

# Enhanced expression of activation-induced cytidine deaminase in human gastric mucosa infected by *Helicobacter pylori* and its decrease following eradication

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Received: 4 February 2013 / Accepted: 2 April 2013 / Published online: 18 April 2013  
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## Abstract

**Background** Recent studies have shown important roles for activation-induced cytidine deaminase (AID), an intrinsic genome mutator, in *H. pylori*-associated gastric cancer development. Here, we evaluated the relationship between *H. pylori*-induced gastritis and AID expression from human biopsy specimens.

**Methods** In 109 patients with dyspeptic symptoms who had undergone endoscopy and received biopsy of the antrum, angulus, and corpus, *H. pylori* infection was diagnosed by serologic test, <sup>13</sup>C urea breath test, and histological examination. Histological scores of *H. pylori*,

neutrophils, mononuclear cells, atrophy, and intestinal metaplasia (IM) were assessed using the updated Sydney system (USS). Immunohistochemical AID expression of the biopsy specimens was scored.

**Results** Sixty of 109 (55.0 %) patients were positive for *H. pylori* and eradication was successful in 48 patients. AID expression in *H. pylori*-infected mucosa was significantly higher ( $p < 0.01$ ) than in non-infected mucosa. AID expression was highest in the antrum and was significantly ( $p < 0.01$ ) reduced toward the proximal portion of the stomach. For USS, multivariate analysis using linear regression revealed that mononuclear cell infiltration ( $p < 0.01$ ) and IM ( $p < 0.05$ ) correlated independently with AID expression. After eradication of *H. pylori*, AID expression was significantly decreased ( $p < 0.01$ ), but was still higher than that in *H. pylori*-negative patients in all sites of the stomach.

**Conclusions** AID expression is elevated in *H. pylori*-positive patients and is reduced following *H. pylori* eradication. Moreover, AID expression is highest in the antrum and correlated with severity of chronic inflammation and IM, suggesting an important role for AID in gastric cancer development through gastritis.

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**Keywords** Activation-induced cytidine deaminase · *Helicobacter pylori* · Updated Sydney system · Intestinal metaplasia · Endoscopic biopsy

## Abbreviations

*H. pylori* *Helicobacter pylori*  
IM Intestinal metaplasia  
USS Updated Sydney system  
AID Activation-induced cytidine deaminase  
NSAIDs Non-steroidal anti-inflammatory drugs  
PPI Proton pump inhibitor

$^{13}\text{C}$ -UBT	$^{13}\text{C}$ urea breath test
DU	Duodenal ulcer
GU	Gastric ulcer

## Introduction

Since the discovery of *Helicobacter pylori* by Warren and Marshall in 1982, it has been well established that *H. pylori* infection plays a key role in the development of gastric cancer [1–3]. Development of gastric cancer by *H. pylori* infection involves a multi-step process of gastritis, which starts with active inflammation and neutrophilic infiltration and progresses to chronic inflammation with mononuclear cell infiltration and subsequent glandular atrophy, intestinal metaplasia (IM), and dysplasia, eventually leading to cancer [4].

The updated Sydney system (USS) has been widely used for the histological evaluation of *H. pylori*-associated gastritis [5–8], and severity on the scale has been shown to be useful for predicting gastric cancer development [3, 5–8]. However, the molecular mechanisms underlying gastric cancer development through progression of gastritis have not been fully studied.

Various mechanisms have been considered for gastric cancer development following *H. pylori* infection. Among them, an important characteristic feature of cancer development is the stepwise accumulation of genetic alterations such as gene mutations and gene deletions [9, 10]. It has been shown that *H. pylori* can induce gene mutation in the gastric mucosa. Indeed, the mucosa in *H. pylori*-infected gastritis shows a considerable level of gene mutations [9–12]. We recently reported that activation-induced cytidine deaminase (AID) plays a critical role as an intrinsic genome mutator in *H. pylori*-induced gastric cancer development [13, 14]. In vivo, AID is exclusively expressed in B cells and contributes to diversification of antibody production by inducing both somatic hypermutation and class-switch recombination of immunoglobulin genes [11, 13–15]. Interestingly, we found that AID is expressed aberrantly in the gastric mucosa in response to *H. pylori* infection through NF $\kappa$ B activation and that it enhances genetic aberrations such as gene mutations and deletions [13, 14]. Moreover, we showed that *H. pylori* induces AID expression, not only directly but also indirectly, through enhancing gastric inflammation, suggesting that AID expression may reflect the risk for gastric cancer development in the gastric mucosa [13]. However, the histological relationship between AID expression and *H. pylori*-associated gastritis has not been well characterized.

In the present study, to elucidate the relationship between *H. pylori*-induced gastritis and AID expression

more precisely, we assessed histological findings of USS and AID expression using a large number of biopsy specimens from the gastric mucosa of dyspeptic patients. Furthermore, we also examined the changes of AID expression in the gastric mucosa after the eradication of *H. pylori*.

## Methods

### Patients

We recruited 253 consecutive dyspeptic patients who agreed to receive upper gastrointestinal endoscopy and biopsy at three sites of the stomach to assess *H. pylori*-associated gastritis between 2002 and 2008 at the National Center for Global Health and Medicine (NCGM), Japan.

We excluded the following 144 patients from analysis: 3 who had a severe underlying disease; 12 who had taken non-steroidal anti-inflammatory drugs (NSAIDs); 21 who had taken anti-thrombotic drugs; 56 who had taken a proton pump inhibitor (PPI); 12 who had taken an H<sub>2</sub>-blocker; 56 who had undergone eradication of *H. pylori*; and 6 whose histological features could not be evaluated because of insufficient biopsy samples. More than one exclusion criterion was applied to some patients. As a result, the remaining 109 patients were included for analysis. The study protocol was approved by the ethics committee of the National Center for Global Health and Medicine, Tokyo, Japan.

### Assessment of *H. pylori* status and eradication

*H. pylori* infection status was evaluated by the presence of immunoglobulin G antibody against *H. pylori* in the serum (HM-CAP, Enteric Products, Westbury, NY),  $^{13}\text{C}$  urea breath test (UBT; with a cut-off value of 2.5 ‰; Ubit, Otsuka Pharmaceuticals, Tokyo, Japan), and histological examination with toluidine blue staining. When all three methods yielded negative results, *H. pylori* infection was considered negative.

*H. pylori*-positive patients were treated with a 7-day regimen consisting of amoxicillin 750 mg, clarithromycin 200 mg (or 400 mg), and a omeprazole 20 mg or lansoprazole 30 mg or rabeprazole 10 mg twice daily. If eradication was not successful, a second regimen consisting of amoxicillin 750 mg, metronidazole 250 mg, and a omeprazole 20 mg or lansoprazole 30 mg or rabeprazole 10 mg was administered.

Eradication was confirmed by negative histological examination of gastric biopsy specimens, together with a negative  $^{13}\text{C}$ -UBT at least 2 months after the completion of eradication therapy. When all of the tests were negative, a patient was defined as having undergone successful

eradication of *H. pylori* infection. After confirmation of successful eradication for a mean (SD) period of 16.6 (8.8) months, endoscopic biopsy of the same locations (antrum, angulus, and corpus) as those at the initial diagnosis was repeated.

#### Endoscopic assessment

All endoscopies were performed by well-trained endoscopists using a Q240 or Q260H video endoscope (Olympus, Tokyo, Japan). Endoscopic diagnosis included active duodenal ulcer (DU), active gastric ulcer (GU), and hyperplastic polyp.

#### Histological assessment

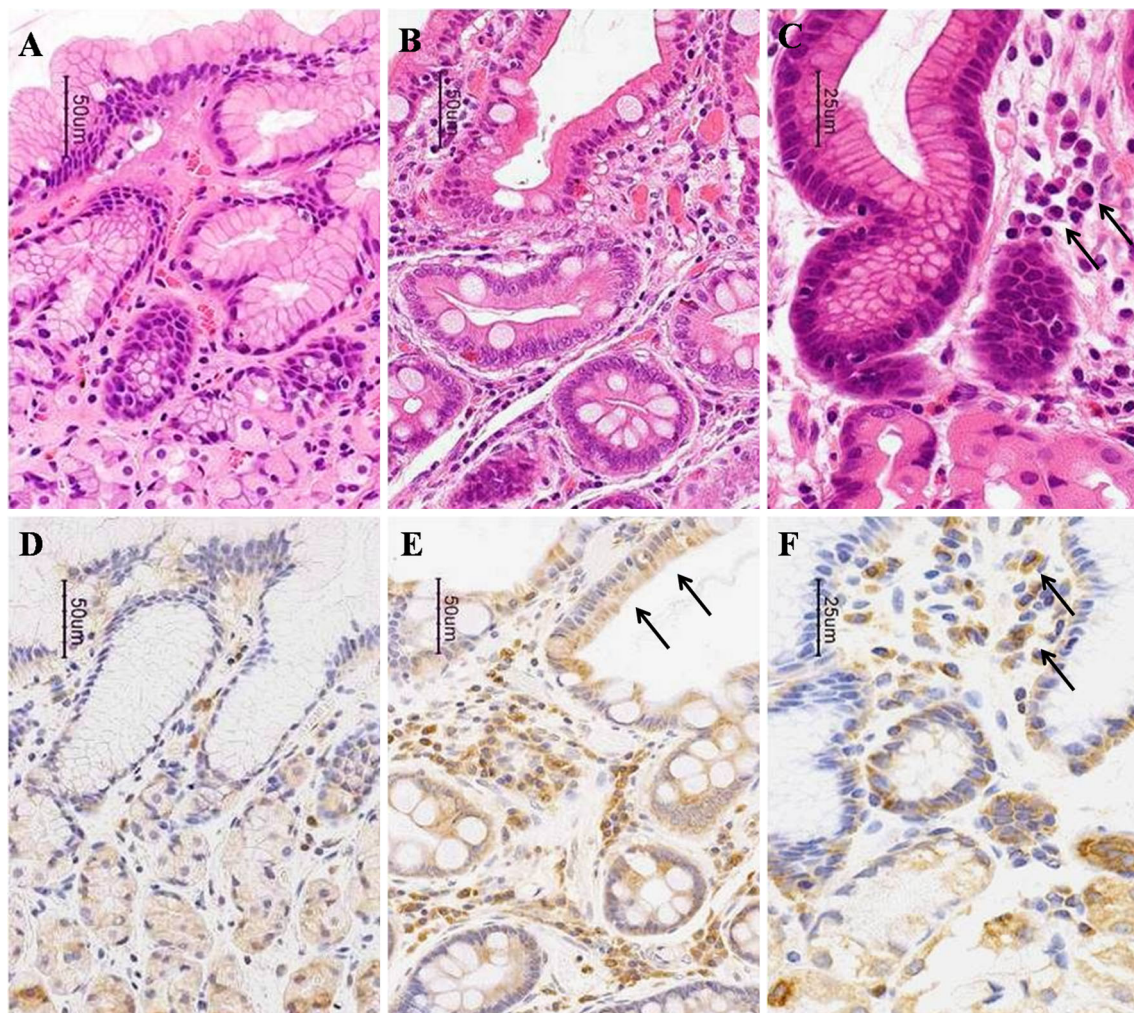
Biopsy specimens were taken from the following three sites of the gastric mucosa: the greater curvature of the

antrum (antrum), the incisura angulus (angulus), and the greater curvature of the upper body (corpus). Thus, a total of 327 biopsy specimens were taken from 109 patients, fixed in formalin, and stained with hematoxylin and eosin and toluidine blue.

According to the USS, a score of 0–3 (0, absent; 1, mild; 2, moderate; 3, marked) was assigned to each of the following parameters: *H. pylori*, neutrophil infiltration, mononuclear cell infiltration, glandular atrophy, and IM [5].

#### Inter-observer agreement of histological findings of USS

Histological features were diagnosed by an expert pathologist (Y.L.) who was blinded to clinical information and endoscopic findings. To validate inter-reliability and the



**Fig. 1** Immunohistochemical analysis of AID expression. Representative images of HE staining (a–c) and AID immunostaining (d–f) are shown. a, d Normal fundic mucosa of a *H. pylori*-negative patient with no AID immunostaining. b, e Fundic mucosa with intestinal

metaplasia in a *H. pylori*-positive patient with strong AID expression (> 30 % cells and strong intensity). Arrows in e indicate epithelial cells with strong AID expression. c, f Some plasma cells were used as internal positive control (arrows)

consensus of the ratings given by judges, another expert pathologist (T.I.) who was also blinded to clinical information and endoscopic findings evaluated each pathological finding and its associated USS score in 74 patients.

#### Assessment of AID expression

AID immunostaining was performed using a specific antibody for AID [13] and was semiquantitatively evaluated for the percentage and intensity of the positively stained gastric epithelial cells because there was no established standard (Fig. 1). We chose a field with best quality, and evaluated AID levels. The percentage score was evaluated as follows: 0 = no staining, 1 =  $\leq 5$  % stained cells, 2 =  $>5$ –10 %; 3 =  $>10$ –30 %; 4 =  $>30$  % stained cells. An intensity score of 0, 1, 2, or 3 corresponded to no, weak, moderate, or strong staining of cells, respectively. We used some plasma cells with strong staining of AID as internal positive controls. We scored 3 when the cytoplasm was stained at the same level or more than that of plasma cells. Accordingly, immunohistochemical AID expression in each biopsy specimen was scored from 0 to 7 by combining percentage score and intensity score. Immunohistochemical analysis was evaluated by a single observer (Y.L.).

#### Statistical analysis

We examined differences in patient characteristics (age, sex, and endoscopic diagnosis) and histological scores between *H. pylori*-positive and -negative patients. Wilcoxon's rank sum test was used to compare age with histological score. The Chi squared test was used to compare sex ratio with endoscopic diagnosis between the two groups.

AID expression was compared between groups by Wilcoxon's rank sum test for *H. pylori* infection (positive or negative), age ( $\geq$  or  $<$  60 years), and sex (male or female) in all patients. In patients with *H. pylori* infection, simple linear regression analysis was used to determine the USS inflammatory parameters correlating with AID expression. Then we used a multiple linear regression model to identify the independent factors correlating with AID expression. Wilcoxon's matched-pairs signed-rank test was used to compare AID expression with intragastric localization (antrum, angulus, and corpus).

To identify changes in AID expression before and after *H. pylori* eradication, we used Wilcoxon's matched-pairs signed-rank test. We also examined the difference in AID expression between *H. pylori*-eradicated patients and *H. pylori*-negative patients using Wilcoxon's rank sum test.

We calculated the value of agreement in pathological findings for the USS score. Inter-observer agreement was measured with kappa statistics [17, 18]. Kappa

values  $> 0.80$  denoted excellent, between 0.60 and 0.80 good, between 0.40 and 0.60 moderate, between 0.20 and 0.40 fair, and  $< 0.20$  poor [17].

Values of  $p < 0.05$  were considered significant. All statistical analysis was performed using Stata version 10 software (StataCorp, Lakeway Drive College Station, TX, USA).

## Results

### Patient characteristics

Patient baseline characteristics are shown in Table 1. Of the 109 patients, 60 (55.0 %) were positive for *H. pylori*. Age and sex did not differ between *H. pylori*-positive and -negative patients. *H. pylori*-positive patients comprised 10 with DU, 4 with GU, and 5 with hyperplastic polyps.

All USS scores of *H. pylori* density, neutrophil infiltration, monocyte infiltration, glandular atrophy, and IM at any portion of the gastric mucosa (antrum, angulus, and corpus) in *H. pylori*-positive patients were significantly higher than those in *H. pylori*-negative patients, except for glandular atrophy and IM in the corpus.

### AID expression according to *H. pylori* infection and intragastric localization

AID expression was significantly ( $p < 0.01$ ) higher in *H. pylori*-positive patients than in *H. pylori*-negative patients in the corpus, angulus, and antrum (Fig. 2a).

In *H. pylori*-positive patients, AID expression was highest in the antrum ( $p < 0.01$ ), and expression significantly ( $p < 0.01$ ) decreased toward the proximal part of the stomach (Fig. 2a). In *H. pylori*-negative patients, AID expression in the antrum was significantly higher than that of the angulus ( $p < 0.01$ ) and corpus ( $p < 0.01$ ) (Fig. 2a). There were no significant differences in AID expression for age group or sex at any sites of the stomach (Fig. 2b, c).

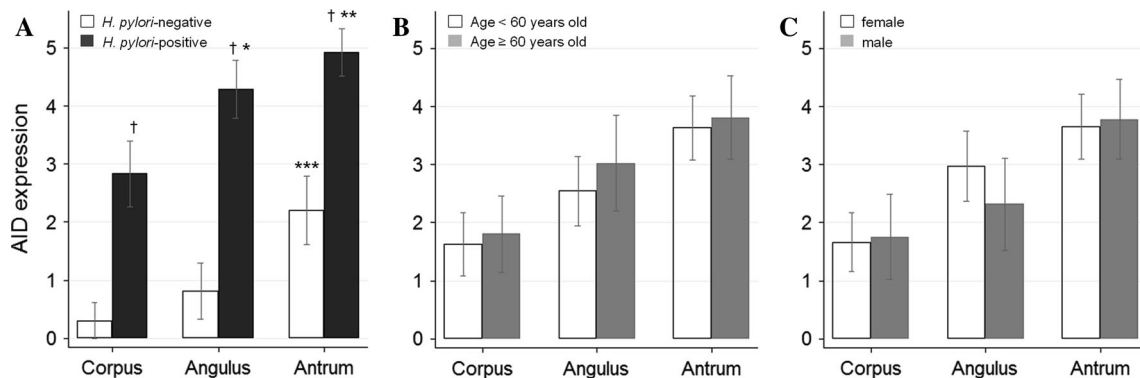
### AID expression and USS scores in *H. pylori*-positive patients

The correlation between AID expression and USS scores in *H. pylori*-positive patients is shown in Table 2. AID expression correlated significantly with neutrophil infiltration ( $p = 0.01$ ), mononuclear cell infiltration ( $p < 0.01$ ), glandular atrophy ( $p < 0.01$ ), and IM ( $p < 0.01$ ) by simple linear regression analysis. However, AID expression did not correlate significantly with *H. pylori* density. Multivariate analysis using linear regression revealed that mononuclear cell infiltration ( $p < 0.01$ ) and IM ( $p < 0.05$ ) were independently correlated with AID expression (Table 2). In agreement with these data, we also found that

**Table 1** Characteristics of patients with or without *H. pylori* infection ( $n = 109$ )

	<i>H. pylori</i> -negative ( $n = 49$ )	<i>H. pylori</i> -positive ( $n = 60$ )	<i>p</i> value
Median age (IQR) years	57.0 (42–67)	55.5 (40–65.5)	0.75
Sex (male/female)	18/31	23/37	0.88
Endoscopic diagnosis			
No findings	49	41	
Active duodenal ulcer	0	10	
Active gastric ulcer	0	4	
Hyperplastic polyp	0	5	<0.01
Histological grading score (0–3) of USS <sup>a</sup>			
<i>Helicobacter pylori</i> density			
Antrum	0.02 (0.14)	1.40 (0.76)	<0.01
Angulus	0.02 (0.14)	1.28 (0.69)	<0.01
Corpus	0	1.52 (0.77)	<0.01
Neutrophil infiltration			
Antrum	0.02 (0.14)	1.48 (0.70)	<0.01
Angulus	0.02 (0.14)	1.33 (0.80)	<0.01
Corpus	0	1.53 (0.96)	<0.01
Mononuclear cell infiltration			
Antrum	0.73 (0.49)	2.03 (0.58)	<0.01
Angulus	0.73 (0.49)	2.20 (0.73)	<0.01
Corpus	0.69 (0.51)	1.83 (0.62)	<0.01
Glandular atrophy			
Antrum	0.04 (0.20)	0.85 (1.02)	<0.01
Angulus	0.18 (0.60)	0.75 (1.08)	<0.01
Corpus	0.04 (0.29)	0.07 (0.31)	0.43
Intestinal metaplasia			
Antrum	0.02 (0.14)	0.57 (0.93)	<0.01
Angulus	0.12 (0.53)	0.58 (1.01)	<0.01
Corpus	0.04 (0.29)	0.07 (0.31)	0.43

IQR inter quartile range,  
USS updated Sydney system  
<sup>a</sup> Mean (SD) values are shown



**Fig. 2** AID expression in different portions of the gastric mucosa. **a** Data from *H. pylori*-negative patients (white bars) and *H. pylori*-positive patients (black bars) are shown. †AID expression in *H. pylori*-positive patients was significantly higher than in *H. pylori*-negative patients at all sites ( $p < 0.01$ ). \*Significantly higher than in the corpus ( $p < 0.01$ ), and \*\*significantly higher than in the angulus ( $p < 0.01$ ) in *H. pylori*-positive patients. \*\*\*Significantly higher than

in the corpus and angulus of *H. pylori*-negative patients ( $p < 0.01$ ). **b** Data of age < 60 years old (white bars) and age  $\geq 60$  years old (gray bars) are shown. No significant differences in AID expression between age groups at any sites were noted ( $p > 0.05$ ). **c** Data from females (white bars) and males (gray bars) are shown. No significant differences in AID expression between age groups at any sites were noted ( $p > 0.05$ ) were noted

**Table 2** Correlation between AID expression and grading of USS by univariate and multivariate analysis in *H. pylori*-positive patients

Factor	Univariate analysis		Multivariate analysis	
	Regression coefficient (95 % CI)	<i>p</i> value	Regression coefficient (95 % CI)	<i>p</i> value
<i>Helicobacter pylori</i> density	0.20 (−0.21 to 0.62)	0.34	0.27 (−0.13 to 0.67)	0.19
Neutrophil infiltration	0.47 (0.10–0.83)	0.01	0.11 (−0.57 to 0.35)	0.63
Mononuclear cell infiltration	0.81 (0.35–1.26)	<0.01	0.81 (0.26–1.36)	<0.01
Glandular atrophy	0.80 (0.50–1.10)	<0.01	0.30 (−0.29 to 0.90)	0.32
Intestinal metaplasia	0.92 (0.58–1.26)	<0.01	0.67 (0.01–1.34)	<0.05

CI confidence interval

AID expression is significantly higher in gastritis with IM than that without IM (mean AID score, gastritis with IM 5.5 versus without IM 3.6,  $p < 0.01$ ).

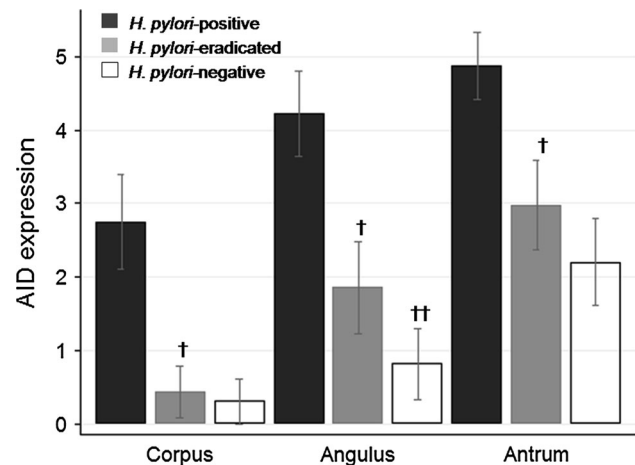
Changes in AID expression before and after *H. pylori* eradication

Of the 60 *H. pylori*-positive patients, 50 agreed to undergo eradication therapy, and successful eradication was confirmed in 48 of these patients. USS scores of *H. pylori* density, neutrophil infiltration, and mononuclear cell infiltration were significantly decreased in all portions of the gastric mucosa ( $p < 0.01$ ), while atrophy and IM scores were not significantly decreased after eradication (Table 3).

**Table 3** Changes in USS scores after eradication of *H. pylori* ( $n = 48$ )

Factor	Before eradication	After eradication	<i>p</i> value
<i>H. pylori</i> density			
Antrum	1.40 (0.76)	0	<0.01
Angulus	1.25 (0.73)	0	<0.01
Corpus	1.52 (0.80)	0	<0.01
Neutrophils infiltration			
Antrum	1.46 (0.74)	0.04 (0.20)	<0.01
Angulus	1.25 (0.84)	0.02 (0.14)	<0.01
Corpus	1.46 (0.97)	0	<0.01
Mononuclear cell infiltration			
Antrum	2.02 (0.60)	1.04 (0.20)	<0.01
Angulus	2.10 (0.75)	1.06 (0.43)	<0.01
Corpus	1.77 (0.63)	0.98 (0.25)	<0.01
Atrophy			
Antrum	0.67 (0.88)	0.70 (0.81)	0.36
Angulus	0.72 (1.07)	0.83 (0.99)	0.49
Corpus	0.08 (0.35)	0.04 (0.20)	0.16
Intestinal metaplasia			
Antrum	0.50 (0.83)	0.44 (0.77)	0.60
Angulus	0.54 (1.00)	0.60 (0.96)	0.94
Corpus	0.08 (0.35)	0.04 (0.20)	0.16

Mean (SD) values are shown

**Fig. 3** Changes in AID expression in the gastric mucosa following *H. pylori* eradication. †AID expression was significantly decreased by *H. pylori* eradication ( $p < 0.01$ ; Wilcoxon's matched paired signed-rank test) in all three sites of the stomach. However, it was still higher than in *H. pylori*-negative patients in all sites of the stomach, ††especially in the angulus ( $p = 0.01$ ; Wilcoxon's rank sum test)

AID expression in *H. pylori*-infected patients ( $n = 48$ ) was significantly ( $p < 0.01$ ) decreased following eradication of *H. pylori* in all sites of the stomach, but expression in eradicated patients ( $n = 48$ ) was still higher than that in *H. pylori*-uninfected patients ( $n = 49$ ) in all sites of the stomach, with a significant difference in the angulus ( $p = 0.01$ ) (Fig. 3).

Inter-observer agreement of USS scores

Kappa values of *H. pylori* scores were moderate in the antrum and angulus (0.50 and 0.56, respectively) and good (0.61) in the corpus. The kappa value of neutrophil infiltration scores was good in the antrum, angulus, and corpus (0.60, 0.70, and 0.74, respectively). The kappa value of mononuclear cell infiltration scores was good in the antrum, angulus, and corpus (0.63, 0.68, and 0.65, respectively). The kappa value of glandular atrophy scores was fair (0.35) in the antrum and moderate in the angulus

and corpus (0.43 and 0.52, respectively). The kappa value of IM scores was good in the antrum, angulus, and corpus (0.77, 0.80, and 0.80, respectively).

## Discussion

Recent studies have revealed that AID plays a critical role in inflammation-associated cancer development in various digestive organs by inducing genome instability, including gene mutations and aberrations [11, 13, 15, 19]. Importantly, AID has been shown to be strongly expressed in *H. pylori*-positive gastritis mucosa as well as gastric cancer tissue in humans, suggesting involvement of AID in gastric cancer development [11, 13, 14]. However, the precise relationship between gastritis and AID expression has not been evaluated. In this study, we examined AID expression in the gastric mucosa of 109 patients who had undergone endoscopy with biopsy of three locations. We then confirmed that AID expression was significantly higher in *H. pylori*-positive patients than in *H. pylori*-negative patients, but it was significantly reduced by *H. pylori* eradication. Furthermore, USS enabled us to demonstrate that AID expression in the gastric mucosa of *H. pylori*-positive patients correlated significantly with gastritis severity. These data strongly suggest important roles for AID in the gastritis-to-gastric cancer sequence.

In this study, we used USS to evaluate the relationship between the degree and characteristics of gastritis and AID expression. Simple linear regression analysis showed that AID expression correlated significantly with neutrophil infiltration, mononuclear cell infiltration, glandular atrophy, and IM. Multivariate analysis revealed that only mononuclear cell infiltration and IM scores are independent factors correlating significantly with AID expression. Recently, Takeda et al. [20] demonstrated that AID expression in the non-cancerous mucosa adjacent to the tumor is associated with the severity of mononuclear cell activity, which is consistent with our study. In contrast, we could not find a significant correlation between *H. pylori* density and AID expression. Data regarding *H. pylori* density are in agreement with those of a previous study by Goto et al. [21]. However, their data, like ours, are rather unexpected, because previous studies have clearly shown direct and potent effects of *H. pylori* on AID expression in gastric mucosal cells [13–15]. Nevertheless, it has also been reported that not only *H. pylori* but also various cytokines induced during inflammation, such as tumor necrosis factor (TNF) $\alpha$ , interleukin (IL)1- $\beta$ , and IL4, enhance AID expression [10, 11, 13, 15, 22]. Thus, the present findings that inflammatory cell infiltration, particularly mononuclear cell infiltration, correlated significantly

with AID expression suggest that cytokines produced by mononuclear cells such as macrophages [23, 24] during chronic gastric inflammation may be more important stimuli than *H. pylori* for AID expression in the clinical setting.

The finding that IM scores correlated independently with AID expression is also somewhat surprising because inflammation is generally reduced in the gastric mucosa with severe IM [25]. Furthermore, it is believed that *H. pylori* colonization cannot occur in the gastric mucosa with IM [26]. Thus, the reason for the significant correlation between AID expression and IM scores is unknown. However, it is well established that IM reflects persistence of chronic gastritis [4, 25]. Therefore, it can be speculated that when chronic gastritis continues in response to long-term *H. pylori* infection, gastric epithelial cells switch on autonomous AID expression. The present data which showed that AID expression was still present in the gastric mucosa after *H. pylori* eradication at higher levels than in non-infected patients might support such a possibility. In agreement with this notion, we have often found autonomous high expression of AID in human gastric cancer cell lines [13, 21, 27].

The present study clearly showed that the expression of AID in *H. pylori*-positive patients was most prominent in the antrum and exhibited a linear reduction toward the proximal portion of the stomach, angulus, and corpus. *H. pylori* first colonizes the antral mucosa [1], and inflammation subsequently spreads to the proximal stomach [3, 28]. The mean age of our *H. pylori*-positive patients was 56, thus most of our patients appear to have had long-term *H. pylori* infection and chronic gastritis. In Japan, corpus predominant gastritis is the most prevalent form of *H. pylori*-induced chronic gastritis [29]. In this sense, the higher expression of AID in the antrum than in the corpus in our patients is intriguing. The reason for this phenomenon is not clear at present. *H. pylori* infection induces NF $\kappa$ B activation, and NF $\kappa$ B has been reported to play a central role in *H. pylori*-induced gastric inflammation [11, 13, 15]. Notably, NF $\kappa$ B has been shown to be strongly activated in the antrum [22], which might result in high AID expression in the antrum. Alternatively, the finding that AID expression in the antrum is higher than in other parts of the stomach, even in *H. pylori*-negative patients, may indicate that the antrum is the site where AID expression is easily induced. In this regard, it might be possible that different cellular composition between the corpus and the antrum; ex. presence or absence of parietal cells and chief cells resulted in different AID expression levels.

It has been suggested that a high concentration of bile acids plays a role in the progression of premalignant conditions in *H. pylori*-negative cases [30]. In addition, our

previous study revealed that bile acid is a potent enhancer of AID expression [31], which might also explain, at least in part, the strong AID expression in the antrum. In any case, our data clearly showed that strong AID expression persists in the antrum during long-term *H. pylori* infection, which is the possible reason for the antrum being the site where gastric cancer develops most frequently [32].

The present study clearly demonstrated that AID expression was significantly reduced by *H. pylori* eradication. This finding appears to be compatible with data showing that *H. pylori* eradication reduces the risk for gastric cancer development [16, 33]. However, recent reports have also revealed that several patients developed gastric cancer even after *H. pylori* eradication [16, 34]. Such a result may also be in agreement with our results which showed that although reduced, AID expression after eradication was still higher in *H. pylori*-positive patients than in *H. pylori*-negative patients. In this regard, we found that although both scores of active and chronic inflammation were reduced, scores of atrophy and IM remained unchanged after eradication. Thus, the decrease of AID expression by *H. pylori* eradication may have been derived mainly from the reduction of chronic inflammation, while the residual AID expression may be related to atrophy and IM. Remaining atrophy and IM in the gastric mucosa after *H. pylori* eradication may suggest that even eradicated patients still have the risk for gastric cancer development, and thus the subsequent endoscopic follow-up may be needed. However, since the number of the patients was rather small in our study, further study with a larger number of patients will be required to clarify precise change of AID expression by *H. pylori* eradication.

Here, we demonstrated that AID expression is elevated in *H. pylori*-positive patients and is reduced following *H. pylori* eradication. Moreover, AID expression correlated significantly with chronic inflammation and IM and thus appears to be involved in *H. pylori*-induced gastric carcinogenesis. Recent studies have shown that inflammation promotes epigenetic changes during gastric carcinogenesis [35]. In addition, it has also been reported that AID expression is associated with epigenetic alteration [36]. These data further support important roles of AID in inflammation-associated gastric carcinogenesis.

**Acknowledgments** We thank Ms. Hisae Kawashiro, Clinical Research Coordinator for help with data collection. This work was supported by JSPS KAKENHI 21229009, 24229005, and 24659363, Research program of the Project for Development of Innovative Research on Cancer Therapeutics (P-Direct) from Ministry of Education, Culture, Sports, Science and Technology of Japan, and by Health and Labour Sciences Research Grants for Research of Japan, and a grant from the National Center for Global Health and Medicine.

**Conflict of interest** The authors declare that they have no conflict of interest.

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