Claudin-18

Patterns of Expression in the Upper Gastrointestinal Tract and Utility as a Marker of Gastric Origin in Neuroendocrine Tumors

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• ***Context.***—**Claudin-18 is expressed in some gastric cancers. Clinical trials are evaluating it as a therapeutic target.**

Objectives.—**To evaluate claudin-18 expression in intestinal metaplasia, dysplasia, and adenocarcinoma of the distal esophagus/gastroesophageal junction and stomach and to evaluate claudin-18 expression in gastric and nongastric neuroendocrine tumors as a marker of gastric origin.**

Design.—**Samples included gastroesophageal junction with intestinal metaplasia (n = 40), dysplasia (n = 54), and adenocarcinoma (n = 20) and stomach with intestinal metaplasia (n = 79), dysplasia (n = 43), and adenocarcinoma (n = 25). Additionally, gastric (n = 40) and nongastric (n = 322) neuroendocrine tumors were included. Claudin-18 expression was evaluated for any staining as positive and by meeting clinical trial inclusion criteria (≥2+ intensity in ≥50% of tumor).**

Results.—**Claudin-18 staining was not significantly different across dysplasia categories in the gastroesophageal junction ($P = .11$) or stomach ($P = .12$). The rate of positive staining was higher in gastroesophageal junction than stomach for intestinal metaplasia (37 of 40 [92.5%] versus 37 of 79 [46.8%]; $P < .001$) and high-grade dysplasia (33 of 38 [86.8%] versus 9 of 16 [56.3%]; $P = .03$). Intestinal metaplasia showed staining in 7 of 37 autoimmune gastritis samples (18.9%) compared with 30 of 42 samples without autoimmune gastritis (71.4%) ($P < .001$). Adenocarcinoma showed similar staining in gastroesophageal junction (15 of 20; 75.0%) and stomach (17 of 25; 68.0%) ($P = .85$). Eighty percent (32 of 40) of gastric neuroendocrine tumors were positive for claudin-18 expression, with 57.5% (23 of 40) meeting clinical trial inclusion criteria. Comparatively, 0.62% (2 of 322) of nongastric neuroendocrine tumors showed staining ($P < .001$).**

Conclusions.—**Claudin-18 staining was similar in intestinal metaplasia, dysplasia, and adenocarcinoma. Claudin-18 was negative in most cases of intestinal metaplasia in autoimmune gastritis, indicating that intestinal metaplasia in this setting may differ from other forms. Claudin-18 was sensitive and specific for gastric origin in neuroendocrine tumors.**

(*Arch Pathol Lab Med.* 2023;147:559–567; doi: 10.5858/arpa.2021-0428-OA)

Gastric cancer is the fifth most common cancer and the fourth leading cause of cancer death worldwide, but gastric cancer mortality has significantly decreased during the last half century.[1] Intestinal metaplasia (IM), gastric dysplasia, and chronic gastritis (eg, *Helicobacter pylori* gastritis and autoimmune metaplastic atrophic gastritis [AMAG]) have been shown to be risk factors for gastric cancer.[2–4] Despite advances in targeted therapy and immunotherapy in various cancers, the survival of patients with advanced gastric cancer has remained dismal, with a median overall survival of approximately 10 months.[5,6]

Antibody-based therapy has become an emerging area of research in the treatment of cancers as targeted agents such as trastuzumab, ramucirumab, and bevacizumab have become treatment options for advanced gastric cancers during the last decade.[7–10] One active area of advanced gastric cancer treatment development is the discovery of

Accepted for publication March 28, 2022.
Published online August 17, 2022.
Supplemental digital content is available for this article at https://meridian.allenpress.com/aplm in the May 2023 table of contents.

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This research was funded through Cedars-Sinai Department of Pathology and Laboratory Medicine internal grants. The authors have no relevant financial interest in the products or companies described in this article.

Presented as 2 posters at the United States and Canadian Academy of Pathology (USCAP) annual meeting; March 2, 2020; Los Angeles, California.

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monoclonal antibodies that are specific to proteins exclusively expressed on tumor cells, thereby decreasing the risk of side effects.[11] One such protein family is the claudins, which are surface proteins that are important components of tight cell junctions and control the flow of molecules between cells.[12] Different subtypes of claudin proteins are expressed differentially across tissue types, including various malignancies.[13]

Claudin-18 was first identified as a novel downstream target gene of the T/EBP/NKX2.1 homeodomain transcription factor, which was found in the lung and stomach of mice.[14] The downregulation of claudin-18 isoform 2 was then observed in gastric cancer with intestinal phenotype and is correlated with reduced expression and poor survival.[15] However, Sahin et al[16] identified isoform 2 of claudin-18 (claudin 18.2) as a highly selective lineage marker for the epithelial cells of gastric mucosa and found that this isoform is expressed in a significant proportion of gastric cancers. Anti–claudin 18.2 antibody IMAB362 (zolbetuximab) was subsequently developed as a potential targeted therapy for gastric adenocarcinomas and displayed antitumor activity by eliminating claudin-18.2–expressing tumor cells through antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity.[11,17] Currently, anti–claudin 18.2 therapy (zolbetuximab) is in phase II/III clinical trials for treatment of advanced gastric and gastroesophageal junction (GEJ) adenocarcinoma.[18–20]

In addition to gastric adenocarcinoma, well-differentiated neuroendocrine tumors (WDNETs) also occur in the stomach. These tumors are rare, but the incidence has been increasing, comprising 1.77% of gastric neoplasms and 8.7% of gastrointestinal (GI) WDNETs.[21] Currently, there are no commonly available immunohistochemical markers of gastric origin for WDNETs.

In this retrospective descriptive study, claudin-18 expression in IM, dysplasia, and adenocarcinoma of the distal esophagus/GEJ and stomach was evaluated for differential expression in these processes. In the course of the present study, it was noted that the hyperplastic enterochromaffin-like cells (ECL cells) in AMAG showed diffuse claudin-18 expression. Therefore, expression of claudin-18 protein in gastric and nongastric WDNETs was also evaluated to assess claudin-18 as a marker of gastric origin in WDNETs. The goal of this study was to describe patterns of claudin-18 expression in precursor lesions, adenocarcinoma, and WDNETs of the upper GI tract.

MATERIALS/SUBJECTS AND METHODS

This study was approved by the Cedars-Sinai Medical Center (Los Angeles, California) and University of Pittsburgh (Pittsburgh, Pennsylvania) institutional review boards. The Cedars-Sinai Medical Center departmental surgical pathology archives were searched to identify biopsy and surgical resection cases of nondysplastic distal esophagus/GEJ IM (n = 40), distal esophagus/GEJ with dysplasia (n = 54; 16 with low-grade dysplasia [LGD] and 38 with high-grade dysplasia [HGD]), and adenocarcinoma (n = 20) in the distal esophagus/GEJ. Resection cases included 10 cases of LGD, 23 cases of HGD, and all 20 cases of adenocarcinoma.

In the stomach, cases of IM without dysplasia (n = 79, including 37 with IM in a background of AMAG), dysplasia (n = 43; 27 with LGD and 16 with HGD), and adenocarcinoma (n = 25) were identified. Resection cases included 6 cases of LGD, 8 cases with HGD, and all 25 cases of adenocarcinoma. Stomach cases were categorized as AMAG based on pathologic diagnosis using histologic features that included oxyntic mucosa with parietal cell loss/atrophy, metaplastic changes (pseudopyloric, intestinal, and pancreatic), lymphoplasmacytic inflammation, and ECL-cell hyperplasia along with relative sparing of the antral mucosa.[22,23] Grade of dysplasia for both the distal esophagus/GEJ and stomach cases was determined based on consensus of 2 fellowship-trained GI pathologists (M.T.W., K.M.W.). Grade of differentiation and Lauren classification of adenocarcinoma were also noted.[24]

In the course of the present study, it was noted that the hyperplastic ECL cells in a slide with AMAG showed diffuse claudin-18 expression. Based on this observation, whole slide samples from 19 gastric WDNETs, including 1 that also had resected tissue from a liver and lymph node metastasis from the Cedars-Sinai Medical Center surgical pathology archives, were added to the study. Tissue microarrays (TMAs) with WDNETs from both Cedars-Sinai Medical Center (n = 101) and the University of Pittsburgh (n = 242) were also used that included 21 additional gastric WDNETs (for a total of 40 gastric WDNETs; average 1.67 cores per case; 35 total cores). These TMAs also included 322 WDNETs (average 1.89 cores per case; 608 total cores) from nongastric sites (pancreas, n = 102; duodenum/ampulla, n = 7; small intestine, n = 99; colorectum, n = 20; appendix, n = 16; and lung, n = 78).

Immunohistochemical detection of claudin-18 was performed on 4-μm tissue sections (a section from each case and TMA) using predilute mouse monoclonal antibody (clone 43-14A, Roche Ventana Medical Systems, Tucson, Arizona). Clone 43-14A detects both claudin 18.1 and 18.2 isoforms. Staining was done on the Ventana Benchmark Ultra (Roche Ventana Medical Systems) automated slide stainer using an onboard heat-induced epitope retrieval method in high-pH buffer. The staining was visualized using the Ventana Optiview DAB Detection System. Membranous staining of claudin-18 was evaluated based on intensity (ranging from 0 to 3+) and proportion of staining (in percentages) in 2 ways. Cases with any membranous staining were considered positive. Cases were also evaluated for whether claudin-18 expression met criteria for inclusion in clinical trials: 2+ or higher staining intensity with reactivity in 50% of lesional cells or more as per the MONO study and the ongoing phase II ILUSTRO trial.[19,25,26] Aberrant cytoplasmic staining of claudin-18 was noted in a small subset of cases, but was not counted as positive.

Statistical analysis was performed using the R statistical programming language (R Foundation, Vienna, Austria). Tests of proportion were used to test for differences in proportion. χ^2 tests were used to test for differences in proportion across multiple categories. Correlation coefficients (r) were calculated to test for correlation between different levels of dysplasia/neoplasia when present on the same slide.

RESULTS

Claudin-18 Expression in the Distal Esophagus/GEJ and Stomach

Normal squamous epithelium of the esophagus did not stain with claudin-18, whereas normal columnar epithelium of both the distal esophagus/GEJ and stomach had strong

Table 1. Claudin-18 Expression in the Distal Esophagus/Gastroesophageal Junction (GEJ) and Stomach			
	Distal Esophagus/GEJ, % (No./Total)	Stomach, % (No./Total)	*P* Value[a]
Positive claudin-18 staining			
Nondysplastic intestinal metaplasia	92.5 (37/40)	46.8 (37/79)	<.001
Dysplasia	77.8 (42/54)	60.5 (26/43)	.10
Low grade	56.3 (9/16)	63.0 (17/27)	.91
High grade	86.8 (33/38)	56.3 (9/16)	.03
Adenocarcinoma	75.0 (15/20)	68.0 (17/25)	.85
P value[b]	.11	.12	
Differentiation of adenocarcinomas			
Well	88.9 (8/9)	100 (1/1)	>.99
Moderate	50.0 (3/6)	90.9 (10/11)	.19
Poor	80.0 (4/5)	46.2 (6/13)	.44
P value[b]	.22	.05	
Lauren classification of adenocarcinomas			
Intestinal	NA	70.0 (14/20)	NA
Diffuse	NA	60.0 (3/5)	NA
P value[c]	NA	>.99	
Positive claudin-18 staining using clinical trial inclusion criteria[19,25,26]			
Nondysplastic intestinal metaplasia	72.5 (29/40)	29.1 (23/79)	<.001
Dysplasia	51.9 (28/54)	27.9 (12/43)	.30
Low grade	37.5 (6/16)	18.5 (5/27)	.31
High grade	57.9 (22/38)	43.8 (7/16)	.51
Adenocarcinoma	50.0 (10/20)	44.0 (11/25)	.92
P value[b]	.09	.32	
Differentiation of adenocarcinomas			
Well	44.4 (4/9)	0.0 (0/1)	>.99
Moderate	50.0 (3/6)	63.6 (7/11)	.98
Poor	60.0 (3/5)	30.8 (4/13)	.77
P value[b]	.69	.18	
Lauren classification of adenocarcinomas			
Intestinal	NA	45.0 (9/20)	
Diffuse	NA	40.0 (2/5)	
P value[c]	NA	>.99	

Abbreviation: NA, not applicable.

[a] Test of proportions comparing distal esophagus/GEJ with stomach.
[b] χ^2 test comparing proportion positive across categories of dysplasia (nondysplasia, dysplasia, and adenocarcinoma) and differentiation.
[c] Test of proportions comparing proportion positive between intestinal and diffuse-type gastric adenocarcinomas.

membranous staining. In the distal esophagus/GEJ, significant differences in staining were not noted across the categories of nondysplastic IM (37 of 40; 92.5%), dysplasia (42 of 54 [77.8%]; 9 of 16 [56.3%] in LGD and 33 of 38 [86.8%] in HGD), and adenocarcinoma (15 of 20; 75.0%) ($P = .11$) or grade of differentiation ($P = .22$; Table 1; Figure 1, A through D). Similarly, no significant differences were noted in the stomach, as there was staining in 37 of 79 nondysplastic IM cases (46.8%), 26 of 43 of dysplasia cases (60.5%; 17 of 27 [63.0%] in LGD, 9 of 16 [56.3%] in HGD), and 17 of 25 adenocarcinoma cases (68.0%) ($P = .12$; Figure 2, A through D), and there were no significant differences noted in gastric adenocarcinoma across grade of differentiation ($P = .05$) or by the Lauren classification ($P > .99$). There were also no significant differences in staining across these categories when using clinical trial criteria in the distal esophagus/GEJ ($P = .09$) and stomach ($P = .32$). More detailed tabulation of the intensity and extent of staining is provided in the supplemental digital content at https://meridian.allenpress.com/aplm in the May 2023 table of contents.

Nondysplastic IM showed staining in 37 of 40 samples (92.5%) from the distal esophagus/GEJ, but in only 37 of 79 samples (46.8%) from the stomach ($P < .001$). This difference was also seen when using clinical trial inclusion criteria (29 of 40 [72.5%] versus 23 of 79 [29.1%]; $P < .001$). Claudin-18 staining in dysplasia was more common in the distal esophagus/GEJ (42 of 54; 77.8%) than in the stomach (26 of 43; 60.5%) ($P = .10$). This finding was largely due to differences in HGD (33 of 38 [86.8%] versus 9 of 16 [56.3%]; $P = .03$). Adenocarcinoma showed similar levels of claudin-18 staining between the distal esophagus/GEJ (15 of 20; 75.0%) and stomach (17 of 25; 68.0%) ($P = .85$). There was also similar staining in adenocarcinoma of the distal esophagus/GEJ (10 of 20; 50.0%) and stomach (11 of 25; 44.0%) when using clinical trial inclusion criteria ($P = .92$).

A portion of the difference in staining of nondysplastic IM between the distal esophagus/GEJ and stomach can be accounted for by the presence or absence of background AMAG in the stomach. Nondysplastic gastric IM showed staining in 7 of 37 samples with AMAG (18.9%) and 30 of 42 samples without AMAG (71.4%) ($P < .001$; Table 2). This difference was limited to nondysplastic IM. There were no significant differences between dysplasia (7 of 14 [50.0%] versus 19 of 29 [65.5%]; $P = .52$) and adenocarcinoma (4 of 6 [66.7%] versus 13 of 19 [68.4%]; $P > .99$) with and without

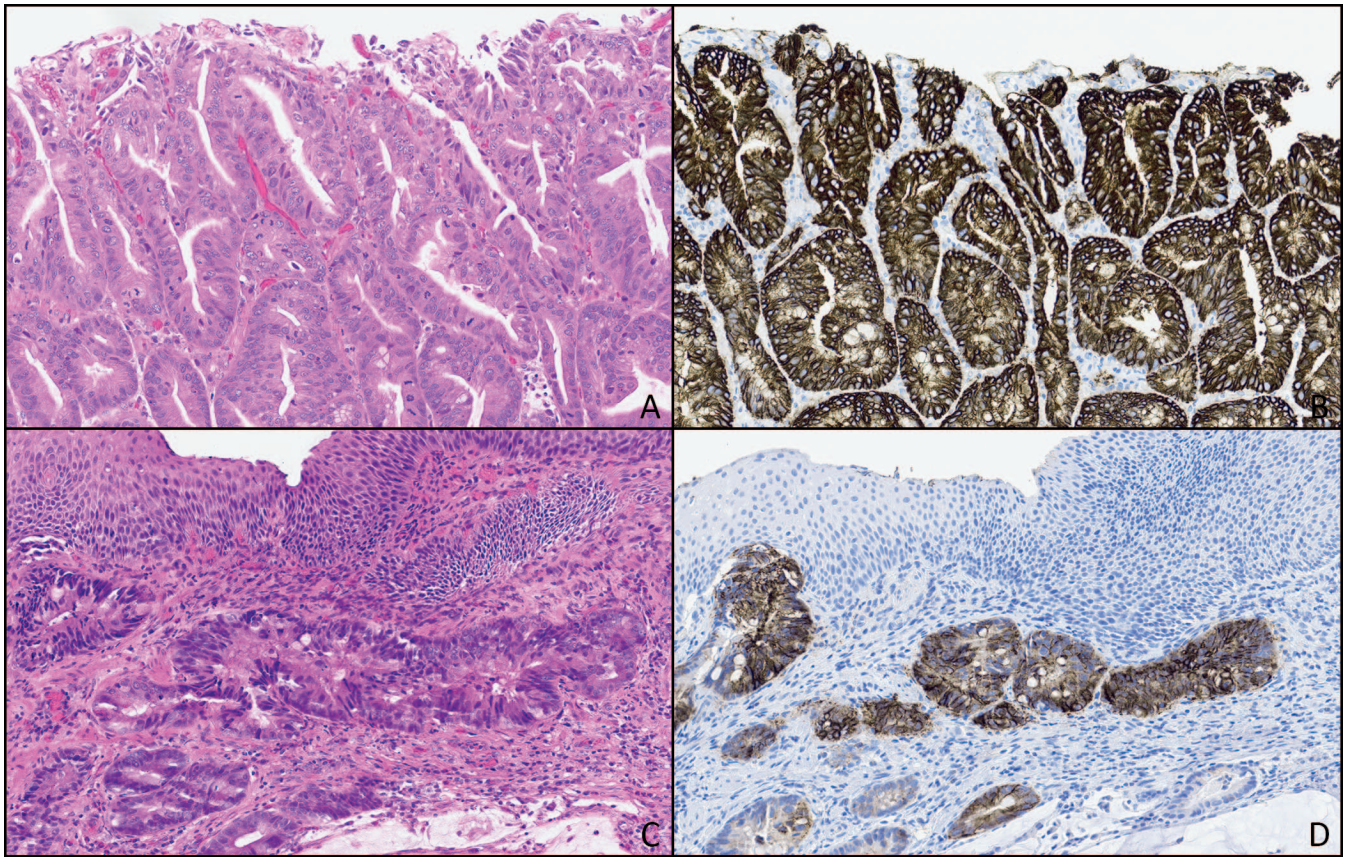

Figure 1. *Claudin-18 expression in the distal esophagus/gastroesophageal junction. Barrett esophagus with high-grade dysplasia (A) and 3+ claudin-18 expression (B). Esophageal adenocarcinoma underlying squamous epithelium (C) with 3+ expression in the majority of glands and no claudin-18 staining in the squamous epithelium (D) (hematoxylin-eosin, original magnifications ×200 [A] and ×100 [C]; claudin-18, original magnifications ×100 [D] and ×200 [B]).*

Table 2. Effect of Background Autoimmune Metaplastic Atrophic Gastritis (AMAG) on Claudin-18 Expression in the Stomach

	AMAG, % (No./Total)	No AMAG, % (No./Total)	*P* Value[a]
Positive claudin-18 staining			
Nondysplastic intestinal metaplasia	18.9 (7/37)	71.4 (30/42)	<.001
Complete intestinal metaplasia	18.9 (7/37)	61.3 (19/31)	
Incomplete intestinal metaplasia	NA (0/0)	100 (11/11)	
P value[b]		.04	
Dysplasia	50.0 (7/14)	65.5 (19/29)	.52
Low grade	37.5 (3/8)	73.7 (14/19)	.18
High grade	66.7 (4/6)	50.0 (5/10)	.90
Adenocarcinoma	66.7 (4/6)	68.4 (13/19)	>.99
P value[c]	.02	.87	
Positive claudin-18 staining using clinical trial inclusion criteria[19,25,26]			
Nondysplastic intestinal metaplasia	10.8 (4/37)	45.2 (19/42)	.002
Complete intestinal metaplasia	10.8 (4/37)	38.7 (12/31)	
Incomplete intestinal metaplasia	NA (0/0)	63.6 (7/11)	
P value[b]		.28	
Dysplasia	21.4 (3/14)	31.0 (9/29)	.77
Low grade	0.0 (0/8)	26.3 (5/19)	.29
High grade	50.0 (3/6)	40.0 (4/10)	>.99
Adenocarcinoma	66.7 (4/6)	36.8 (7/19)	.42
P value[c]	.006	.47	

Abbreviation: NA, not applicable.

[a] Test of proportions comparing AMAG with no AMAG.
[b] Test of proportions comparing complete intestinal metaplasia with incomplete intestinal metaplasia.
[c] χ^2 test comparing proportion positive in nondysplastic intestinal metaplasia, dysplasia, and adenocarcinoma.

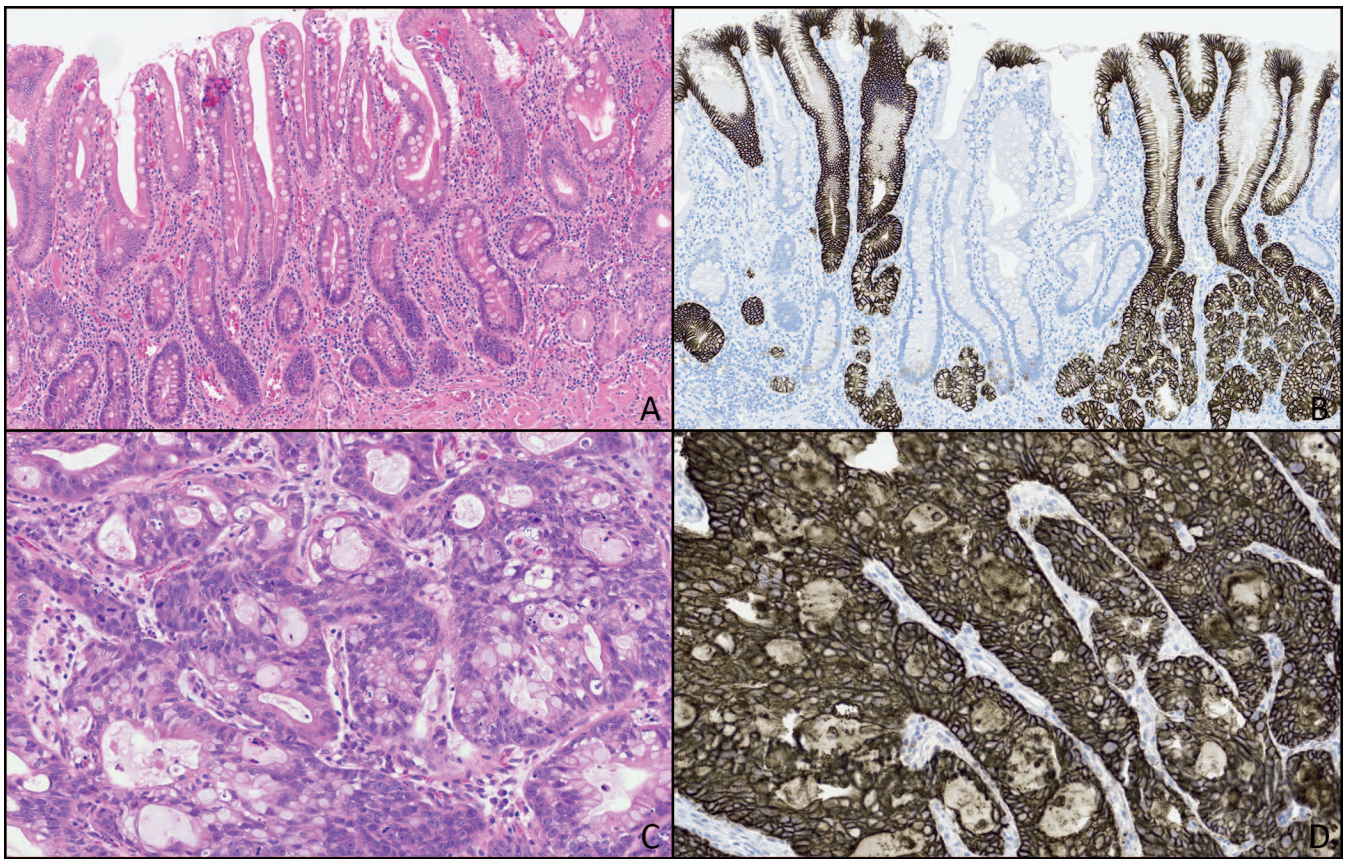

Figure 2. *Claudin-18 expression in the stomach. Gastric intestinal metaplasia in the setting of autoimmune metaplastic atrophic gastritis (A) with no claudin-18 expression in the intestinal metaplasia and 3+ membranous expression in the background gastric foveolar epithelium (B). Gastric adenocarcinoma (C) with 3+ claudin-18 expression (D) (hematoxylin-eosin, original magnifications ×100 [A] and ×200 [C]; claudin-18, original magnifications ×100 [B] and ×200 [D]).*

background AMAG, respectively. The cases with nondysplastic IM in AMAG all had complete IM. Of the cases with nondysplastic IM without AMAG, 31 had entirely complete IM, whereas 11 had a component of incomplete IM. Sixty-one percent (19 of 31) of the cases with entirely complete IM showed staining, compared with 100% (11 of 11) of the cases with a component of incomplete IM ($P = .04$). This difference was not statistically significant when using clinical trial inclusion criteria (12 of 31 [38.7%] versus 7 of 11 [63.6%]; $P = .28$).

Correlation of claudin-18 expression was examined in slides with 2 or more dysplasia/adenocarcinoma categories (Table 3). Claudin-18 staining in nondysplastic IM was not significantly correlated with claudin-18 staining in areas of at least LGD ($r = 0.05$; $P = .79$). This correlation was also not statistically significant when using clinical trial inclusion criteria ($r = 0.16$; $P = .13$). There was weak correlation of claudin-18 staining between areas of dysplasia (LGD plus HGD) and adenocarcinoma that did not reach statistical significance ($r = 0.32$; $P = .09$). This correlation did reach statistical significance when using clinical trial inclusion criteria ($r = 0.52$; $P = .002$). This correlation was stronger and statistically significant when limiting the comparison to HGD and adenocarcinoma for both claudin-18 staining ($r = 0.75$; $P < .001$) and when using clinical trial inclusion criteria ($r = 0.73$; $P < .001$).

Claudin-18 Expression in WDNETs

Staining of gastric WDNETs was performed after it was noted that neuroendocrine cell hyperplasia was positive for claudin-18 in 100% of 26 gastric samples with AMAG. Eighty percent (32 of 40) of gastric WDNETs were positive for claudin-18, with 57.5% (23 of 40) meeting clinical trial inclusion criteria (Table 4; Figure 3, A through H). The rate of staining in cases from whole slides was compared with that in cases from TMAs to assess for possible underestimation of staining because of limited tumor sampling in TMAs. WDNETs with whole slide staining had a slightly higher rate of positivity (16 of 19; 84.2%) than those from TMAs (16 of 21; 76.2%), but this difference did not reach statistical significance ($P = .81$). In gastric WDNET cases where the type was known ($n = 22$), there was no significant difference in the rate of claudin-18 staining in type 1 (13 of 16; 81.3%) and type 3 (6 of 6; 100%) WDNETs ($P = .66$). The rate of staining was also similar in type 1 (11 of 16; 68.8%) and type 3 (4 of 6; 66.7%) WDNETs when using clinical trial criteria ($P > .99$). The single available liver metastasis from a type 3 gastric WDNET showed 3+ staining in 100% of the tumor cells. The lymph node from the same patient's metastasis showed 3+ staining in 30% of the tumor cells. Claudin-18 staining was present in 2 of 322 nongastric WDNETs (0.62%; $P < .001$ versus gastric WDNETs). The remaining 320 nongastric WDNETs were completely negative. No nongastric WDNETs (0 of 322 [0%]; $P < .001$) had staining that met clinical trial inclusion criteria. One lung

Table 3. Paired Claudin-18 Expression in the Upper Gastrointestinal Tract (All Sites)										
	Positive Claudin-18 Staining			r	*P*	Positive Claudin-18 Staining Using Clinical Trial Inclusion Criteria[a]			r	*P*
						≥Low-grade dysplasia				
		Yes	No				Yes	No		
Non-dysplastic intestinal metaplasia	Yes	67	26	0.05	.79	Yes	35	35	0.16	.13
	No	16	8			No	16	31		
						Adenocarcinoma				
		Yes	No				Yes	No		
Dysplasia (low and high grade)	Yes	21	6	0.32	.09	Yes	15	5	0.52	.002
	No	7	8			No	5	17		
High-grade dysplasia	Yes	17	3	0.75	<.001	Yes	12	4	0.73	<.001
	No	0	6			No	0	10		

[a] Data derived from ClinicalTrials.gov,[19] Klempner et al,[25] and Türeci et al.[26]

WDNET had 3+ staining in 35% of tumor cells, and 1 pancreatic WDNET had 2+ staining in 1% of tumor cells. Therefore, the estimated diagnostic sensitivity for gastric origin in WDNETs was 80.0% (95% CI, 63.8%–90.4%), and the specificity was 99.4% (95% CI, 97.5%–99.9%).

DISCUSSION

This study describes claudin-18 expression in adenocarcinomas, WDNETs, and precursor lesions of the upper GI tract, including distal esophagus/GEJ and stomach. Despite gastric IM being suggested as a positive control for claudin-18, claudin-18 staining was observed in only a minority of gastric IM samples (37 of 79; 46.8%), and fewer (23 of 79; 29.1%) stained strongly enough to be considered positive using clinical trial inclusion criteria. An even smaller proportion of nondysplastic gastric IM showed claudin-18 staining in the setting of AMAG. Background IM also had higher of rates of staining when there was a component of incomplete IM compared with samples with entirely complete IM. A much higher proportion of claudin-18 staining was present in nondysplastic IM of the distal esophagus/GEJ (37 of 40; 92.5%). All cases exhibited claudin-18 staining in the background gastric mucosa, which may be a more reliable control tissue for claudin-18 immunohistochemistry. Strategic and careful selection of positive control tissue will be needed by laboratories if this stain eventually becomes a companion test for determining eligibility for anticlaudin therapy, as there are multiple ongoing phase II/III clinical trials of zolbetuximab with chemotherapy in advanced GEJ/gastric cancer.[18–20] The possibility of internal laboratory error to explain the lack of staining in some samples of gastric IM was considered, but internal controls (eg, neuroendocrine hyperplasia and background gastric tissue) stained appropriately in all samples, and the positivity rate in adenocarcinoma was similar to that seen in previous reports.[26–29]

Table 4. Claudin-18 Expression in Well-Differentiated Neuroendocrine Tumors (WDNETs)		
	Positive Claudin-18 Staining, % (No./Total)	Positive Claudin-18 Staining Using Clinical Trial Inclusion Criteria, % (No./Total)[19,25,26]
Gastric WDNETs	80.0 (32/40)	57.5 (23/40)
Nongastric WDNETs	0.62 (2/322)[a]	0.0 (0/322)
P value	<.001	<.001

[a] One lung WDNET and 1 pancreas WDNET showed claudin-18 staining.

Meyer et al[30] postulated that IM in the esophagus is derived from the progenitor cells of the squamous epithelium. Ormsby et al[31] also demonstrated a distinctly different pattern of CK7/CK20 expression in IM of the esophagus versus IM of the stomach. The observed differences in claudin-18 expression are supportive of these hypotheses that the development and pathophysiology of IM in the distal esophagus/GEJ differ from those in the stomach and also indicate that IM in AMAG has a distinct phenotype from other types of gastric IM. There was also some evidence that cases with only complete IM were more likely to lose claudin-18 expression than cases with a component of incomplete IM.

IM and dysplasia are known risk factors for adenocarcinoma of the distal esophagus/GEJ and stomach.[3,4] The current study showed no significant correlation between claudin-18 staining in nondysplastic IM and dysplastic/neoplastic (≥LGD) epithelium. This analysis was also limited to sections where both levels of dysplasia were present on a single slide. This correlation may be even weaker when the nondysplastic IM is more distant from the dysplastic/neoplastic epithelium. Therefore, it cannot be recommended to use claudin-18 staining in nondysplastic IM as a proxy for staining in dysplastic/neoplastic epithelium. The correlation was stronger, though not perfect, when comparing dysplasia (LGD + HGD) to adenocarcinoma, especially when limiting the dysplasia to HGD, both by claudin-18 staining and by claudin-18 positivity by the clinical trials inclusion criteria. This further supports the finding in the literature that HGD itself is a strong risk factor for adenocarcinoma as well as a precursor lesion to adenocarcinoma.[32–34] The higher levels of staining in HGD and adenocarcinoma compared with LGD could indicate that claudin-18 expression changes over time as lesions progress to higher grades.

Rohde et al[35] and Coati et al[36] reported a significant association between claudin-18 expression and diffuse-type gastric adenocarcinoma as well as high-grade (G3) gastric adenocarcinoma (by Rohde et al[35] only). However, Arnold et al[37] and Dottermusch et al[38] reported no significant association between claudin-18 expression and histomorphologic subtype, including grade of differentiation and

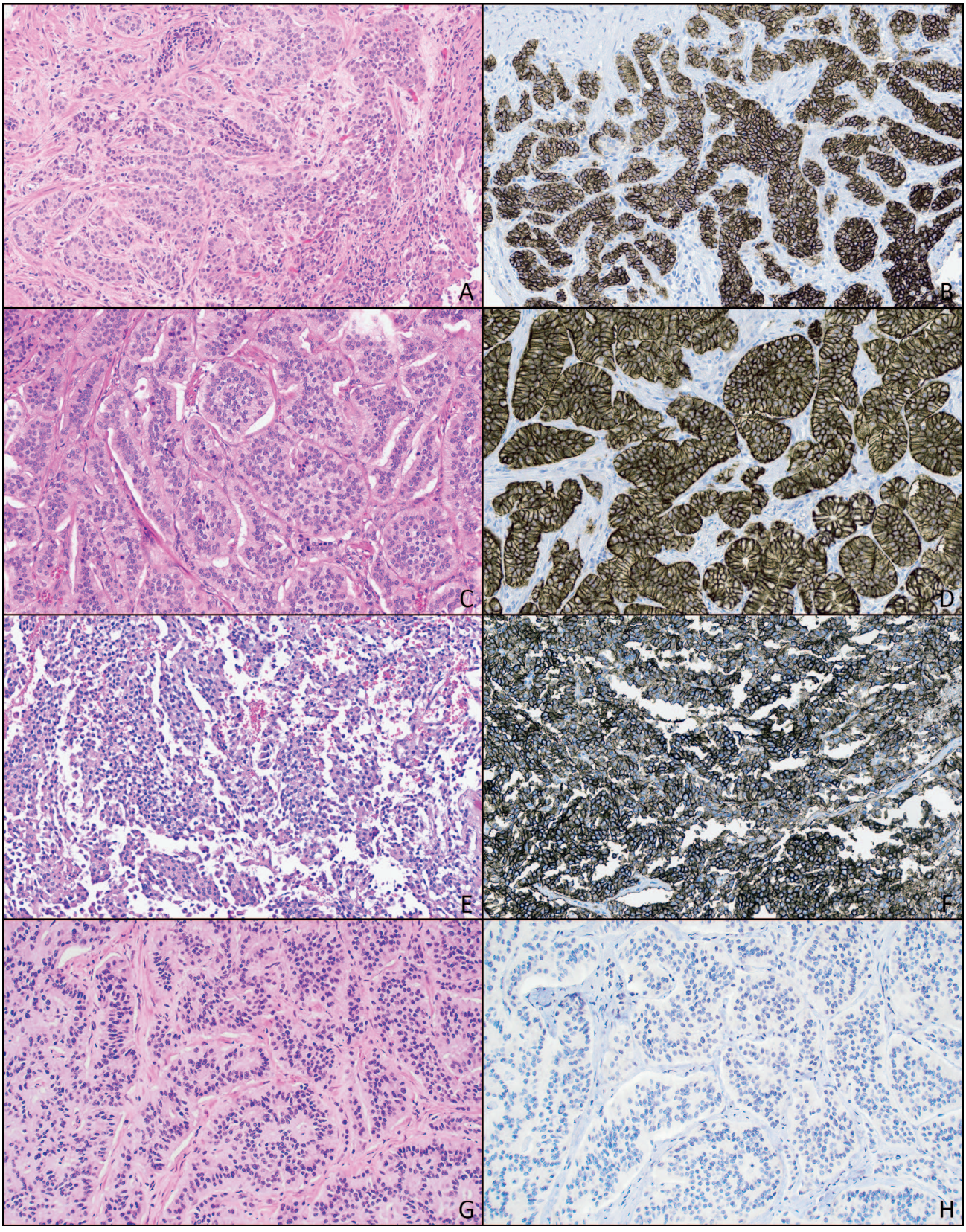

Figure 3. *Claudin-18 expression in gastric well-differentiated neuroendocrine tumors. Type 1 well-differentiated neuroendocrine tumor (A) with 3+ claudin-18 expression (B). Type 3 well-differentiated neuroendocrine tumor (C) with 3+ claudin-18 expression (D). Type 3 metastatic well-differentiated neuroendocrine tumor in liver (E) with 3+ claudin-18 expression (F). Nongastric (colorectal) well-differentiated neuroendocrine tumor (G) with no claudin-18 expression (H) (hematoxylin-eosin, original magnification ×200 [A, C, E, and G]; claudin-18, original magnification ×200 [B, D, F, and H]).*

Lauren classification. In this study, no association was identified between claudin-18 expression and grade of differentiation or Lauren classification of gastric adenocarcinoma. The different rates and association of claudin-18 expression across various studies may be attributed to the methods (eg, any positivity, semiquantitative H-score formula, immunoreactivity score formula) that were used to determine positive claudin-18 expression.[35–38] In addition, different clones of antibodies were used in different studies (eg, clone EPR19202 by Abcam, clone 34H14L15 by Invitrogen, clone 43-14A by Ganymed Pharmaceuticals and Roche Ventana).[35–38] However, Arnold et al[37] reported that there was no significant difference in claudin-18 expression between clone EPR19202 and clone 43-14A. Additional studies are needed to compare the clones of claudin-18 antibodies to determine if these various clones play a role in the different reported rates of claudin-18 expression in gastric and gastroesophageal adenocarcinoma.

Claudin-18 expression is frequently present in cases of primary (29.4%) and metastatic (34.1%) gastric and gastroesophageal adenocarcinoma.[36] Although a study recently found that claudin-18 was a sensitive (79%) and specific (93%) marker for adenocarcinoma of the stomach and pancreaticobiliary tract, it has not been investigated as a marker of gastric origin in WDNETs.[39] Currently, there are no commonly used markers of gastric origin in WDNETs. Immunolabeling for CDX2 is usually patchy and weak and sometimes even negative in gastric WDNETs.[40,41] CDX2 also stains patchily and weakly in other foregut, hindgut, and pancreatic WDNETs and strongly and diffusely in midgut WDNETs.[40,41]

In this study, expression of claudin-18 was both a sensitive (80.0%; 32 of 40) and specific (99.4%; 320 of 322) marker of gastric origin in WDNETs. Although positive staining was present in liver and lymph node metastases from a single case, this finding will need to be shown in a larger sample of metastatic gastric WDNETs to ensure that expression is maintained in metastatic disease. Use of TMAs allowed for testing of a large sample of WDNETs, but a limitation of TMAs is that it is possible that sampling a small portion of a tumor with heterogeneous staining patterns could cause sensitivity to be underestimated and specificity to be overestimated. Comparing the rate of staining in gastric WDNET cases from whole slides with those from TMAs did not provide strong evidence that the sensitivity was underestimated, as there was only a small difference that did not reach statistical significance. Only TMAs were used for the nongastric WDNET samples, but staining being limited to just 2 cases of a large sample (n = 322 with 608 total cores) makes it unlikely that the specificity was overestimated by a large amount.

Although this study shows claudin-18 staining with high sensitivity and specificity for gastric WDNETs, Wöll et al[42] demonstrated 20% (n = 5 of 25) of pancreatic neuroendocrine neoplasms with claudin-18 expression, whereas our study showed 1.0% (n = 1 of 102) of pancreatic neuroendocrine neoplasms with claudin-18 expression. The difference in the rate of claudin-18 expression may be due to the different claudin-18 antibodies that were used (Wöll et al[42] with diagnostic monoclonal mouse anti-CLND18.2 antibody aGC182 and this study with clone 43-14A [Roche Ventana Medical Systems]). In addition, Wöll et al[42] used predominantly paraffin-embedded tissue with some TMAs of pancreatic tumors, whereas the current study used only TMAs of pancreatic WDNETs. Thus, sampling may also play a small role in the different rates of claudin-18 expression. Additional studies using this clone may be helpful in further investigating this difference.

The strong membranous expression of claudin-18 in a significant portion of gastric WDNETs (23 of 40 [57.5%] met clinical trial exclusion criteria) raises the possibility that they may be amenable to anti–claudin-18 therapy. This possibility is less important in the more common, typically indolent, type 1 gastric WDNETs. However, similar staining was observed in a limited sample of type 3 gastric WDNETs, which are more aggressive as they tend to be deeply invasive and may metastasize.[43] Although all 6 type 3 gastric WDNETs and a single type 3 gastric WDNET liver metastasis in this cohort were positive for claudin-18 staining, additional staining in a larger sample of type 3 WDNETs and in metastases will be necessary to more precisely determine the rates of positivity in these tumors. It should also be noted that the clinical trial inclusion criteria used in the current study were designed for adenocarcinoma and may not be applicable to WDNETs.

The current study shows that rates of claudin-18 staining in IM and dysplasia were not significantly different from those of adenocarcinomas. However, significant correlation in cases with 2 adjacent categories of dysplasia could be detected only between HGD and adenocarcinoma. Nondysplastic IM in the background of AMAG showed predominantly negative staining for claudin-18, indicating that IM in this setting may differ from other forms of IM. Furthermore, claudin-18 is expressed in the vast majority of gastric WDNETs. Diagnostically, claudin-18 appears to be a very sensitive and specific marker of gastric origin in WDNETs. The membranous expression of claudin-18 in gastric WDNETs, including in the more aggressive type 3 cases, indicates that they may be a candidate for anti–claudin-18 therapy.

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