original report

Unexpected *CDH1* Mutations Identified on Multigene Panels Pose Clinical Management Challenges

See accompanying editorial DOI: 10.1200/PO.17.00006

Katrina Lowstuter
Carin R. Espenschied
Duveen Sturgeon
Charité Ricker
Rachid Karam
Holly LaDuca
Julie O. Culver
Jill S. Dolinsky
Elizabeth Chao
Julia Sturgeon
Virginia Speare
Yanling Ma
Kerry Kingham
Marilena Melas
Gregory E. Idos
Kevin J. McDonnell
Stephen B. Gruber

Author affiliations appear at the end of this article.
K.L., C.R.E., and D.S. contributed equally to this work.
Supported by the National Cancer Institute, University of Southern California Norris Cancer Center Core Grant (P30CA014089); American Cancer Society (RSGT 1020301); Avon Foundation (052011057); Anton B. Burg Foundation; and Lynne Cohen Foundation.
Corresponding author: Duveen Sturgeon, MSN, ACNP, AGN, USC Norris Comprehensive Cancer Center, 1441 Eastlake Ave, Los Angeles, CA 90033; e-mail: duveen.sturgeon@med.usc.edu.

abstract

Purpose **Mutations in the *CDH1* gene confer up to an 80% lifetime risk of diffuse gastric cancer and up to a 60% lifetime risk of lobular breast cancer. Testing for *CDH1* mutations is recommended for individuals who meet the International Gastric Cancer Linkage Consortium (IGCLC) guidelines. However, the interpretation of unexpected *CDH1* mutations identified in patients who do not meet IGCLC criteria or do not have phenotypes suggestive of hereditary diffuse gastric cancer is clinically challenging. This study aims to describe phenotypes of *CDH1* mutation carriers identified through multigene panel testing (MGPT) and to offer informed recommendations for medical management.**

Patients and Methods **This cross-sectional prevalence study included all patients who underwent MGPT between March 2012 and September 2014 from a commercial laboratory (n = 26,936) and an academic medical center cancer genetics clinic (n = 318) to estimate *CDH1* mutation prevalence and associated clinical phenotypes. *CDH1* mutation carriers were classified as IGCLC positive (met criteria), IGCLC partial phenotype, and IGCLC negative.**

Results **In the laboratory cohort, 16 (0.06%) of 26,936 patients were identified as having a pathogenic *CDH1* mutation. In the clinic cohort, four (1.26%) of 318 had a pathogenic *CDH1* mutation. Overall, 65% of mutation carriers did not meet the revised testing criteria published in 2015. All three *CDH1* mutation carriers who had risk-reducing gastrectomy had pathologic evidence of diffuse gastric cancer despite not having met IGCLC criteria.**

Conclusion **The majority of *CDH1* mutations identified on MGPT are unexpected and found in individuals who do not fit the accepted diagnostic testing criteria. These test results alter the medical management of *CDH1*-positive patients and families and provide opportunities for early detection and risk reduction.**

Precis Oncol 00. © 2017 by American Society of Clinical Oncology

INTRODUCTION

Mutations in the *CDH1* gene cause hereditary diffuse gastric cancer (HDGC), which confers up to an 80% lifetime risk of diffuse gastric cancer (DGC) and up to a 60% lifetime risk of female invasive lobular carcinoma (ILC), with the average age at diagnosis being 38 and 53 years, respectively.[1-3] These high risks, coupled with the difficulty of diagnosing early-stage DGC and current clinical recommendations to consider prophylactic gastrectomy in *CDH1* carriers,[4,5] highlight the importance and clinical challenges of identifying and managing individuals with *CDH1* mutations (*CDH1*+).

Traditionally, genetic testing for cancer susceptibility is performed after the development of a differential diagnosis, including Mendelian syndromes caused by a few highly penetrant genes suggested by personal/family history. Genetic testing then proceeds in a stepwise, targeted manner to evaluate the differential diagnosis. With the advent of multigene panel testing (MGPT), clinicians are able to analyze multiple genes simultaneously, including those lower on the differential diagnosis or not considered at all. The International Gastric Cancer Linkage Consortium (IGCLC) published guidelines in 2010 (updated in 2015) for identifying individuals as appropriate for *CDH1* gene testing[4,5] (Table 1); however, multigene panels are now used to test increasing numbers of individuals for *CDH1*. We describe a cross-sectional prevalence study of *CDH1*+ individuals identified by MGPT. Implications for medical management and testing criteria are featured.

© 2017 by American Society of Clinical Oncology

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

Table 1. International Gastric Cancer Linkage Consortium Guidelines

2010 Guidelines	2015 Revised Guidelines
1. Two or more documented cases of DGC in first- or second-degree relatives, with at least one being diagnosed before the age of 50 years, or	1. Families with two or more patients with gastric cancer at any age, one confirmed DGC
2. Three or more cases of documented DGC in first- or second-degree relatives independent of age of onset	2. Individuals with DGC before the age of 40 years
3. Individual with DGC diagnosed before the age of 40 years	3. Families with diagnoses of both DGC and ILC (one diagnosis before the age of 50 years)
4. Personal or family history of DGC and ILC, one diagnosed before the age of 50 years	Consideration of genetic testing for the following individuals:
	1. Patients with bilateral or familial ILC before the age of 50 years
	a. Patient with bilateral ILC diagnosed at younger than 50 years of age or
	b. Multiple close relatives with ILC with at least two diagnosed at younger than 50 years of age
	2. Patients with DGC and cleft lip/palate
	3. Patients with precursor lesions for signet ring cell carcinoma

Abbreviations: DGC, diffuse gastric cancer; ILC, invasive lobular carcinoma.

PATIENTS AND METHODS

This collaborative, institutional review board–approved study between Ambry Genetics and the University of Southern California (USC) investigated the prevalence and describes the phenotypes of *CDH1* mutation carriers identified through MGPT. A retrospective review of two cohorts was performed: The laboratory cohort included all probands, unrelated to our knowledge, who underwent MGPT for *CDH1* (genes analyzed, five to 43) at Ambry Genetics between March 16, 2012, and September 30, 2014 (n = 26,936), and the clinic cohort consisted of all patients who underwent MGPT with *CDH1* included in the panel (genes analyzed, five to 110) from April 15, 2013, to May 29, 2014, at USC (n = 318). For all the patients in the clinic cohort, MGPT was ordered because one or more of the genes included in the panel was the primary target and part of the differential diagnosis. For the laboratory cohort, data were abstracted from test request forms, and information about the targeted genes or the differential diagnosis was not available. No overlap existed between the cohorts in patients who tested positive for *CDH1*, and patients who tested negative for *CDH1* mutations shared by USC and Ambry were excluded from Ambry's count. Panel testing in the clinic cohort was performed at the following laboratories: Ambry Genetics, Myriad Genetics, University of Washington, and Fulgent Diagnostics. For the laboratory cohort, data were abstracted from test requisition forms and by contacting the ordering provider to obtain further pathology, personal and family history, testing information on family members, and outcomes data. For the clinic cohort, data were abstracted directly from the patient's medical record. USC clinicians (genetic counselors and physician cancer geneticists) classified patients in the clinic cohort; the laboratory cohort was classified by an Ambry genetic counselor. Subsequent blinded classifications were repeated by clinicians at Ambry and USC.

CDH1+ cases were classified into three categories on the basis of the 2010 IGCLC guidelines[4]: IGCLC positive (IGLCC-Pos) was defined as mutation carriers who met IGCLC criteria; IGCLC partial phenotype (IGCLC-PP) was defined as mutation carriers who did not meet IGCLC criteria, but HDGC was in their differential diagnosis because of the presence of gastric cancer, age older than 40 years, or ILC at any age in the family; and IGCLC negative (IGCLC-Neg) was defined as mutation carriers who did not meet IGCLC criteria and had no gastric cancer or ILC present in the family. Each

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

Table 2. *CDH1* Mutations and Phenotype Description

Proband ID	IGCLC Classification	*CDH1* Mutation	Panel Tested	Proband Diagnosis, Age (years)	Proband Pathology	Family Cancer Status/ Familial Testing, Age (years)*
CC1	IGCLC-Neg	EX1_EX2del	MyRisk	Breast, 44	Ductal	None
LC2	IGCLC-Neg	c.1565+1G>A	ColoNext	CRC, 54, 59	Unknown	Mother breast, 63
						Sister *CDH1*+, polyps, 61
						Sister RCC, 60
LC9	IGCLC-Neg	c.387+1G>A	BRCAplus version 1	UA		Mat GM breast, 45
						Mat GF brain, 49
LC12	IGCLC-Neg	c.1147C>T	BRCAplus version 1	Breast, 36	Ductal	Brother *CDH1*+, UA
						Mat aunt *CDH1*+, breast (LCIS)
						Mat GGF GI cancer NOS
						Mat GF lung, 62
						Mat cousin *CDH1*+, UA
						Pat GM breast, 70
LC13	IGCLC-Neg	c.1999delC	BRCAplus version 1	Breast neoplasm of unknown behavior	Unknown	Mother breast, 72
						Sisters × 2 breast, 58, 61
						Sister cancer NOS
						Mat GM breast, 56
CC2	IGCLC-PP	c.2164+1G>A	MyRisk	Breast, 48	Lobular	Son, *CDH1*+, UA
						Sister ovarian, 29
						Mat aunt breast, 25
						Mat uncle CRC, 70s
CC3	IGCLC-PP	EX1_EX2del	MyRisk	Breast, 43	Ductal with lobular features	None
LC3	IGCLC-PP	c.1565+1G>A	BreastNext	Bil breast, 40	Lobular	Father sarcoma, 27
						Pat aunt × 3 breast, 40s
LC4	IGCLC-PP	c.2064_2065delTG	BRCAplus version 1	Breast, 54	Lobular	Sister *CDH1*+, breast (IDC), 44
						Mat GM breast, 30
						Mat aunt pancreatic, 65
LC6	IGCLC-PP	c.1003C>T	ColoNext	Gastric, 68	Diffuse	Daughter, *CDH1*+, 2-5 polyps, 38
						Sister *CDH1*−, melanoma, 40
						Sister *CDH1*+, 1-2 polyps, 47
						Sister rectal, 55
						Mother skin NOS
						Mat aunt skin NOS
LC14	IGCLC-PP	c.202delT	BRCAplus version 1	Breast	Lobular	Mother breast, 50
						Mat GF liver, 62

(Continued on following page)

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

Table 2. *CDH1* Mutations and Phenotype Description (Continued)

Proband ID	IGCLC Classification	*CDH1* Mutation	Panel Tested	Proband Diagnosis, Age (years)	Proband Pathology	Family Cancer Status/ Familial Testing, Age (years)*
LC15	IGCLC-PP	c.1565+1G>A	BRCAplus version 1	Breast, 60	Lobular	Father prostate, 75; testicular, 80
						Son sarcoma, 41
						Pat aunt breast, < 80
						Pat aunt breast, 50
						Pat aunt breast, < 90
						Mat uncle CRC, 50s
						Mat uncle CRC, < 70
						Mat GM breast, 70s
LC16	IGCLC-PP	c.1979dupT	BRCAplus version 2	Breast, 43	Lobular	Mother breast, 56
						Mat aunt ovarian, 22
						Mat uncle throat, 40s
						Mat uncle throat, 60s
LC17	IGCLC-PP	5′UTR_IN2del	BRCAplus version 2	Bil breast, 45	Lobular	Father gastric, 65
						Pat GM lung, 70s
						Mat aunt breast, 67
CC4	IGCLC-Pos	c.504del	MyRisk	Breast, 44	Ductal	Mother ovarian, 40; CRC, 71
						Sister × 2 gastric, 38, 46
						Sister CRC, 43
						Brother prostate, 44
						Mat aunt gastric, 71
						Mat great aunt gastric, 75
						Pat uncle gastric, 52
LC8	IGCLC-Pos	c.1565+1G>T	CancerNext	Breast, 53	Lobular	Mother breast, 70
						Brother *CDH1*+, UA
						Sister *CDH1*+, UA
						Daughter *CDH1*+, UA
						Pat cousin brain, 40
						Niece gastric, 30
LC1	IGCLC-Pos	c.521dupA	ColoNext	Gastric, 49, 58	Signet cell	Sister × 2 gastric, 38, 39
						Mother gastric, 59
						Brother esophageal, 60s
						Brother *CDH1*+, UA, 49
						Son × 2 *CDH1*+, UA
						Sister *CDH1*+, UA, 52
						Sister *CDH1*+, UA, 67
						Niece × 2 *CDH1*+, UA
						Niece *CDH1*+, UA, 26
						Nephew × 2 *CDH1*+, UA

(Continued on following page)

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

Table 2. *CDH1* Mutations and Phenotype Description (Continued)

Proband ID	IGCLC Classification	*CDH1* Mutation	Panel Tested	Proband Diagnosis, Age (years)	Proband Pathology	Family Cancer Status/ Familial Testing, Age (years)*
LC10	IGCLC-Pos	c.1921C>T	BRCAplus version 1	Gastric, 25	Diffuse, signet cell	Father *CDH1*+, melanoma, 60
						Pat GM GYN, 60s; esophageal, 90s
						Pat GF CRC, 89
						Pat aunt H/N, 50s
						Pat aunt × 3 gastric, 40s, 50, 50
						Pat aunt throat
						Pat uncle lung, 40s
						Pat uncle prostate
						Pat GGM gastric, 82
						Pat great uncle CRC, 44
LC11	IGCLC-Pos	IN2_EX5del	CancerNext	Breast, 38	Ductal	Father *CDH1*+, CRC, 67
						Sister *CDH1*+, UA, 47
						Sister *CDH1*+, UA, 49
						Pat aunt gastric, 47
						Pat GM breast, 72
						Pat uncle *CDH1*+, prostate, 76
						Pat cousin × 2 gastric, 42, 50
						Cousin × 3 *CDH1*+, UA
						Cousin brain, 8
						Nephew × 3 *CDH1*+, UA
LC18	IGCLC-Pos	c.2064_2065delTG	ColoNext	Gastric, 30	Unknown	Father gastric, 65
						Mother breast, 56
						Mat aunt breast, 67
						Mat aunt ovarian, 20
						Mat uncle throat, 40
						Mat uncle throat, 60
						Mat cousin ovarian, 22
						Mat cousin breast, 38
						Mat GM lung, 70
						Pat GM gastric, 70

NOTE. MyRisk manufactured by Myriad Genetics (Salt Lake City, UT); ColoNext, BRCAplus, BreastNext, and CancerNext manufactured by Ambry Genetics (Aliso Viejo, CA). Abbreviations: BC, breast cancer; Bil breast, bilateral breast cancer; CRC, colorectal cancer; GF, grandfather; GGF, great grandfather; GGM, great grandmother; GM, grandmother; GYN, gynecologic cancer; H/N, head and neck cancer; IDC, invasive ductal breast cancer; IGCLC, International Gastric Cancer Linkage Consortium; LCIS, lobular carcinoma in situ; Mat, maternal; Neg, negative; NOS, not otherwise specified; Pat, paternal; Pos, positive; PP, partial phenotype; RCC, renal cell carcinoma; UA, unaffected.

*Family members are untested unless otherwise indicated. Unaffected relatives who tested negative were excluded from this table.

case was further reviewed on the basis of the 2015 guidelines.[5] E-cadherin immunohistochemistry (IHC) staining was conducted on invasive ductal carcinoma (IDC) tissue when feasible.

RESULTS

In the laboratory cohort, 0.06% (16 of 26,936) of patients were *CDH1*+. Five were classified as IGCLC-Pos, seven as IGCLC- PP, and four as IGCLC-Neg. In the clinic cohort, 1.26% (four of 318) were *CDH1*+ (Table 2). Case CC4 was classified as IGCLC-Pos as a result of family history; however, the proband's presentation was atypical because she was given a diagnosis of IDC at age 44 years. IHC staining revealed a lack of expression of E-cadherin in the tumor; thus, it was

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

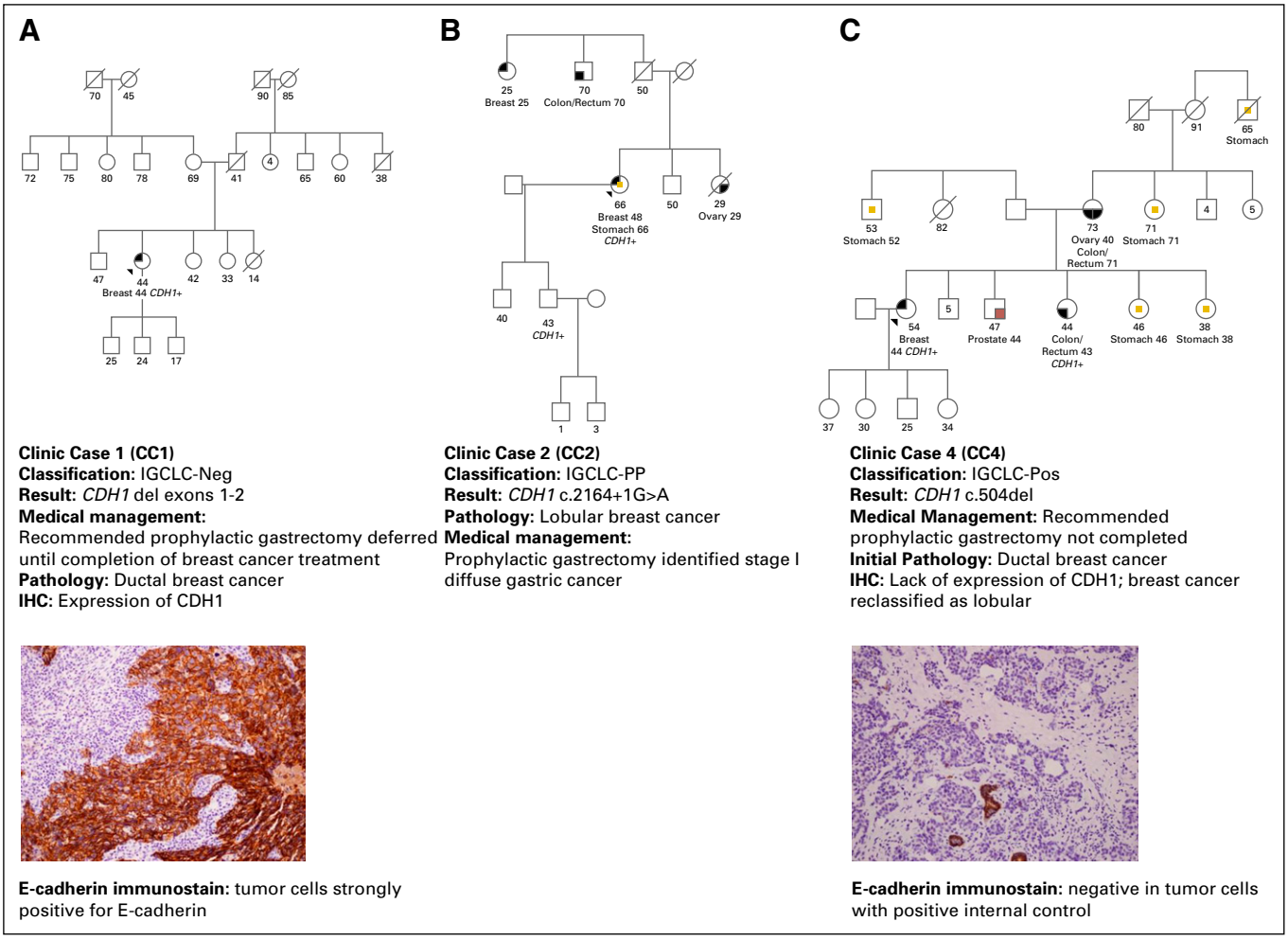

Fig 1. International Gastric Cancer Linkage Consortium (IGCLC)-negative (IGCLC-Neg), IGCLC-partial phenotype (IGCLC-PP), and IGCLC-positive (IGCLC-Pos) pedigrees. IHC, immunohistochemistry.

reclassified as ILC. Two cases were IGCLC-PP, and one was IGCLC-Neg because the proband had IDC (confirmed on IHC), no family history of cancer, and complete family structure (Fig 1).

Overall, 20 pathogenic *CDH1* mutations were identified. One of the probands was unaffected. Of patients with cancer, 42% (n = 8) presented with ILC and 21% (n = 4) with gastric cancer consistent with the expected phenotype; however, 21% (n = 4) presented with IDC, 5% (n = 1) with IDC with lobular features, 5% (n = 1) with breast neoplasm not otherwise specified, and 5% (n = 1) with colon cancer (Fig 2A). Breast cancer was the most prevalent overall (73% of affected probands; Fig 2A). The average age of onset for breast and gastric cases is presented in Figs 2B and 2C. *BRCA1* and *BRCA2* mutations were ruled out in patients with breast cancer (Data Supplement). Six (30%) of 20 *CDH1*+ cases were classified as IGCLC-Pos. In this study, 14 (70%) of 20 cases did not meet the 2010 IGCLC criteria (nine IGCLC-PP and five IGCLC-Neg). Cases were reviewed to determine whether they met the 2015 guidelines, and case LC17, initially classified as IGCLC-PP, met the revised testing criteria as a result of a diagnosis of bilateral ILC at age 45 years.[5] Therefore, 65% of cases did not meet the 2015 guidelines for consideration of testing.

Mutation Description

Sixteen distinct pathogenic/likely pathogenic *CDH1* alterations were detected in 20 *CDH1*+ probands, with three recurrent mutations identified (Fig 3; Data Supplement). Mutations were classified according to the American College of Medical Genetics and Genomics guidelines.[6] All 16 mutations are predicted to result in a premature termination codon. A variety of mechanisms lead to pathogenicity, including nonsense-mediated decay[6] of mRNAs that contain premature termination codons.[7,8] For *CDH1*, nonsense-mediated decay has been shown to cause downregulation of alleles, which results in loss of function.[7]

Of the mutations described, seven are novel to our knowledge (Fig 3). Five of the novel mutations

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

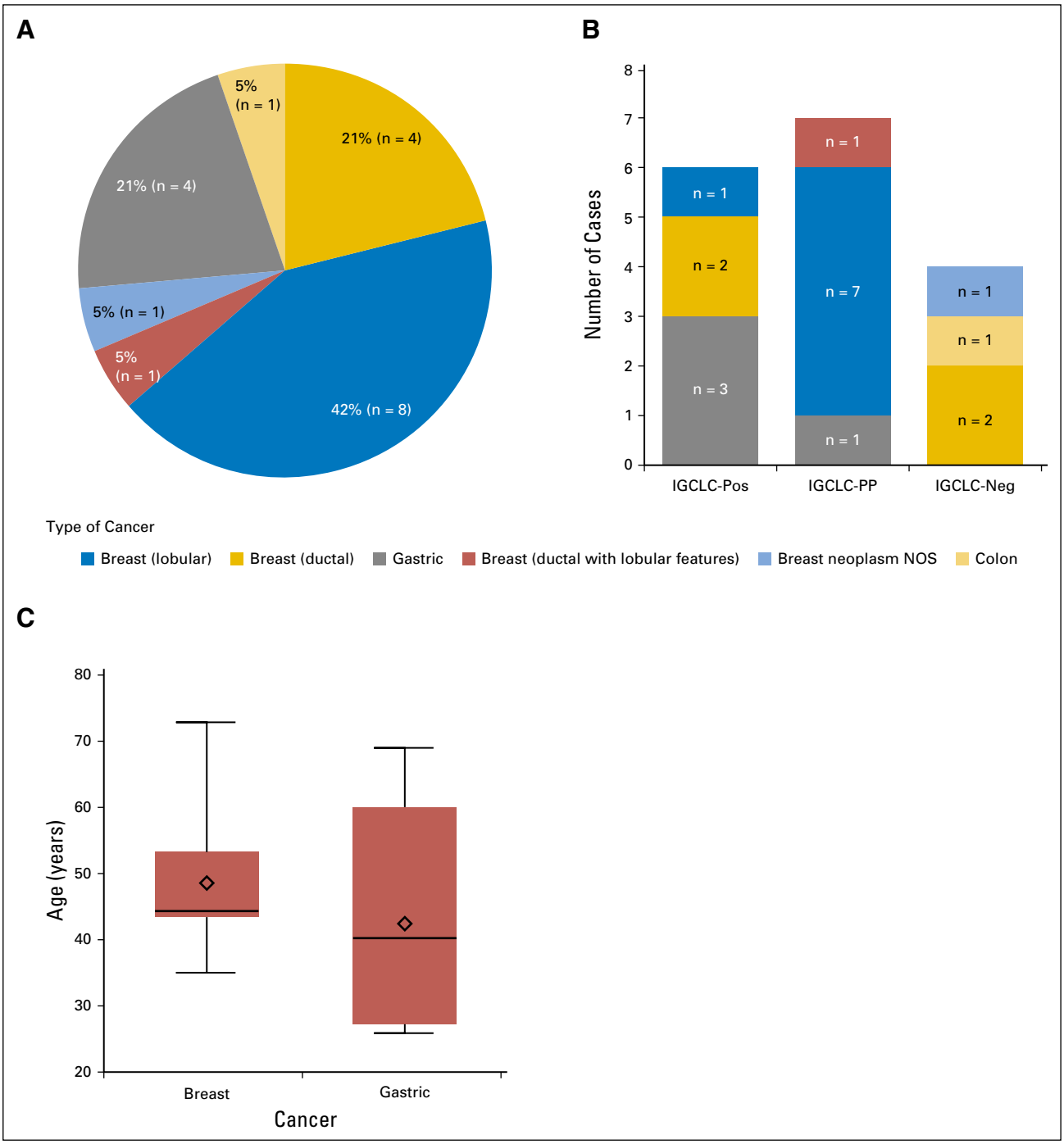

Fig 2. Cancer diagnoses among *CDH1* mutation carriers. (A) Cancer diagnoses of probands in the current study. One proband did not have a cancer diagnosis. (B) Proband cancer diagnoses categorized by International Gastric Cancer Linkage Consortium

were classified as pathogenic, including three out-of-frame deletion mutations (c.202delT, c.1979dupT, and c.1999delC), one in-frame deletion mutation (c.504del), and one nonsense mutation (c.1921C>T p.Q641*).[6] The remaining two novel mutations are splice site variants that affect the canonical donor site (c.387+1G>A and c.2164+1G>A). Both canonical variants are predicted to abolish the native donor splice site by four different splicing prediction tools (Human Splicing Finder [HSF], MaxEntScan, Berkeley Drosophila Genome Project [BDGP], and ESE-finder), and were classified as likely pathogenic as a result of lack of additional clinical evidence.

The six previously described mutations were classified as pathogenic. The c.521dupA mutation has been reported in a family with only early-onset ILC. No other breast or gastric cancers were known in that family.[9] In the current cohort, this mutation was seen in an IGCLC-Pos family with

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

(IGCLC) classification as follows: IGCLC-positive (IGCLC-Pos), IGCLC-partial phenotype (IGCLC-PP), or IGCLC-negative (IGCLC-Neg). (C) Box and whiskers plot of proband breast and gastric cancer age at diagnosis. NOS, not otherwise specified.

multiple gastric cancers. The nonsense mutations c.1003C>T (p.R335*) and c.1147C>T (p.Q383*) have been reported in multiple families with HDGC,[3,8,10-14] whereas they were found in an IGCLC-PP and IGCLC-Neg family in the current cohort. The frameshift mutation c.2064_2065delTG was previously identified in highly selected families with a history of early-onset DGC,[3,15] whereas it was seen in an IGCLC-PP family in the current cohort. We detected two previously described splice site mutations that affected the first nucleotide of intron 10. The mutation c.1565+1G>T was first reported in a family with multiple DGCs,[16] and the mutation c.1565+1G>A was initially reported in a patient with ILC whose family history was significant for multiple breast cancers and one early-onset gastric cancer.[17] In the current cohort, c.1565+1G>T was seen in an IGCLC-Pos family, and c.1565+1G>A was seen in two families, one IGCLC-Neg and the other IGCLC-PP. The four different splicing prediction tools (HSF, MaxEntScan, BDGP, and ESEfinder) predict that these two variants will abolish the native donor site, but we cannot exclude that the use of alternative cryptic donor sites may affect the expressivity of the phenotype in these cases.

We detected four multiexon deletions: three (5′UTR_IN2del and EX1_2del twice) include the transcription and translation start sites, and the fourth (IN2_EX5del) is predicted to include 1,237 nucleotides of coding sequence and to be out of frame. Two different deletions encompassing exons 1 to 2 were previously reported in three families with HDGC; however, we cannot confirm whether the deletions of this region in the current cohort have the same breakpoints as those previously reported.[18] To our knowledge, these deletions contain the only functional translation start sites in the gene and therefore are predicted to abrogate protein synthesis. The two families in this cohort with the deletion of exons 1 to 2 were classified as IGCLC-Neg, the family with the deletion that spans from the 5′UTR through intron 2 was classified as IGCLC-PP, and the family with the intron 2 through exon 5 deletion was classified as IGCLC-Pos (Data Supplement).

Clinical Follow-up

All *CDH1*+ clinic cohort patients underwent a discussion of management consistent with HDGC guidelines,[5] and the testing of at-risk family members for the *CDH1* mutation was recommended (subsequent family testing data listed in Table 2). One of the four clinic cohort patients elected to proceed with prophylactic gastrectomy. For the other three clinical cases, two patients declined prophylactic surgery at this time, and one elected to wait until cancer treatment was completed to make a decision about prophylaxis. For the laboratory cohort patients, the ordering clinicians for *CDH1*+ patients were contacted to obtain follow-up information, and the available information is provided here. For one of the laboratory cohort patients who underwent a prophylactic gastrectomy, a family member was seen at USC after identification of the mutation, which allowed additional information to be obtained (LC12). In 15% of patients (three of 20 [CC2, LC12, and LC6]), either the proband or the family member is known to have pursued prophylactic gastrectomy and were found to have DGC (Table 3). None of the three who underwent gastrectomy met current IGCLC criteria. The clinical details of these three cases reflect the diversity and challenges of clinical presentation and management. Patient CC2 was suspicious for HDGC (ICGCL-PP) and was offered, and chose, prophylactic gastrectomy. Pathology revealed stage I DGC. Patient LC12 (proband had IDC at age 36 years) was initially classified as IGCLC-Neg on the basis of a family history of lobular carcinoma in situ in a second-degree relative (maternal aunt) with unknown age of onset and no documented family history of gastric cancer. After genetic testing, the family history was clarified in more depth, and a maternal great grandfather's unspecified GI cancer was confirmed as stomach cancer. Given this updated information, the family's posterior classification is IGCLC-PP. The maternal aunt with lobular carcinoma in situ underwent genetic testing, which was positive for the known *CDH1* mutation. She also elected prophylactic gastrectomy at age 55 years and was found to have DGC. The proband has not undergone gastrectomy to our knowledge. Patient LC6 was suggestive of HDGC because the proband had DGC at age 68 years (IGCLC-PP), and predictive testing in the proband's unaffected daughter identified the known familial mutation. The daughter elected prophylactic gastrectomy at age 39 years and was found to have gastric cancer.

DISCUSSION

In this study, 65% of *CDH1*+ cases did not meet the 2015 revised testing criteria,[4,5] which is consistent with a study where *CDH1* mutations were identified by MGPT in patients without DGC or family history of DGC.[19] A number of potential explanations exist, including limited or underascertained family history, incomplete appreciation of the histologic subtype of breast cancer,[20-22]

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

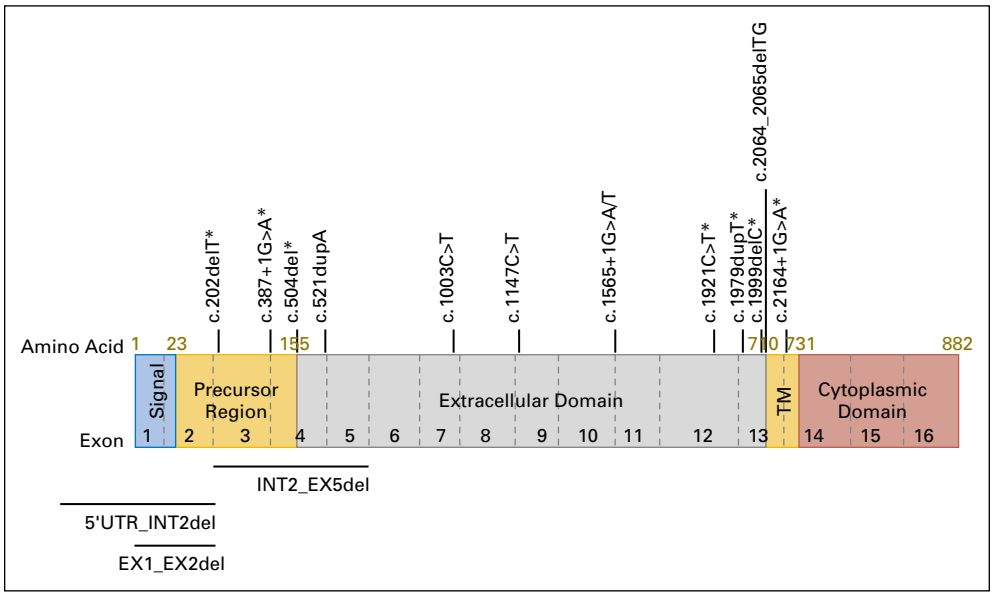

Fig 3. *CDH1* mutations and related impact on E-cadherin. *Novel mutations. TM, transmembrane domain.

incomplete penetrance and variable expressivity of mutations, and de novo germline mutation.[23,24]

Current published penetrance estimates have been derived from families ascertained by stringent HDGC guidelines. Although some of the mutations in the current study have been documented in highly penetrant families, others have not and, therefore, may represent less-penetrant mutations. Because the threshold for testing for *CDH1* changes over time, it is reasonable to anticipate that the cancer risks will have a wider range as families who present outside the high-risk criteria are ascertained, which is consistent with what has been derived in other well-studied cancer predisposition syndromes, such as hereditary breast and ovarian cancer syndrome.[25]

Variable expressivity for *CDH1* mutations has been demonstrated in studies of *CDH1*+ patients with ILC and/or a family history of ILC in the absence of DGC.[9,14,26] Consideration of ILC was added to the revised guidelines published in 2015[5] (Table 1). Detailed family histories that require deep investigation and retrieval of pathology reports from distant or deceased relatives are helpful when available, and post hoc review of family histories in this study identified previously unconfirmed gastric cancer and lobular breast cancers that changed the classification of risk for some patients who had undergone testing.

Current management guidelines encourage prophylactic gastrectomy between ages 20 and 30 years and annual breast magnetic resonance imaging starting at age 30 years.[4,5] These recommendations are based on the cancer risk estimates derived from high-penetrance *CDH1*+ families. The unexpected *CDH1* mutations may reflect an

Table 3. Results of Risk-Reducing Surgery in Family Members

Proband ID	IGCLC Classification	Family Member Who Had Gastrectomy	Outcome
CC2	IGCLC-PP	Proband (history of ILC)	Stage I DGC identified in prophylactic gastrectomy
LC6	IGCLC-PP	Daughter (unaffected)	Gastric cancer identified from prophylactic gastrectomy (unknown stage/pathology) performed at age 39 years
LC12	IGCLC-Neg*	Maternal aunt (history of LCIS)	DGC identified from prophylactic gastrectomy (unknown stage) performed at age 55 years

Abbreviations: DGC, diffuse gastric cancer; ILC, invasive lobular breast cancer; IGCLC, International Gastric Cancer Linkage Consortium; LCIS, lobular carcinoma in situ; Neg, negative; PP, partial phenotype.

*Proband LC12 (IGCLC-Neg) maternal aunt had LCIS with age of onset unknown and no documented cases of gastric cancer; therefore, the family was initially classified as IGCLC-Neg. When additional family members presented to the University of Southern California for testing, the maternal great grandfather's unspecified GI cancer was confirmed as stomach cancer. Given this updated information, the family's posterior classification is best described as IGCLC-PP. The maternal aunt with LCIS underwent genetic testing, which was positive for the known *CDH1* mutation. She subsequently underwent a prophylactic gastrectomy at age 55 years and was found to have DGC.

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

underlying population of families with reduced penetrance, and appropriately powered epidemiologic studies are required to investigate this hypothesis. These types of studies also will help to inform clinical decision making. In the meantime, our clinical approach has been to manage incidentally identified *CDH1*+ patients as classic HDGC families and offer prophylactic gastrectomy. Counseling and support balanced by considerations of risks, benefits, and costs are integral to partnering with these patients for shared decision making about optimal management strategies and their timing. The option of gastrectomy in IGCLC-PP families typically is framed in the context of the family history of cancer, which underscores the importance of obtaining a complete family history and review of pathology reports when available. For IGCLC-Neg families, consideration of what is known about each mutation and full exploration of the family history is key. To date, uptake of prophylactic gastrectomy has been low in families who do not exhibit a highly penetrant phenotype. As genomics is further integrated into clinical practice, the gathering of families into research registries is important for long-term follow-up to expand the clinical understanding of *CDH1* mutations, including range of cancer risk, and appropriate medical management across the phenotypic spectrum.

In conclusion, MGPT identifies *CDH1*+ individuals who do not meet criteria for *CDH1* testing. In the absence of MGPT, these patients would not likely have undergone *CDH1* testing. MGPT may provide an opportunity to identify individuals with an increased risk for a highly morbid cancer (DGC) before they present with advanced disease, even in the absence of suggestive family history. Many of these families presented with cancer histories more suggestive of hereditary breast and ovarian cancer because of early-onset IDC, and testing previously may have been limited to the *BRCA* genes, which raises the question of whether genetic testing criteria should be broadened to identify more *CDH1*+ patients. At this time, whether the cancer risks in the incidentally identified carriers match the levels reported for classic HDGC families is unclear, although in this series, 100% of *CDH1*+ patients who underwent gastrectomy had gastric cancer. In the absence of comprehensive data on penetrance, we currently recommend classic HDGC management guidelines for all *CDH1* mutation carriers.[4,5]

DOI: 10.1200/PO.16.00021
Published online on po.ascopubs.org on March 29, 2017.

AUTHOR CONTRIBUTIONS

Conception and design: Katrina Lowstuter, Carin R. Espenschied, Duveen Sturgeon, Charité Ricker, Julie O. Culver, Jill S. Dolinsky, Elizabeth Chao, Stephen B. Gruber

Financial support: Stephen B. Gruber

Administrative support: Katrina Lowstuter, Duveen Sturgeon, Virginia Speare, Stephen B. Gruber

Provision of study materials or patients: Carin R. Espenschied, Charité Ricker, Rachid Karam, Elizabeth Chao, Kerry Kingham, Gregory E. Idos, Stephen B. Gruber

Collection and assembly of data: Katrina Lowstuter, Carin R. Espenschied, Duveen Sturgeon, Charité Ricker, Holly LaDuca, Julie O. Culver, Jill S. Dolinsky, Elizabeth Chao, Yanling Ma, Marilena Melas, Gregory E. Idos, Kevin J. McDonnell, Stephen B. Gruber

Data analysis and interpretation: Katrina Lowstuter, Carin R. Espenschied, Duveen Sturgeon, Charité Ricker, Rachid Karam, Julie O. Culver, Julia Sturgeon, Virginia Speare, Kerry Kingham, Stephen B. Gruber

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Unexpected *CDH1* Mutations Identified on Multigene Panels Pose Clinical Management Challenges

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or po.ascopubs.org/site/ifc.

Katrina Lowstuter
No relationship to disclose

Carin R. Espenschied
Employment: Ambry Genetics

Duveen Sturgeon
No relationship to disclose

Charité Ricker
No relationship to disclose

Rachid Karam
Employment: Ambry Genetics
Research Funding: Ambry Genetics

Holly LaDuca
Employment: Ambry Genetics

Julie O. Culver
No relationship to disclose

Jill S. Dolinsky
Employment: Ambry Genetics
Stock and Other Ownership Interests: Ambry Genetics

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

Elizabeth Chao
Employment: Ambry Genetics
Leadership: Ambry Genetics
Stock and Other Ownership Interests: Ambry Genetics
Consulting or Advisory Role: Premier Genomics

Julia Sturgeon
No relationship to disclose

Virginia Speare
Employment: Ambry Genetics

Yanling Ma
No relationship to disclose

Kerry Kingham
No relationship to disclose

Marilena Melas
No relationship to disclose

Gregory E. Idos
Research Funding: Myriad Genetics

Kevin J. McDonnell
No relationship to disclose

Stephen B. Gruber
Research Funding: Myriad Genetics

ACKNOWLEDGMENT
We thank the ordering clinicians of and patients who had testing at Ambry Genetics. We also acknowledge Jennifer Thompson and Patrick Reineke for assistance with data curation.

Affiliations

Katrina Lowstuter, **Duveen Sturgeon**, **Charité Ricker**, **Julie O. Culver**, **Julia Sturgeon**, **Yanling Ma**, **Marilena Melas**, **Gregory E. Idos**, **Kevin J. McDonnell**, and **Stephen B. Gruber**, University of Southern California, Los Angeles; **Carin R. Espenschied**, **Rachid Karam**, **Holly LaDuca**, **Jill S. Dolinsky**, **Elizabeth Chao**, and **Virginia Speare**, Ambry Genetics, Aliso Viejo; and **Kerry Kingham**, Stanford University School of Medicine, Stanford, CA.

REFERENCES

1. Oliveira C, Sousa S, Pinheiro H, et al: Quantification of epigenetic and genetic 2nd hits in CDH1 during hereditary diffuse gastric cancer syndrome progression. Gastroenterology 136:2137-2148, 2009
2. Pharoah PD, Guilford P, Caldas C: Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 121:1348-1353, 2001
3. Kaurah P, MacMillan A, Boyd N, et al: Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. JAMA 297:2360-2372, 2007
4. Fitzgerald RC, Hardwick R, Huntsman D, et al: Hereditary diffuse gastric cancer: Updated consensus guidelines for clinical management and directions for future research. J Med Genet 47:436-444, 2010 [Erratum: J Med Genet 48:216, 2011]
5. 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 52:361-374, 2015
6. Richards S, Aziz N, Bale S, et al: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405-424, 2015
7. Karam R, Carvalho J, Bruno I, et al: The NMD mRNA surveillance pathway downregulates aberrant E-cadherin transcripts in gastric cancer cells and in CDH1 mutation carriers. Oncogene 27:4255-4260, 2008
8. Karam R, Wengrod J, Gardner LB, et al: Regulation of nonsense-mediated mRNA decay: Implications for physiology and disease. Biochim Biophys Acta 1829:624-633, 2013
9. Masciari S, Larsson N, Senz J, et al: Germline E-cadherin mutations in familial lobular breast cancer. J Med Genet 44: 726-731, 2007
10. Jonsson BA, Bergh A, Stattin P, et al: Germline mutations in E-cadherin do not explain association of hereditary prostate cancer, gastric cancer and breast cancer. Int J Cancer 98:838-843, 2002
11. Suriano G, Yew S, Ferreira P, et al: Characterization of a recurrent germ line mutation of the E-cadherin gene: Implications for genetic testing and clinical management. Clin Cancer Res 11:5401-5409, 2005
12. Norton JA, Ham CM, Van Dam J, et al: CDH1 truncating mutations in the E-cadherin gene: An indication for total gastrectomy to treat hereditary diffuse gastric cancer. Ann Surg 245:873-879, 2007
13. 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 12:503-507, 2013
14. Benusiglio PR, Malka D, Rouleau E, et al: CDH1 germline mutations and the hereditary diffuse gastric and lobular breast cancer syndrome: A multicentre study. J Med Genet 50:486-489, 2013
15. 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 41:508-517, 2004

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.

16. Humar B, Toro T, Graziano F, et al: Novel germline CDH1 mutations in hereditary diffuse gastric cancer families. Hum Mutat 19:518-525, 2002

17. Schrader KA, Masciari S, Boyd N, et al: Hereditary diffuse gastric cancer: Association with lobular breast cancer. Fam Cancer 7:73-82, 2008

18. Oliveira C, Senz J, Kaurah P, et al: Germline CDH1 deletions in hereditary diffuse gastric cancer families. Hum Mol Genet 18:1545-1555, 2009

19. Kurian AW, Hare EE, Mills MA, et al: Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. J Clin Oncol 32:2001-2009, 2014

20. Schneider KA, DiGianni LM, Patenaude AF, et al: Accuracy of cancer family histories: Comparison of two breast cancer syndromes. Genet Test 8:222-228, 2004

21. Mitchell RJ, Brewster D, Campbell H, et al: Accuracy of reporting of family history of colorectal cancer. Gut 53: 291-295, 2004

22. Sijmons RH, Boonstra AE, Reefhuis J, et al: Accuracy of family history of cancer: Clinical genetic implications. Eur J Hum Genet 8:181-186, 2000

23. Sugimoto S, Yamada H, Takahashi M, et al: Early-onset diffuse gastric cancer associated with a de novo large genomic deletion of CDH1 gene. Gastric Cancer 17:745-749, 2014

24. Shah MA, Salo-Mullen E, Stadler Z, et al: De novo CDH1 mutation in a family presenting with early-onset diffuse gastric cancer. Clin Genet 82:283-287, 2012

25. Antoniou A, Pharoah PD, Narod S, et al: Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: A combined analysis of 22 studies. Am J Hum Genet 72: 1117-1130, 2003

26. Petridis C, Shinomiya I, Kohut K, et al: Germline CDH1 mutations in bilateral lobular carcinoma in situ. Br J Cancer 110:1053-1057, 2014

Downloaded from ascopubs.org by 108.90.43.98 on April 8, 2017 from 108.090.043.098
Copyright © 2017 American Society of Clinical Oncology. All rights reserved.