Original article

No association between *AICDA* 7888 C/T polymorphism, *Helicobacter pylori* seropositivity, and the risk of atrophic gastritis and gastric cancer in Japanese

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Abstract

Background. The aberrant expression of activation-induced cytidine deaminase (*AICDA*) was reportedly induced in gastric epithelial cells infected with cytotoxin-associated gene A (*cagA*)-positive *Helicobacter pylori*, resulting in the accumulation of alterations in the *TP53* tumor suppressor gene in gastric cells. We investigated the association of the *AICDA* 7888 C/T polymorphism with *H. pylori* infection and the risk of gastric cancer and atrophic gastritis in Japanese subjects.

Methods. The study subjects were 583 histologically diagnosed gastric cancer patients (cases) and 1637 age- and sex-frequency-matched control outpatients, who visited Aichi Cancer Center Hospital from the years 2001 to 2005. In the controls, serum anti-*H. pylori* IgG antibody and pepsinogens were measured to evaluate *H. pylori* infection and atrophic gastritis, respectively. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by a logistic model.

Results. *H. pylori* seropositivity in the controls was not significantly associated with the *AICDA* 7888 C/T genotypes. Among the *H. pylori* seropositive control subjects, the age- and sex-adjusted ORs of atrophic gastritis were not statistically significant: 0.84 (95% CI, 0.62–1.13) for C/T, 0.82 (95% CI, 0.56–1.21) for T/T, and 0.83 (95% CI, 0.63–1.11) for C/T+T/T, relative to the C/C genotype. The age- and sex-adjusted ORs of gastric cancer relative to atrophic gastritis were also not statistically significant, at 1.17 (95% CI 0.89–1.54), 1.21 (95% CI, 0.85–1.71), and 1.18 (95% CI, 0.91–1.53), respectively. The OR of gastric cancer cases compared with the whole cohort of control subjects was also not significant. **Conclusion.** The hypothetical association of the *AICDA* 7888 C/T polymorphism with the risk of gastric cancer or gastric atrophy was not shown in this study.

Key words *AICDA* · Atrophic gastritis · Gastric cancer · Genetic polymorphism · *Helicobacter pylori*

Introduction

Helicobacter pylori infection has been established as a risk factor for gastric cancer through the development of atrophic gastritis and subsequent precancerous lesions. In particular, *H. pylori* strains with the cytotoxin-associated gene A (*cagA*) gene are strongly associated with an increased risk of gastric adenocarcinoma [1]. Severe atrophic gastritis and corpus-predominant gastritis, together with intestinal metaplasia, are well established as predominant predispositions to gastric cancer [2]. Host genetic factors, along with bacterial virulence factors such as CagA, have been shown to determine the severity of gastric damage and the eventual clinical outcome of *H. pylori* infection [3–6]. The risk of gastric cancer increases several fold if both host genetic factors and certain bacterial virulence factors are present [7, 8]. In East Asian populations, the great majority of *H. pylori* strains are *cagA*-positive. CagA is divided into two major subtypes; namely, East Asian type and Western type [9]. The grade of atrophic gastritis and gastric cancer risk is higher in patients infected with East Asian *cagA*-positive strains than in those infected with *cagA*-negative or Western *cagA*-positive strains [10].

Activation-induced cytidine deaminase (*AICDA*), a member of the cytidine-deaminase family that acts as an editor of DNA and RNA, is an enzyme essential for somatic hypermutation (SHM) and class-switch recombination (CSR), in immunoglobulin genes [11–13]. The aberrant expression of *AICDA* is also shown to act as a genomic mutator that leads to tumorigenesis. Recently, Matsumoto et al. [14] showed that infection of gastric epithelial cells with *cagA*-positive *H. pylori* induced aberrant expression of *AICDA* via the inhibitor of nuclear factor- κ B kinase-dependent nuclear factor (NF)- κ B activation pathway. *H. pylori*-mediated over-

expression of *AICDA* resulted in the accumulation of nucleotide alterations in the *TP53* tumor suppressor gene in gastric cells in vitro, providing evidence that the aberrant *AICDA* expression induced by *H. pylori* infection might be a mechanism of mutation accumulation in the gastric epithelium during *H. pylori*-associated gastric carcinogenesis. Meanwhile, one study in Japanese subjects has suggested that the 7888 C/T polymorphism or its associated *AICDA* polymorphisms contribute to the development of asthma and the regulation of total IgE levels, suggesting that this *AICDA* polymorphism is functional [15].

In *H. pylori*-related gastric cancer, the process leading to disease has three steps; *H. pylori* infection, the development of atrophic gastritis, and carcinogenesis. At each step, genetic traits and their interactions with lifestyle may influence the process [16]. Considering the biological significance of *AICDA* in *H. pylori*-induced gastric cancer, it seems intriguing to investigate the role of this *AICDA* 7888 C/T polymorphism in this process. This study aimed to examine the hypothetical association of the *AICDA* polymorphism with the risk of *H. pylori* seropositivity, atrophic gastritis (measured with serum pepsinogens [PGs]), and gastric cancer in a large number of Japanese subjects.

Subjects, materials, and methods

Subjects

The subjects were participants in the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC) study, in which first-visit outpatients were consecutively invited to provide lifestyle data and blood samples, after informed consent was obtained [17]. Among the participants who visited Aichi Cancer Center Hospital from the years 2001 to 2005, 583 patients diagnosed with gastric cancer (cases) and 1637 age- and sex-frequency matched cancer-free outpatients (controls) were selected. Informed consent was obtained from all the subjects and the study protocol was approved by the Ethics Committees of Aichi Cancer Center and Nagoya University Graduate School of Medicine.

Samples and diagnostic criteria

Serum samples from participants were immediately stored at -20°C until analysis. Anti-*H. pylori* IgG antibody was measured with a direct enzyme-linked immunosorbent assay (ELISA) kit (E plate Eiken *H. pylori* Antibody; Eiken Kagaku, Tokyo, Japan). According to the kit instructions, 10.0 units or higher was regarded as seropositive. Serum PGs were measured by chemiluminescence enzyme immunoassay (CLEIA). Gastric

mucosal atrophy was grouped into the categories of: “none” ($\text{PG I} > 70 \text{ ng/ml}$ or $\text{PG I} / \text{PG II} > 3$), “mild” ($\text{PG I} \leq 70 \text{ ng/ml}$ and $\text{PG I} / \text{PG II} \leq 3$, excluding “severe” cases), or “severe” ($\text{PG I} \leq 30 \text{ ng/ml}$ and $\text{PG I} / \text{PG II} \leq 2$). Because we planned to use the serum samples of the gastric cancer patients for a study with higher priority, the *H. pylori* IgG antibody and PG levels of the gastric cancer patients were not measured.

Genotyping

DNA was extracted from the buffy coat with a BioRobot M48 Workstation (QIAGEN Group, Tokyo, Japan). The *AICDA* 7888 C/T polymorphism (rs2028373) was genotyped with a polymerase chain reaction with confronting two-pair primers (PCR-CTPP) [18]. The primers were: F1, GCC GAA ATT AAA GAT TAG AAG CAG; R1, CCA GGC TTT GAA AGT TCT TTC A; F2, TGG AAT ACT TTT GTA GAA AAC CAC; and R2, CGA AAC TCC AAA AAA GAC TGT AG. Amplification conditions were 10 min of initial denaturation at 95°C , followed by 35 cycles of 1 min at 95°C , 1 min at 63°C , and 1 min at 72°C , then a 5-min final extension at 72°C . The amplified DNA was visualized on a 2% agarose gel with ethidium bromide staining. The amplified DNA was 165 bp for the C allele, 225 bp for the T allele, and 344 bp for the common band. Representative gel used for genotyping is shown in Fig. 1.

Fig. 1. Agarose gel (2%) showing the different genotypes for the *AICDA* polymorphism. Lane M, 100-bp marker; lane 1, T/T genotype (225- and 344-bp bands); lane 2, C/T genotype (165-, 225-, and 344-bp bands); lane 3, C/C genotype (165- and 344-bp bands)

Statistical analysis

Differences in proportions were examined with Fisher's exact test. The 95% confidence intervals (CIs) for percentages were calculated based on binomial distributions. Odds ratios (ORs) were applied to assess the strength of associations of the *AICDA* genotype with the risks of *H. pylori* seropositivity, atrophic gastritis, and gastric cancer. Logistic regression analysis was performed to estimate the ORs and 95% CIs after adjustment for sex and age as continuous variables. The ORs of atrophic gastritis or severe atrophic gastritis among the controls were calculated relative to those without each disease defined as a reference. The calculations were done using STATA version 7 (Stata, College Station, TX, USA).

Results

Characteristics of the subjects and allele frequency of the *AICDA* polymorphism

The characteristics of the subjects are summarized in Table 1. The mean age $\pm$ SD was 58.7 ± 10.6 years for the controls and 58.8 ± 10.5 years for the cases. The proportions of females were 26.5% in the controls and 26.4% in the cases; 57.3% of the controls were infected with *H. pylori*, while 30.3% of the controls had atrophic gastritis. *H. pylori* seropositivity was not associated with sex, but increased with age. The genotype frequency of the *AICDA* polymorphism among the controls was 30.7% for *C/C*, 50.7% for *C/T*, and 18.6% for *T/T*,

which was in Hardy-Weinberg equilibrium ($\chi^2 = 1.402$; $P = 0.237$). The allele frequency was 56.1% for the *C* allele and 43.9% for the *T* allele. Among the cases the genotype frequency was 29.3% for *C/C*, 50.8% for *C/T*, and 19.9% for *T/T*. The allele frequency was 54.7% for the *C* allele and 45.3% for the *T* allele.

AICDA polymorphism, *H. pylori* seropositivity, atrophic gastritis, and gastric cancer

There was no significant association between the *AICDA* polymorphism and *H. pylori* seropositivity among the controls; the OR of the *T/T* genotype was 0.91 and that of the *C/T+T/T* genotype was 0.94 relative to the *C/C* genotype (Table 2).

Among the 938 *H. pylori* seropositive control subjects, 496 (52.9%) subjects had gastric atrophy and 168 (17.9%) subjects had severe atrophy. On the other hand, there were only 45 (6.4%) subjects with gastric atrophy and 28 (4.0%) subjects with severe atrophy among the 699 *H. pylori* seronegative control subjects. The difference in the prevalence was statistically significant ($P < 0.001$; χ^2 test). The genotype distribution among the seronegative control subjects was not significantly associated with gastric atrophy by the χ^2 test ($P = 0.077$).

The age- and sex-adjusted ORs of atrophic gastritis among the *H. pylori* seropositive control subjects were 0.84 (95% CI, 0.62–1.13) for *C/T*, 0.82 (95% CI, 0.56–1.21) for *T/T*, and 0.83 (95% CI, 0.63–1.11) for *C/T+T/T*, compared with the *C/C* genotype. The age- and sex-adjusted ORs of severe atrophic gastritis among the

Table 1. Characteristics of the subjects and the *AICDA* 7888 C/T polymorphism

	Controls <i>n</i> = 1637			Cases <i>n</i> = 583
	<i>H. pylori</i> (–)	<i>H. pylori</i> (+)		
		GA (–)	GA (+)	
<i>n</i>	699	442	496	583
Sex				
Male	479 (68.5%)	363 (82.1%)	362 (73.0%)	429 (73.6%)
Female	220 (31.5%)	79 (17.9%)	134 (27.0%)	154 (26.4%)
Age (years)				
<30	6 (0.9%)	1 (0.2%)	1 (0.2%)	2 (0.3%)
30–39	67 (9.6%)	11 (2.5%)	3 (0.6%)	31 (5.3%)
40–49	138 (19.7%)	54 (12.2%)	31 (6.3%)	64 (11.0%)
50–59	195 (27.9%)	151 (34.2%)	141 (28.4%)	214 (36.7%)
60–69	211 (30.2%)	153 (34.6%)	222 (44.8%)	166 (28.5%)
70–	82 (11.7%)	72 (16.3%)	98 (19.8%)	106 (18.2%)
Genotype				
<i>C/C</i>	213 (30.5%)	127 (28.7%)	163 (32.9%)	171 (29.3%)
<i>C/T</i>	353 (50.5%)	231 (52.3%)	246 (49.6%)	296 (50.8%)
<i>T/T</i>	133 (19.0%)	84 (19.0%)	87 (17.5%)	116 (19.9%)

GA (–), subjects with no gastric atrophy; GA (+), subjects with gastric atrophy (prostaglandin [PG] I $\leq$ 70 ng/ml and PG I / PG II $\leq$ 3)

Table 2. Age- and sex-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of the *AICDA* 7888 C/T polymorphism for *H. pylori* seropositivity among controls

Genotype	<i>n</i>	<i>H. pylori</i> +	Percentage	OR ^a	95% CI
<i>C/C</i>	503	290	57.7	1	Reference
<i>C/T</i>	830	477	57.4	0.94	0.74–1.18
<i>T/T</i>	304	171	56.3	0.91	0.68–1.23
<i>C/T+T/T</i>	1,133	647	57.1	0.94	0.75–1.16

^aAdjusted for age and sex**Table 3.** Genotype frequencies for the *AICDA* 7888 C/T polymorphism, age- and sex-adjusted odds ratios (ORs), and 95% confidence intervals (CIs) of atrophic gastritis in the *H. pylori* seropositive control subjects (a) and *H. pylori*-infected control subjects (b)

Genotype	<i>n</i>	All gastric atrophy (%)	OR	95% CI	<i>P</i>	Severe gastric atrophy (%)	OR ^a	95% CI	<i>P</i>
a									
<i>C/C</i>	290	163 (56.2)	1	Reference		54 (18.6)	1	Reference	
<i>C/T</i>	477	246 (51.6)	0.84	0.62–1.13	0.245	87 (18.2)	0.94	0.64–1.38	0.735
<i>T/T</i>	171	87 (50.9)	0.82	0.56–1.21	0.320	27 (15.8)	0.83	0.49–1.39	0.477
<i>C/T + T/T</i>	648	333 (51.4)	0.83	0.63–1.11	0.208	114 (17.6)	0.91	0.63–1.32	0.605
b									
<i>C/C</i>	305	178 (58.4)	1	Reference		61 (20.0)	1	Reference	
<i>C/T</i>	492	260 (52.8)	0.79	0.59–1.07	0.124	100 (20.3)	0.97	0.67–1.40	0.874
<i>T/T</i>	183	99 (54.1)	0.85	0.58–1.24	0.391	34 (18.6)	0.90	0.56–1.45	0.667
<i>C/T + T/T</i>	675	359 (53.2)	0.81	0.61–1.07	0.134	134 (19.9)	0.95	0.67–1.35	0.780

^aAdjusted for age and sex**Table 4.** Genotype frequencies for the *AICDA* 7888 C/T polymorphism, age- and sex-adjusted odds ratios (ORs), and 95% confidence intervals (CIs) of gastric cancer in subjects with atrophic gastritis

Genotype	All gastric atrophy <i>n</i> = 541	Gastric cancer <i>n</i> = 583	OR ^a	95% CI	<i>P</i>
<i>C/C</i>	179 (33.1%)	171 (29.3%)	1	Reference	
<i>C/T</i>	263 (48.6%)	296 (50.8%)	1.17	0.89–1.54	0.259
<i>T/T</i>	99 (18.3%)	116 (19.9%)	1.21	0.85–1.71	0.288
<i>C/T + T/T</i>	362 (66.9%)	412 (70.7%)	1.18	0.91–1.53	0.208

^aAdjusted for age and sex

H. pylori seropositive control subjects were 0.94 (95% CI, 0.64–1.38) for *C/T*, 0.83 (95% CI, 0.49–1.39) for *T/T*, and 0.91 (95% CI, 0.63–1.32) for *C/T+T/T* (Table 3a). When the *H. pylori* seropositive controls and seronegative controls with atrophic gastritis were regarded as *H. pylori*-infected controls, the age- and sex-adjusted OR of atrophic gastritis among the *H. pylori* infected controls was 0.81 (95% CI, 0.61–1.07) for *C/T+T/T* (Table 3b).

To investigate how this polymorphism of *AICDA* contributes to the gastric carcinogenesis arising from atrophic gastritis, we next compared the genotype distribution between the gastric cancer cases and atrophic gastritis subjects. The age- and sex-adjusted ORs of gastric cancer were 1.17 (95% CI, 0.89–1.54) for *C/T*, 1.21 (95% CI, 0.85–1.71) for *T/T*, and 1.18 (95% CI, 0.91–1.53) for *C/T+T/T*, relative to the *C/C* genotype, none of which were statistically significant (Table 4).

Discussion

Our study results in a Japanese cohort did not show the hypothesized association of the *AICDA* 7888 C/T polymorphism with the risk of atrophic gastritis among *H. pylori*-infected control subjects or the risk of gastric cancer among control subjects with gastric atrophy. *AICDA* was initially identified as a gene selectively expressed in B cells that are activated to undergo CSR. The *AICDA* sequence has the closest homology to the apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (*APOBEC1*), an mRNA cytidine deaminase involved in lipid metabolism. Soon after its identification, *AICDA* was shown to be essential for both CSR and SHM in mice, and mutations in the *AICDA* gene were found to be associated with hyper-IgM syndrome type 2 (HIGM2) [19].

Atrophic gastritis and intestinal metaplasia are well-known histological precursors of intestinal-type distal

gastric adenocarcinoma. *H. pylori* colonizes the gastric epithelium, inducing a chronic inflammatory reaction that may persist throughout the patient's lifetime in spite of a strong local immune reaction. The extent and severity of the gastric mucosal inflammation, as well as the clinical outcome of the infection, depend on a number of factors, including the virulence of the bacterium, such as the presence of CagA; the presence of oxygen-derived free radicals; host genetic susceptibility; immune response; age at which the infection was acquired; and environmental factors including dietary factors such as vegetable intake [20–22].

In the present study our hypothesis that gastric cancer development after *cagA*-positive *H. pylori* infection would be influenced by the *AICDA* polymorphism was not confirmed. As initially mentioned, *H. pylori* infection is a well-established risk factor for gastric cancer [22], and has been shown to induce aberrant *AICDA* expression in the presence of CagA in a NF κ B-dependent way in gastric epithelial cells, leading to nucleotide alterations in the *p53* gene, which is one of the most critical events in gastric carcinogenesis [14, 23, 24]. Taking this pivotal role of *AICDA* in *H. pylori*-induced gastric carcinogenesis, it would be plausible to hypothesize that functional polymorphisms in *AICDA* might influence this important process. In addition, considering the biological mechanisms in the genesis of gastric mucosal inflammation mentioned above, in addition to the fact that *p53* mutations have been detected early in the histopathologic sequence of gastric cancer or metaplasia [23, 25], it would also seem possible that alterations in immunological responses by B cells or in the effects of aberrantly expressed *AICDA* in gastric epithelial cells due to this *AICDA* polymorphism might induce atrophic changes in the gastric mucosa and subsequent gastric carcinogenesis. These hypotheses, however, were not confirmed in the present study.

To date, several reports about the relationship of this *AICDA* 7888C/T polymorphism and diseases have been published. First, a Japanese group reported that the 7888C allele was transmitted preferentially to asthmatic children a finding which was statistically significant by the transmission disequilibrium test (TDT; $P = 0.007$), and they also found that serum IgE levels were significantly associated with *AICDA* 7888 C/T genotypes, where those with the C/C genotype had the highest IgE levels [15]. Subsequently, one Japanese report showed no association with childhood asthma [26], and one Danish report also showed no association with common variable immunodeficiency (CVID) [27]. One Chinese report demonstrated an inverse correlation of this polymorphism with adult allergic asthma, where those with T/T genotypes confer susceptibility to asthma and are associated with higher plasma IgE [28]; we speculate

that these differing findings could be attributable to the small numbers of subjects in the studies or to differences in gene-environment or gene-gene interactions between the people of different ethnicities. In the present study, no statistically significant associations were observed between this *AICDA* 7888 C/T polymorphism and the risk of *H. pylori* infection, gastric atrophy, or gastric carcinogenesis, indicating that the influence of this *AICDA* polymorphism on *H. pylori*-induced gastric carcinogenesis is limited in Japanese people.

The 7888 C/T polymorphism of *AICDA* is a synonymous single nucleotide polymorphism (SNP) in exon 4. Although the biological role of the present polymorphism is yet to be clarified, the polymorphism might have some influence on the formation of *AICDA* mRNA splicing variants, of which eight forms have been reported to date (SpliceMinor homepage developed by The Genomics & Bioinformatics Group (GBG) of NCI: <http://www.tigerteamconsulting.com/SpliceMiner/>) through a linkage to another still unknown SNP. In the Japanese study described above [26], the authors tried to evaluate the relationship between *AICDA* mRNA and the polymorphism, where they found novel *AICDA* splicing variants all lacking part of exon 4, and these variants were not associated with this *AICDA* 7888 C/T polymorphism. According to Revy et al. [29], only subjects with homozygous missense mutation or a stop codon in exon 4 developed HIGM2, suggesting that this splice variant has no enzyme activity. The effect of the *AICDA* 7888 C/T polymorphism or its linked polymorphisms on the function of *AICDA* itself, and the interaction between *AICDA* and CagA require further investigation. There may also still be a possibility of gastric carcinogenesis occurring via *AICDA* SNPs other than the 7888 C/T polymorphism, which also warrants further examination.

The T allele frequencies of the *AICDA* polymorphism (rs2028373) among people of different ethnicities are as follows: 0.575 of 120 chromosomes in Caucasians, 0.433 of 90 chromosomes in Japanese, 0.589 of 90 chromosomes in Chinese, and 0.317 of 120 chromosomes in Africans. The T allele frequency in our controls (0.438) was similar to the reported allele frequency in Japanese subjects.

The present study has several limitations. Although the *H. pylori* status of the control subjects was examined with a serology test, we did not check the CagA status. As reported in Japan, nearly 100% of *H. pylori* strains possess a functional *cag* pathogenicity island (*cagPAI*), which encodes and produces the CagA protein [30]. A previous study also confirmed that almost all strains isolated from our Japanese subjects were East Asian *cagA*-positive strains [31, 32], indicating that the *H. pylori* strains in our study subjects also possessed CagA. Another limitation is related to the diagnosis of

atrophic gastritis. This was done entirely on the basis of serum PG levels and not through histological assessment. However, the PG method is well established as a surrogate marker of atrophic gastritis [33–35]. The validated criterion for atrophic gastritis is PG I 70 ng/ml or less and a PG I/II ratio of 3.0 or less, and that for severe atrophic gastritis is PG I 30 ng/ml or less and a PG I/II ratio of 2.0 or less, both of which are presumed to be reliable because they are widely used in practice in Japan [36, 37]. The *H. pylori* seropositivity and PG levels were not examined in the gastric cancer cases in this study, based on the assumption that most of the gastric cancer cases would be *H. pylori*-positive with atrophic gastritis [21, 38]. Considering that the intestinal type of gastric cancer, the predominant type of gastric cancer in Japan, arises from gastric atrophy caused by *H. pylori* infection, and the diffuse type of gastric cancer occurs regardless of gastric atrophy [39, 40], and non-cardia gastric cancer is associated with *H. pylori* infection and gastric atrophy, while cardia gastric cancer is not [41], it would be intriguing to perform a subgroup analysis according to these two histological types or tumor locations. These analyses might help unveil the association of this *AICDA* polymorphism with the steps of gastric carcinogenesis more clearly. However, we could not perform such analyses because of the unavailability of histological data and data about tumor location.

In summary, the present study has revealed that the *AICDA* 7888 C/T polymorphism is not significantly associated with the risk of *H. pylori* infection, atrophic gastritis, or gastric cancer in Japanese subjects, suggesting that the influence of this *AICDA* polymorphism on *H. pylori*-induced gastric carcinogenesis is limited in Japanese people. Further examinations with larger numbers of study subjects, along with the elucidation of the function of this *AICDA* polymorphism, are expected to verify our findings.

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