensive family history of GC as well as a single case of liver cancer (Table 2). Frequency of somatic *PRSS1* mutations is infrequent in sporadic GC (2%) but relatively high in liver cancers (9%).[49] Further analyses are required to define the extent of cancer susceptibility conferred by these genes and justify interventions.

Apart from 2 *CTNNA1* mutations, HDGC families were found to carry mutations in genes associated with other cancer-predisposition syndromes, some with established management strategies, or genes of uncertain clinical significance. The data from our genetic screening of *CDH1* mutation–negative HDGC families suggest that HDGC may be better defined by genetics rather than clinical criteria. In such a system, mutations in *CDH1* and *CDH1*-like genes (eg, *CTNNA1*) define HDGC. Despite meeting phenotypic criteria for HDGC or familial intestinal gastric cancer (FIGC), families with mutations in other genes would most likely benefit from carrier risk reduction strategies based on the mutated gene (ie, *BRCA2*) rather than the cancer types that lead to the referral. The penetrance data from this study will enable genetics professionals to provide more accurate relative risk estimates to *CDH1* mutation–positive carriers, which will help in making more informed clinical management decisions. In addition, in HDGC families, clinicians can provide genetic testing through a broader panel of cancer predisposition genes, which may help to identify the causative underlying mutation in a greater number of these families.

Regarding the limitations of our study, our assay cannot detect copy number alterations within targeted amplicons. It is also likely that environmental factors are genetic modifiers in multicase families. We acknowledge that lifestyle and environment factors that affect risk in the general population can also modify risk in *CDH1* carriers; then the penetrance would be expected to be higher in countries with a high incidence. However, we had insufficient data to evaluate this possibility.

Also, cryptic abnormalities within the *CDH1* locus may account for many cases of HDGC, as suggested by allele-specific expression[50]; lack of available RNA precluded the exclusion of this cause. Limited availability of additional materials from family members prevented complete validation of novel variants. Further work is needed to accurately assess risk associated with mutations in identified genes. Finally, any inaccuracies in a retrospective review of pathology reports would have confounded associations between genetic abnormalities and the distinct clinical entities (ie, diffuse vs intestinal GC). It is often difficult to collect tissue blocks for all cases for histopathologic review owing to the high mortality rate of the disease, but this would be advisable.

Conclusions

To our knowledge, this study represents the most robust and thorough description of HDGC-novel *CDH1* mutations and the penetrance in *CDH1* mutation carriers to date. These data should assist in the genetic counseling and management of at-risk individuals from *CDH1*-positive HDGC families. Using multiplexed panel sequencing, we identified mutations associated with a clinically heterogeneous set of cancer predisposition syndromes. Applying broader screening for families with phenotypic history of GC will increase the number of families who can benefit from targeted risk reduction procedures. The genetic basis of unexplained cases of familial GC is likely some combination of mutations in genes yet to be determined, phenocopies among families or, in the case of HDGC families, other abnormalities at the *CDH1* locus or pathway. Targeted panel sequencing is an efficient way to triage candidate families for broader whole-genome sequencing analysis.

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Author Affiliations: Centre for Translational and Applied Genomics, British Columbia Cancer Agency, Vancouver, British Columbia, Canada (Hansford, Li-Chang, Woo, Senz, Padilla, Lum, Talhouk, Huntsman); Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada (Hansford, Li-Chang, Woo, Senz, Nielsen, Talhouk, Huntsman); Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada (Kaurah); Expression Regulation in Cancer Group, IPATIMUP–Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal (Pinheiro, Carvalho, Oliveira); Hereditary Cancer Program, BC Cancer Agency, Vancouver, British Columbia, Canada (Kaurah, Schrader); Division of Anatomical Pathology, Vancouver General Hospital, University of British Columbia, Vancouver, British Columbia, Canada

(Schaeffer); Department of Molecular Oncology, BC Cancer Agency, Vancouver, British Columbia, Canada (Shumansky, Nielsen, Shah, Huntsman); The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada (Zogopoulos); Rosalind and Morris Goodman Cancer Research Centre, Montreal, Quebec, Canada (Zogopoulos); Human Reproduction Service, University Hospitals of Coimbra, Coimbra, Portugal (Santos); Faculty of Medicine, University of Coimbra, Coimbra, Portugal (Santos); Instituto Português de Oncologia de Lisboa Francisco Gentil E.P.E., Lisbon Portugal (Claro); Frauenshuh Cancer Center, Park Nicollet Clinic, St Louis Park, Minnesota (Baker-Lange); Cancer Research UK Cambridge Institute, Cambridge, England (Richardson, Caldas); Provincial Medical Genetics Program, St John's, Newfoundland, Canada (Lewis, MacMillan, Fernandez); Department of Health Science Research, Mayo Clinic, Scottsdale, Arizona (Lindor); Cancer Care Program, Dr. H. Bliss Murphy Cancer Centre Eastern Health, St John's, Newfoundland, Canada (Pennell); Institute of Pathology, Technische Universität München, München, Germany (Keller); Creighton's Hereditary Cancer Center, Omaha, Nebraska (Lynch); Cancer Genetics Laboratory, Department of Biochemistry, University of Otago, Dunedin, New Zealand (Guilford); Division of General Surgery, Department of Surgery, University of Toronto, Toronto, Ontario, Canada (Gallinger); Samuel Lunenfeld Research Institute, Mount Sinai Hospital Toronto, Ontario, Canada (Gallinger); Department of Experimental Oncology, European Institute of Oncology, Milano, Italy (Corso); Department of Medical Surgical Sciences and Neurosciences, Section of General Surgery and Surgical Oncology, University of Siena, Siena, Italy (Corso, Roviello); Istituto Toscano Tumori (ITT), University Hospital of Siena, Siena, Italy (Roviello); Faculty of Medicine, University of Porto, Porto, Portugal (Oliveira); Department of Oncology, University of Cambridge, Strangeway's Research Laboratory, Wort's Causeway, Cambridge, England (Pharoah); Department of Public Health and Primary Care, University of Cambridge, Strangeway's Research Laboratory, Wort's Causeway, Cambridge, England (Pharoah); Department of Obstetrics and Gynecology (Huntsman), University of British Columbia, Vancouver, British Columbia, Canada (Huntsman).

Author Contributions: Ms Hansford and Ms Kaurah had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Ms Hansford, Ms Kaurah, and Dr Li-Chang contributed equally to this work.
Study concept and design: Hansford, Kaurah, Woo, Schrader, Zogopoulos, Oliveira, Pharoah, Huntsman.
Acquisition, analysis, or interpretation of data: Hansford, Kaurah, Li-Chang, Woo, Senz, Pinheiro, Schaeffer, Shumansky, Zogopoulos, Almeida Santos, Claro, Carvalho, Nielsen, Padilla, Lum, Talhouk, Baker-Lange, Richardson, Lewis, Lindor, Pennell, MacMillan, Fernandez, Keller, Lynch, Shah, Guilford, Gallinger, Corso, Roviello, Caldas, Oliveira, Pharoah, Huntsman.
Drafting of the manuscript: Hansford, Kaurah, Li-Chang, Woo, Shumansky, Nielsen, Padilla, Pennell, Oliveira, Pharoah, Huntsman.
Critical revision of the manuscript for important intellectual content: Hansford, Kaurah, Li-Chang, Woo, Senz, Pinheiro, Schrader, Schaeffer, Zogopoulos, Almeida Santos, Claro, Carvalho, Lum, Talhouk, Baker-Lange, Richardson, Lewis, Lindor, MacMillan, Fernandez, Keller, Lynch, Shah, Guilford, Gallinger, Corso, Roviello, Caldas, Oliveira, Huntsman.
Statistical analysis: Shumansky, Talhouk, Shah, Pharoah.
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Study supervision: Zogopoulos, Fernandez, Shah, Roviello, Oliveira, Huntsman.

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