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ORIGINAL REPORT

Somatic Mutations and Deletions of the E-Cadherin Gene Predict Poor Survival of Patients With Gastric Cancer

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See accompanying editorial on page 838; listen to the podcast by Dr Ford at www.jco.org/podcasts

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ABSTRACT

Purpose
The prognosis of gastric cancer (GC) is poor, and the molecular pathogenesis players are vastly unknown. Surgery remains the primary option in GC treatment. The aim of this study was to investigate the impact of somatic *CDH1* alterations in prognosis and survival of patients with GC.

Patients and Methods
A series of patients with sporadic and familial GC (diffuse and intestinal; n = 246) were analyzed for somatic *CDH1* mutations, promoter hypermethylation, and loss of heterozygosity (LOH) by polymerase chain reaction sequencing. E-cadherin protein expression was determined by immunohistochemistry. Associations between molecular, clinicopathologic, and survival data were analyzed.

Results
CDH1 somatic alterations were found in approximately 30% of all patients with GC. Both histologic types of sporadic GC displayed LOH in 7.5%, mutations in 1.7%, and hypermethylation in 18.4% of patients. Primary tumors from hereditary diffuse GC, lacking germline *CDH1* alterations, showed exclusively *CDH1* promoter hypermethylation in 50% of patients. Familial intestinal GC (FIGC) tumors showed LOH in 9.4% and hypermethylation in 17.0%. *CDH1* alterations did not associate with a particular pattern of E-cadherin expression. Importantly, the worst patient survival rate among all GCs analyzed was seen in patients with tumors carrying *CDH1* structural alterations, preferentially those belonging to FIGC families.

Conclusion
CDH1 somatic alterations exist in all clinical settings and histotypes of GC and associate with different survival rates. Their screening at GC diagnosis may predict patient prognosis and is likely to improve management of patients with this disease.

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INTRODUCTION

Gastric cancer (GC) is the second leading cause of cancer-related deaths worldwide, affecting close to one million people per year.[1] The majority of patients with GC present with advanced disease at diagnosis (stages III and IV), rendering the prognosis extremely poor, with a 5-year overall survival rate of less than 25%.[2-4] Most patients with GC are asymptomatic during the early stages of disease, thus delaying the initial diagnosis. Although, some studies have pinpointed improvements on patient survival with perioperative and adjuvant treatment modalities, surgical resection is still the primary curative treatment for localized GC; however, less than 50% of patients are eligible for resection.[5,6] During the last decade, several genetic and epigenetic changes underlying gastric carcinogenesis have been elucidated.[7,8] Nonetheless, their clinical usefulness is limited because of the lack of systematic genetic-clinical correlations.

GC is histologically classified into two major types, intestinal and diffuse.[9] The intestinal type is characterized by well-differentiated glandular structures, whereas the diffuse type consists of individually infiltrating neoplastic cells that may have signet ring morphology, does not form glandular structures, and has a poorer patient prognosis.[10,11] The majority of GCs (90%) appear in a sporadic setting. The remaining 10% of GCs show familial clustering, and of these, only 1% to 3% constitute hereditary forms.[12] Among GCs with familial aggregation and with histology information, the following specific syndromes can be identified: familial intestinal GC

(FIGC) and hereditary diffuse GC (HDGC; Online Mendelian Inheritance in Man No. 137215).[13] So far, no germline defects have been associated with FIGC, whereas germline mutations and deletions of E-cadherin (*CDH1*) are the underlying genetic defect in 45% of families with clinical diagnosis of HDGC.[14-16]

Furthermore, 70% of *CDH1* mutation–negative HDGC probands display germline monoallelic *CDH1* RNA downregulation (allelic imbalance) reinforcing the role of *CDH1* locus in this disease.[17] HDGC tumors appear when complete somatic *CDH1* inactivation is acquired, leading to reduced or absent E-cadherin expression.[18,19] This occurs through second hit mechanisms, pursuing the Knudson's model of tumor suppressor gene inactivation.[20,21] *CDH1* promoter hypermethylation is the most frequent second hit inactivation mechanism in HDGC primary tumors (50% to 70% of tumors), whereas a second mutation or deletion (loss of heterozygosity [LOH]/intragenic deletions) was less frequently identified.[22-24]

In the sporadic context, the frequency of *CDH1* somatic mutations is controversial, and percentages between 3% and greater than 50% have been reported for GCs with a diffuse component.[25-28] LOH at the *CDH1* locus was noted in diffuse and intestinal histotypes, with rates ranging from 11% to 39% and 36% to 46%, respectively.[29-31] Epigenetic silencing through *CDH1* promoter hypermethylation has been reported in frequencies that vary from 50% to 83% in diffuse tumors and from 6.25% to 50% in intestinal tumors.[28-32] Importantly, the presence of concomitant *CDH1* inactivation mechanisms was rarely described in this setting.[28,29,33] Nevertheless, most of these studies have been limited by the series size, the type of molecular mechanisms studied, different methodologic approaches, and the lack of extensive correlations with clinical parameters, thus hampering potential translation of genetic data into medical practice.[34]

The goal of this study was to analyze the impact of somatic *CDH1* alterations in prognosis and survival of patients with GC. In this study, we performed a comprehensive analysis of somatic *CDH1* mutations, LOH, and promoter hypermethylation in 246 patients with sporadic and familial GC. Additionally, we analyzed the relationship between somatic alterations underlying *CDH1* inactivation and survival rates, clinicopathologic characteristics of patients with GC, and E-cadherin expression in tumors.

PATIENTS AND METHODS

Patients

All 246 patients with GC enrolled onto this study were admitted at the Division of General Surgery and Surgical Oncology, University of Siena (Siena, Italy) and were classified as having sporadic (n = 174) or familial (n = 72) GC. Patients with familial GC were classified as having HDGC (n = 19) or FIGC (n = 53) using clinical criteria defined by the International Gastric Cancer Linkage Consortium[13] (Appendix Table A1, online only). The presence of *CDH1* germline mutations was discarded in all patients with HDGC in this cohort. Patients with GC classification more than pT1N+ were candidates for adjuvant chemotherapy using standard protocols. Prospectively collected clinicopathologic and follow-up data were available. The median follow-up time for surviving patients was 92.1 months. Informed consent was obtained from all patients, and the study was approved by the hospital's ethics committee (Appendix, online only).

DNA Extraction

Tissue areas for DNA extraction were histologically verified to contain a minimum of 70% to 80% of neoplastic cells. DNA was isolated from frozen material using the Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN).

CDH1 *Promoter Hypermethylation Analysis*

CDH1 promoter methylation analysis was carried out in 160 base pairs upstream of the translation start site, encompassing 17 CpG sites, as previously described[23] (Appendix, online only). Primer sequences are listed in Appendix Table A2 (online only).

CDH1 *Somatic Mutation Screening*

Fifty nanograms of tumor DNA from 86 diffuse GCs were subjected to mutation screening at the *CDH1* hotspot region (exons 7 to 10), as previously described.[23] Each mutation was confirmed by an independent polymerase chain reaction amplification followed by sequencing analysis. Primer sequences are listed in Appendix Table A2 (online only).

LOH Analysis

LOH analysis was performed using three intragenic *CDH1* markers (the promoter −161C/A [rs16260], the exon 13 2076T/C [rs1801552], and the 3′-untranslated region single-nucleotide polymorphisms [rs1801026]) and three proximal and distal microsatellite markers (D16S3025, D16S496, and D16S3067) flanking the *CDH1* locus, as described.[23] Only informative markers were considered for LOH analysis, and positive samples were repeated twice (Appendix). Primer sequences are listed in Appendix Table A2 (online only).

E-Cadherin Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues comprising 207 GCs were stained with mouse E-cadherin monoclonal antibody HECD-1 (dilution 1:200; Zymed, San Francisco, CA) followed by incubation with avidin-biotin complex and diaminobenzidine. E-cadherin immunoreactivity was evaluated on tumor and normal tissues considering the predominant expression pattern—normal (complete membrane staining), aberrant (cytoplasmic and heterogeneous staining), or absent (no staining).

Statistical Analysis

Analyses were performed using commercially available statistical software (SPSS, version 14.0; SPSS, Chicago, IL). Statistical associations between clinicopathologic characteristics and *CDH1* somatic alterations were assessed using the χ^2 test for categorical variables and the *t* test or analysis of variance for continuous variables. Survival curves were estimated using the Kaplan-Meier method and were compared using the log-rank test. Multivariate analysis was performed using a Cox proportional hazards regression model by considering the following risk factors: sex, age (older *v* the median age or younger), tumor location (other *v* antrum), Lauren histotype (nonintestinal *v* intestinal), depth of tumor invasion (pT2-4 *v* pT1), lymph node involvement (pN1-3 *v* pN0), presence of systemic metastasis (M1 *v* M0), and R category (R1 or R2 *v* R0). Postoperative mortality was assessed, with deaths unrelated to tumor recurrence considered censored observations at the time of death. $P < .05$ was considered statistically significant.

RESULTS

After screening of somatic *CDH1* promoter hypermethylation, LOH, and mutations in exons 7 to 10, GCs were clustered in the following groups regarding *CDH1* alterations: patients with methylation only (named epigenetic); patients with LOH or mutation, with/without methylation (named structural); and patients without *CDH1* alterations (named negative).

CDH1 *Structural and Epigenetic Alterations in Overall GC*

Overall, 77 (31.3%) of 246 GCs carried somatic *CDH1* alterations. Epigenetic alterations were found in 51 (20.7%) of 246 GCs, and structural alterations were detected in 26 (10.6%) of 246 GCs. Specifically within structural alterations, LOH alone was detected in 18

Table 1. Epigenetic and Structural *CDH1* Alterations in Patients With Sporadic and Familial GCs

Setting and Histologic Type	No. of Patients	*CDH1* Alterations											
		Epigenetic Methylation Only		Structural						Overall Frequency of *CDH1* Alterations*		Negative Patients	
				LOH Only		Mutation Only		Concomitant					
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sporadic													
Intestinal	107	15	14.0	10	9.3	0†	0.0	3‡	2.8	28	26.2	79	73.8
Diffuse/mixed	67	17	25.4	3	4.5	3	4.5	0	0.0	23	34.3	44	65.7
Total	174	32	18.4	13	7.5	3	1.7	3	1.7	51	29.3	123	70.7
Familial													
FIGC§	53	9	17.0	5	9.4	0†	0.0	2‡	3.8	16	30.2	37	69.8
HDGC§	19	10	52.6	0	0.0	0	0.0	0	0.0	10	52.6	9	47.4
Total	72	19	26.4	5	6.9	0	0.0	2	2.8	26	36.1	46	63.9
Total	246	51	20.7	18	7.3	3	1.2	5	2.0	77	31.3	169	68.7

Abbreviations: FIGC, familial intestinal gastric cancer; GC, gastric cancer; HDGC, hereditary diffuse gastric cancer; LOH, loss of heterozygosity.
*This frequency was calculated assuming that intestinal GCs were negative for somatic *CDH1* mutations (three of 174 GCs; 1.7%).
†Intestinal GCs were assumed to lack *CDH1* somatic mutations.
‡Methylation and LOH occurred concomitantly.
§A statistically significant association was found when comparing FIGCs or HDGCs for three classes of alterations—patients with epigenetic alterations only, patients with structural alterations, and negative patients ($P = .005$, Fisher's exact test; Appendix Table A3, online only)—because more than 50% of HDGC tumors carry *CDH1* promoter methylation. LOH was defined when the LOH index was greater than or less than 1.04 ± 0.13 for D16S3025, 1.0 ± 0.67 for D16S496, and 1.06 ± 0.11 for D16S3067 as reference.

(7.3%) of 246 GCs, mutation alone was found in three (1.2%) of 246 GCs (c.1109A>G [p.Asp370Gly], c.IVS9+5G>A, and c.1105_1106 insACCAAC), and five (2%) of 246 GCs presented LOH concomitantly with *CDH1* promoter hypermethylation (Table 1, Appendix Figs A1 and A2, online only).

Patients with tumors with *CDH1* structural alterations displayed poorer overall survival ($P = .017$; Fig 1; all cases) than patients with negative tumors or tumors carrying *CDH1* epigenetic alterations.

CDH1 *Structural and Epigenetic Alterations in Different Clinical Settings and Histologic Types of GC*

We evaluated the distribution of somatic *CDH1* alterations among clinical settings (sporadic and familial) and histotypes (intestinal and diffuse) of GC and correlated these findings with overall patient survival. The overall frequency of *CDH1* alterations was similar in sporadic (51 of 174 tumors; 29.3%) and familial (26 of 72 tumors; 36.1%) tumors (Table 1). Specifically within the sporadic

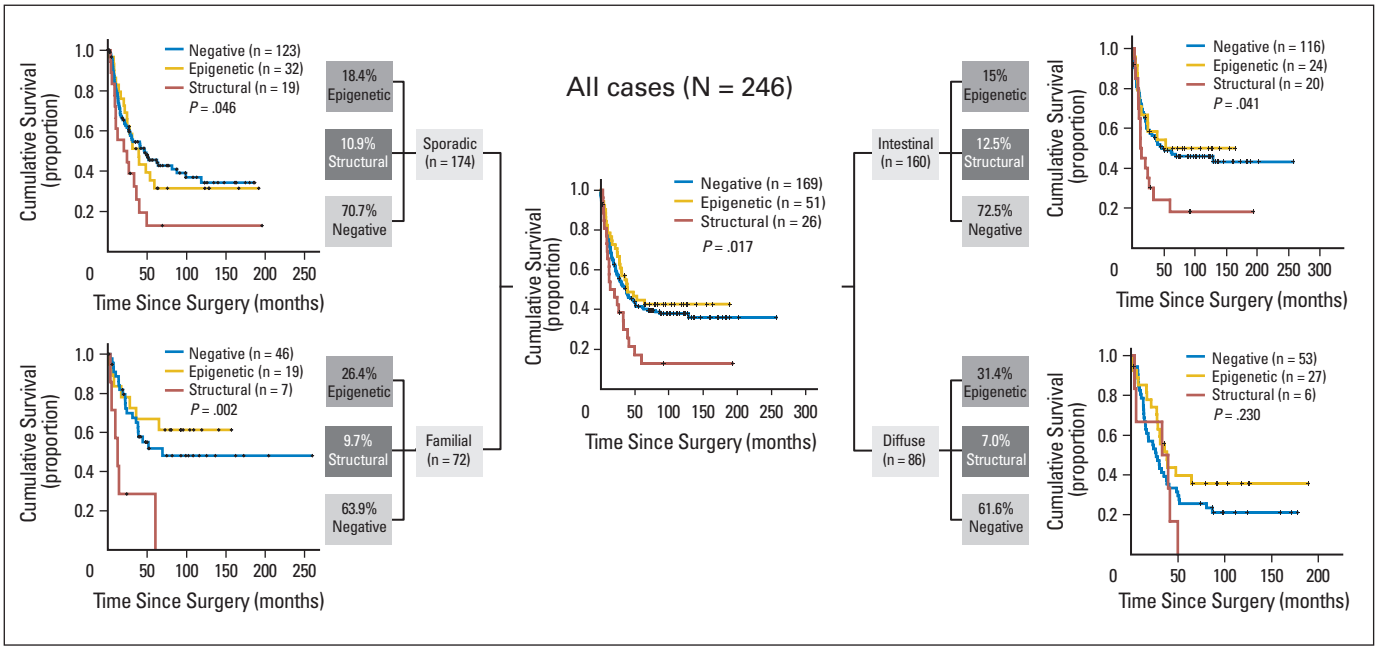

Fig 1. Kaplan-Meier curves showing the probability of overall survival for patients with gastric cancer (GC), according to *CDH1* alterations (epigenetic, structural, or negative) and stratified according to GC clinical settings (sporadic or familial) and histologic type (intestinal or diffuse).

Table 2. Epigenetic and Structural *CDH1* Alterations in Intestinal and Diffuse GCs

		CDH1 Alterations							Overall Frequency of *CDH1* Alterations				
		Epigenetic		Structural		Negative					Negative		
GC Histotype	No. of Patients	No.	%	No.	%	No.	%	*P*	No.	%	No.	%	*P*
Intestinal	160	24	15.0	20	12.5	116	72.5	.007	44	27.5	116	72.5	.108
Diffuse	86	27	31.4	6	7.0	53	61.6		33	38.4	53	61.6	

Abbreviation: GC, gastric cancer.

setting, 32 (18.4%) of 174 GCs displayed epigenetic alterations and 19 (10.9%) of 174 GCs presented structural alterations. Within the familial setting, 19 (26.4%) of 72 GCs harbored epigenetic alterations, whereas seven (9.7%) of 72 GCs displayed structural alterations (Table 1; Appendix Table A3, online only). No significant correlation was observed between GC clinical settings and overall/specific *CDH1* alterations ($P > .05$; Appendix Table A4, online only). However, patients with tumors with *CDH1* structural alterations, from both sporadic and familial settings, had a poorer survival rate than patients with negative tumors or with *CDH1* epigenetic alterations ($P = .046$ and $P = .002$, respectively; Fig 1).

Regarding GC histotypes independent of the clinical setting, the overall frequency of *CDH1* alterations was 27.5% (44 of 160 GCs) in intestinal GCs and 38.4% (33 of 86 GCs) in diffuse GCs (Table 2). Whereas within intestinal tumors, 15% carried epigenetic alterations and 12.5% carried structural alterations, in diffuse tumors, 31.4% carried epigenetic alterations and 7% carried structural alterations. Although diffuse tumors carried epigenetic alterations more often than structural alterations ($P = .007$; Table 2), these molecular features did not confer different overall survival ($P = .230$, Fig 1). In contrast, and although intestinal tumors carried similar frequencies of epigenetic and structural alterations, Kaplan-Meier plots showed that patients with intestinal tumors carrying structural alterations had a lower probability of survival than patients with intestinal tumors negative for or carrying *CDH1* epigenetic alterations ($P = .041$; Fig 1). In fact from the 26 GCs with structural alterations, 20 (76.9%) were of the intestinal type ($P = .007$; Table 3).

We observed that within sporadic and familial settings, patients with tumors carrying structural alterations had worse overall survival. Moreover, we also verified that patients with intestinal-type tumors carrying structural alterations, independent of the clinical setting, had worse overall survival. Therefore, we next assessed whether the worse overall survival of patients with intestinal tumors with structural alterations was a feature of familial and/or sporadic settings.

Intestinal tumors belonging to sporadic and familial (FIGC) settings did not differ in the frequency of epigenetic (14% sporadic, 17% FIGC) and structural alterations (12.1% sporadic, 13.2% FIGC; Table 1, Appendix Table A3, Fig 2). Nonetheless, the Kaplan-Meier plots showed statistical differences in terms of overall survival only for patients belonging to FIGC families. The patients with FIGC carrying tumors with structural alterations had the poorest survival rate ($P < .001$; Fig 2). Although it did not reach statistical significance ($P = .148$), patients with intestinal tumors belonging to the sporadic setting and carrying structural alterations also had a poorer survival rate compared with patients carrying negative/epigenetic alterations (Table 1, Appendix Table A3, Fig 2).

Within diffuse-type tumors, tumors belonging to the sporadic setting displayed epigenetic (25.4%) and structural (8.9%) alterations, whereas HDGC tumors (52.6%) harbored exclusively epigenetic alterations (Table 1, Appendix Table A3). Kaplan-Meier plots showed no association between the type of *CDH1*-specific alterations and overall survival of patients with sporadic diffuse or HDGC tumors ($P = .259$ and $P = .631$, respectively; Fig 2). However, despite the lack of statistical significance, the few patients with sporadic diffuse-type tumors carrying structural alterations had the lowest survival rates ($P = .259$; Fig 2).

CDH1 *Structural and Epigenetic Alterations and Clinicopathologic Features of GC*

We next tried to understand whether the presence of overall or specific *CDH1* alterations correlated with clinicopathologic parameters of tumors and patients (Table 3). This analysis revealed that patients with tumors carrying overall *CDH1* alterations displayed significantly more frequently lymph node metastases ($P = .021$) and more advanced tumors ($P = .029$), in particular invasive stage III. The other clinicopathologic parameters, including age, sex, liver or peritoneal metastases, radicality of resection, extent of gastrectomy, lymphadenectomy, depth of invasion, familial aggregation, and even Lauren histotype, were not significantly associated with the presence of overall *CDH1* alterations (Table 3).

When considering the specific type of *CDH1* alterations in the different GC groups (epigenetic *v* structural *v* negative), we observed that patients carrying tumors displaying epigenetic *CDH1* alterations more frequently had the diffuse histotype ($P = .007$) and 84.3% had lymph node metastases ($P = .02$). We also observed that patients carrying tumors with structural *CDH1* alterations more frequently had intestinal histotype (20 of 26 patients, 76.9%; $P = .007$) and were mainly males (23 of 26 patients, 88.5%; $P = .004$), and 46.1% had been submitted to R1/2 resections (compared with 21.6% with epigenetic alterations and 24.8% negative for alterations; $P = .09$; Table 3).

CDH1 *Structural and Epigenetic Alterations and E-Cadherin Immunoexpression Pattern*

We also evaluated E-cadherin expression and correlated it with tumor histology and *CDH1* alterations. Of 207 patients analyzed, 73.4% showed aberrant expression, 14.5% displayed complete loss of E-cadherin, and 12.1% retained the protein at the cell membrane (Table 4). In both sporadic and familial settings, there was a significant

Table 3. Comparison of Clinicopathologic Variables With *CDH1* Alterations in Patients With GC

Variable	Total No. of Patients (N = 246)	*CDH1* Overall Alterations					*CDH1* Epigenetic and Structural Alterations					
		Positive (n = 77)		Negative (n = 169)			Epigenetic (n = 51)		Structural (n = 26)		Negative (n = 169)		
		No. of Patients	%	No. of Patients	%	*P*	No. of Patients	%	No. of Patients	%	No. of Patients	%	*P*
Age, years													
Mean	68.05	69.1		67.6			69.7		67.9		67.6		
Standard deviation	10.9	11.8		10.4			13.3		8.5		10.4		
Sex						.110							.004
Male	143	51	66.2	92	54.4		28	54.9	23	88.5	92	54.4	
Female	103	26	33.7	77	45.6		23	45.1	3	11.5	77	45.6	
Liver or peritoneal metastases						.920							.218
Present	20	6	7.8	14	8.3		2	3.9	4	15.4	14	8.3	
Absent	226	71	92.2	155	91.7		49	96.1	22	84.6	155	91.7	
Radicality of resection*						.502							.049
R0	181	54	70.1	127	75.1		40	78.4	14	53.8	127	75.1	
R1/2	65	23	29.9	42	24.8		11	21.6	12	46.1	42	24.8	
Extent of gastrectomy						.680							.100
Partial	150	45	58.4	105	62.1		34	66.7	11	42.3	105	62.1	
Total	96	32	41.6	64	37.9		17	33.3	15	57.7	64	37.9	
Lymphadenectomy						1.0							.737
D1	91	28	36.4	63	37.3		17	33.3	11	42.3	63	37.3	
D2/3	155	49	63.6	106	62.7		34	66.7	15	57.7	106	62.7	
Lauren histotype						.108							.007
Intestinal	160	44	57.1	116	68.6		24	47.1	20	76.9	116	68.6	
Diffuse	86	33	42.9	53	31.4		27	52.9	6	23.1	53	31.4	
Depth of invasion						.436							.695
pT1	17	3	3.9	14	8.3		2	3.9	1	3.8	14	8.3	
pT2	93	31	40.3	62	36.7		22	43.1	9	34.6	62	36.7	
pT3-4	136	43	55.8	93	55.0		27	52.9	16	61.5	93	55.0	
Lymph node involvement						.021							.042
Absent (pN0)	72	15	19.5	57	33.7		8	15.7	7	26.9	57	33.7	
Present (pN+)	173	62	80.5	111	65.7		43	84.3	19	73.1	111	65.7	
Stage						.029							.155
I	57	10	13	47	27.8		6	11.8	4	15.4	47	27.8	
II	41	15	19.5	26	15.4		10	19.6	5	19.2	26	15.4	
III	106	41	53.2	65	38.5		28	54.9	13	50.0	65	38.5	
IV	40	10	13	30	17.8		6	11.8	4	15.4	30	17.8	
GC familial aggregation						.371							.372
Positive	72	26	33.8	46	27.2		19	37.3	7	26.9	46	27.2	
Negative	174	51	66.2	123	72.8		32	62.7	19	73.1	123	72.8	
E-cadherin IHC†						.275							.479
Absent	30	12	19.0	18	12.5		8	20	4	17.4	18	12.5	
Aberrant	152	46	73.0	106	73.6		30	75	16	69.6	106	73.6	
Normal	25	5	7.9	20	13.9		2	5	3	13.0	20	13.9	

Abbreviations: GC, gastric cancer; IHC, immunohistochemistry.
*R0 resection indicates a microscopically margin-negative resection.[35] R1/R2 resections indicate different grades of positivity for tumor after surgery (primary tumor, regional nodes, and microscopic/macroscopic margin involvement).[35]
†Only 207 patients were analyzed for IHC; the percentages of *CDH1*-positive, -negative, epigenetic, and structural alterations were calculated by adjusting the total number of alterations to 63, 144, 40, and 23, respectively.

association between complete loss of E-cadherin expression and diffuse histology (sporadic: $P < .001$; familial: $P = .017$; Table 4).

In addition, there were no differences when comparing E-cadherin immunohistochemistry (IHC) pattern in GCs carrying overall or specific *CDH1* alterations with the pattern in those without alterations ($P = .479$ and $P = .275$, respectively; Table 3). These results revealed that the *CDH1* alterations analyzed do not generate a specific E-cadherin IHC pattern and, more importantly, that 60% of GCs without E-cadherin expression and approximately 70% of GCs with aberrant expression are negative for *CDH1* alterations (Appendix Table A5, online only).

DISCUSSION

GC is a highly heterogeneous disease where even similar clinical and pathologic features lead to distinct outcomes.[7,9,11,36] These observations indicated that staging systems, based on clinical and pathologic

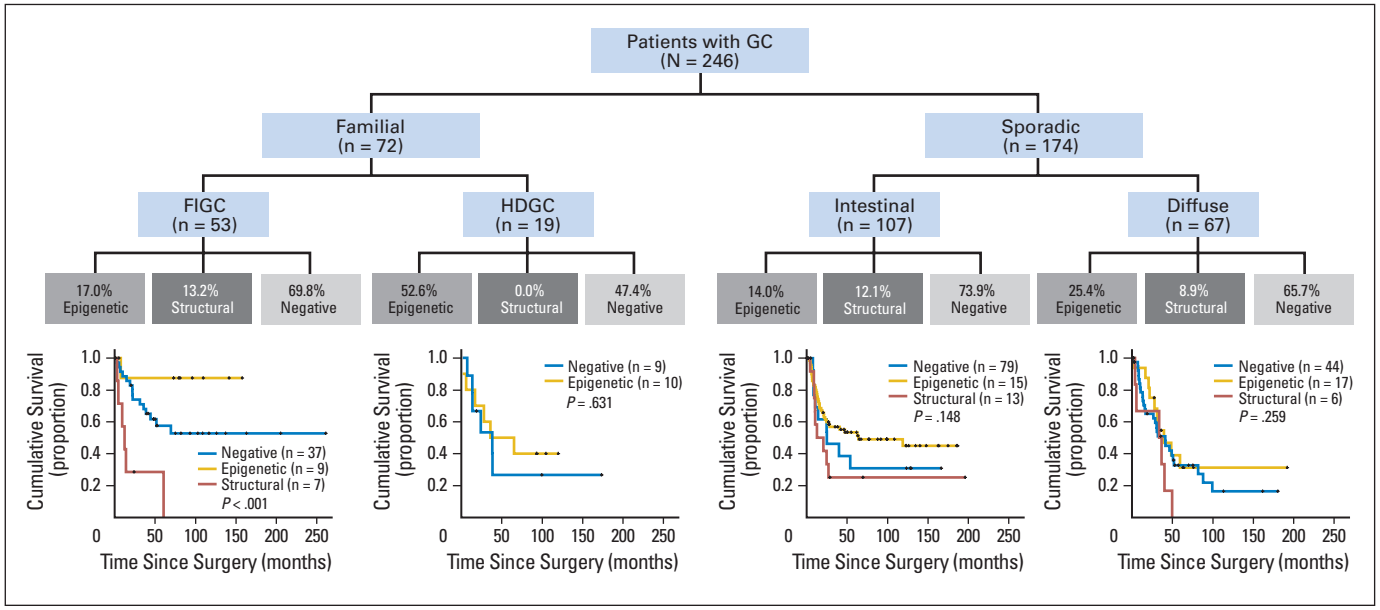

Fig 2. Details of the molecular analysis performed in gastric cancer (GC) samples and survival rates of patients with GC. Kaplan-Meier curves show the probability of overall survival for patients with familial intestinal GC (FIGC), hereditary diffuse GC (HDGC), sporadic intestinal GC, and sporadic diffuse GC according to *CDH1* alterations (epigenetic, structural, or negative).

findings, may have reached their limit of usefulness and impelled the need for molecular biomarkers, as an added value, to predict patients' outcome and treatment.

The present study encompasses a large series of sporadic and familial GCs (negative for CDH1 germline mutations) systematically analyzed for somatic *CDH1* structural and epigenetic inactivating mechanisms. Furthermore, this study systematically correlates the molecular data with patient survival, clinicopathologic parameters, and E-cadherin IHC expression.

The main findings of the present study indicate the following: somatic *CDH1* alterations occur in approximately 30% of all GCs analyzed (approximately 20% epigenetic, approximately 10% structural); *CDH1* structural alterations underlie the worst survival rate of patients with GC overall; patients with FIGC with tumors with structural alterations had the worst overall survival rate; HDGC tumors harbored exclusively *CDH1* promoter hypermethylation (approximately 50%); specific *CDH1* alterations underlie distinct GC clinicopathologic features; and overall or specific *CDH1* alterations are not associated with a distinct pattern of E-cadherin immunoexpression. These and other findings will be discussed in more detail here.

Table 4. E-Cadherin Immunoexpression in Sporadic and Familial GCs

GC Setting and Histologic Type	No. of Patients	E-Cadherin Immunoexpression						*P*
		Absent		Aberrant		Normal		
		No.	%	No.	%	No.	%	
Sporadic	147							<.001
Intestinal	95	4	4.2	74	77.9	17	17.9	
Diffuse/mixed	52	14	26.9	36	69.2	2	3.8	
Familial	60							.017
FIGC	43	5	11.6	32	74.4	6	14	
HDGC	17	7	41.2	10	58.8	0	0	
Total	207	30	14.5	152	73.4	25	12.2	

NOTE. Scoring criteria were as follows: absent, E-cadherin not expressed; aberrant, cytoplasmic or heterogeneous staining of E-cadherin; and normal, complete E-cadherin membrane staining.
Abbreviations: FIGC, familial intestinal gastric cancer; GC, gastric cancer; HDGC, hereditary diffuse gastric cancer.

The results herein reported may change the paradigm thus far described for *CDH1* alterations in GC. In the sporadic context, E-cadherin abnormalities have been, to date, mainly associated with the diffuse type of GC, and *CDH1* somatic mutations and promoter methylation have each been reported in more than 50% of these patients.[26,27,29,33,34,37] In diffuse GCs, we report epigenetic alterations (methylation only) in 25.4%, somatic mutations in 4.5%, and LOH in 4.5% (structural alterations). These frequencies are lower than most earlier reported frequencies, but are similar to those recently reported in several independent series.[28,30,31] Discrepancies are most likely a result of different methodologies used. Interestingly, we demonstrate that *CDH1* somatic epigenetic and structural alterations are as frequent in intestinal as in diffuse GC, suggesting histotype independence.

In the familial setting, tumors from HDGC families lacking germline *CDH1* mutations displayed exclusively *CDH1* promoter methylation in frequencies comparable to those previously published for HDGC carriers of *CDH1* germline mutations.[23] We herein report for the first time, to the best of our knowledge, that tumors from FIGC families also present somatic *CDH1* epigenetic and structural alterations in frequencies similar to those of patients with sporadic GC.

Importantly, GCs with epigenetic or structural *CDH1* alterations or negative for *CDH1* alterations revealed an unequal impact on patient survival. Patients with tumors with *CDH1* structural alterations displayed a significantly poorer survival rate than patients with tumors negative for *CDHI* alterations or patients with tumors with

epigenetic *CDH1* alterations. In line with these findings, Gamboa-Dominguez et al[38] observed that patients with GC displaying *CDH1* exon 8/9 deletions (structural) have a worse clinical evolution and a shorter overall survival. An adverse prognostic effect of *CDH1* promoter hypermethylation in patients with diffuse GC has also been reported, but no structural alterations were reported in that series.[34]

When we explored in depth the survival rates of patients belonging to different clinical settings and histologic types, the most striking finding was that patients with FIGC with tumors with structural alterations had the worst overall survival. This observation indicates that *CDH1* somatic alterations are a novel and unexpected feature of FIGCs and, more importantly, allows the stratification of patients with FIGC into subsets with completely different clinical outcomes. Available data on familial history associated with survival of patients with GC has remained largely inconclusive.[39-41] One of the latest reports addressing this issue demonstrated a favorable prognosis for patients with a positive family history of GC, whereas others did not.[39-41] These controversial results may have been affected by different strategies in selecting the study population and, mainly, by inconsistent definitions and clinical criteria for family history, precluding comparison of trustworthy results. In light of our own findings, molecular variables such as *CDH1* alterations may be crucial to better define the survival of patients with family history.

Apart from the impact of *CDH1*-specific alterations on survival of patients with GC, these alterations also underlie other clinicopathologic features. Tumors with *CDH1* epigenetic alterations were more often of the diffuse histotype, and patients more frequently displayed lymph node metastases. These observations suggest that superextended lymphadenectomy may be an adequate procedure in these patients.[42] However, patients with tumors with *CDH1* structural alterations more often had the intestinal type (76.9%), and despite the generally accepted better prognosis,[43] these patients had the worst survival, as previously discussed. Furthermore, these patients were mainly men and had been more frequently subjected to R1/2 radical resections, indicating the presence of a more aggressive disease. Therefore, screening of *CDH1* alterations at the biopsy stage, concomitantly with histologic classification and both faster and more radical surgery, may bring benefit to the approximately 10% of patients with GC who belong to this specific group (Appendix Fig A3, online only).

In parallel, we also verified that approximately 68% of patients with altered E-cadherin expression were negative for *CDH1* alterations, showing that IHC, per se, is not an efficient method to infer E-cadherin molecular alterations and indicating other transcriptional/post-transcriptional regulatory mechanisms.[44-47] Following this idea, we have recently reported that miR-101 downregulation with consequent EZH2 upregulation constitutes an additional mechanism by which E-cadherin becomes dysfunctional, mainly in intestinal-type GC retaining allele(s) untargeted by classical *CDH1* inactivating mechanisms.[28]

In conclusion, our data provide, for the first time to our knowledge, a detailed analysis of somatic *CDH1* alterations in different clinical settings and histologic types of GC, highlighting subsets of patients with distinct clinical outcomes. In particular, this work defined a group of patients (FIGC with *CDH1* structural alterations) with the worst prognosis among all GCs analyzed. The presence of *CDH1* epigenetic and structural alterations in a diagnostic/preoperative biopsy may provide clinical utility and improve patient management, particularly to infer the prognosis of GC and the pattern of tumor dissemination.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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