SPANDIDOS PUBLICATIONS

Effect of polymorphisms of IL-17A, -17F and MIF genes on CpG island hyper-methylation (CIHM) in the human gastric mucosa

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Abstract. CpG island hyper-methylation (CIHM) is one of the major events in the gastric carcinogenesis and also occurs in non-neoplastic gastric mucosa. IL-17A, -17F and MIF have a crucial role in the gastric inflammationand carcinogenesis. The CIHM status in the non-cancerous gastric mucosa, in relation to IL-17A (-197G>A, rs2275913), -17F (7488T>C, rs763780) and MIF (-173G>C and -794 tetranucleotide repeats) polymorphisms was investigated. Gastric mucosa samples were obtained from 121 cancer free subjects. CIHM of p14, p16, DAP-kinase and CDH1 genes were determined by methylation-specific polymerase chain reaction (MSP). CIHM high was defined as three or all CpG islands methylated. We employed the PCR-SSCP (multiplex PCR for IL-17A and -17F) method to detect the gene polymorphisms. No association were found between CIHM status and L-17A (-197G>A), IL-17F (7488T>C) and MIF (-173G>C) polymorphisms. MIF 5-CATT repeat carrier (5/5+5/6+5/7) held a significantly higher risk of CIHM of DAP-kinase (OR=2.33, 95% CI=1.07-5.09, p=0.03) and CIHM high (OR=3.63, 95% CI=1.31-10.08, p=0.01). Weak association was also found between the same genotype and increased risk of CIHM of p16 (OR=2.45, 95% CI=0.90-6.68, p=0.08) and CDH1 (OR=2.23, 95% CI=0.94-5.32, p=0.07). 6-CATT repeat carrier (5/6+6/6+6/7) was significantly associated with reduced risk of CIHM of p16 (OR=0.31, 95% CI=0.11-0.90, p=0.03), CDH1 (OR=0.40, 95% CI=0.17-0.98, p=0.045), DAP-kinase (OR=0.37, 95% CI=0.17-0.83, p=0.02) and CIHM high (OR=0.25, 95% CI=0.09-0.74, p=0.01). -7-CATT repeat carrier (6/7+7/7) was weakly associated with reduced risk of CIHM of p16 (OR=0.34, 95% CI=0.10-1.13, p=0.08), DAP-kinase (OR=0.43, 95% CI=0.17-1.06, p=0.07) and CIHM high (OR=0.38, 95% CI=0.12-1.20, p=0.098). The present results provided the first evidence that the genetic polymorphisms MIF polymorphism is associated with CIHM status in the human gastric mucosa. Genetic polymorphisms of MIF-794-CATT repeat may be involved in methylation related carcinogenesis in the stomach.

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Abbreviations: CIHM, CpG island hyper-methylation; MIF, macrophage migration inhibitory factor; *H. pylori*, *Helicobacter pylori*; MSP, methylation-specifc PCR

Key words: CpG island hyper-methylation, stomach, interleukin-17, macrophage migration inhibitory factor, genetic polymorphism

Introduction

CpG island hyper methylation (CIHM) has been shown to be an important mechanism in gene silencing. In many kinds of cancer, several genes acquire CIHM. Meanwhile, some genes are also methylated in non-neoplastic tissues with aging, and this alteration is known as age-related methylation (1,2). p16(INK4a) and p14(ARF) are involved in the negative cell cycle regulation via the pRb and p53 pathways, respectively. These two proteins have an independent first exon (exon 1a and 1ß, respectively) but share exon 2 and 3 (3,4). Methylation of p16 and p14 has been shown to be present in gastric cancer as well as premalignant lesion (5,6).

E-cadherin (CDH1) is an adhesion molecule involved in tumour invasion/metastasis. Silencing of E-cadherin by promoter CpG methylation has also been shown in gastric cancer (7). Death-associated protein kinase (DAP-kinase) is a calcium/calmodulin-dependent serine/threonine kinase, and participates in various apoptosis systems. Methylation of DAP-kinase has been reported in many cancers (8) including gastric cancer (9,10). It has also been reported that CIHM of those 4 genes are frequently occurred in non-cancerous gastric mucosa in relation to *H. pylori* infection (7,11,12), histological

or serological severity of gastritis (12) and gastric cancer occurrence (13-15). Therefore, they may be susceptible candidate genes for CIHM in the stomach.

As the possible mechanisms of CIHM, exogenous carcinogens, generated reactive oxygen and host genetic differences may influence its status (16). One of the most important factors causing CIHM in the stomach is *H. pylori* infection (7,11,12), which induces chronic inflammation, causing oxidative stress to the gastric epithelium (16,17). However, there is considerable variation in the extent of gastric inflammation among *H. pylori* infected patients and not all of them show same CIHM status, suggesting that some inter individual genetic difference may contribute to this process.

Interleukin-17 (IL-17) is a relatively newly described cytokine which bridges the adaptive and innate immune systems. IL-17 family members belong to a distinct category of cytokines and play a role in coordinating local tissue inflammation by inducing the release of proinflammatory and neutrophil-mobilizing cytokines (19). In addition, IL-17A and -17F share similar functions in terms of their ability to induce chemokines that are important in neutrophil recruitment and activation. Recently, Kawaguchi *et al* reported that the IL-17F 7488T/C (rs763780), which causes a His-to-Arg substitution at amino acid 161 (H161R) variant, influences the risk of asthma and is a natural IL-17F antagonist in the known polymorphisms of the IL-17 gene (20).

Macrophage migration inhibitory factor (MIF) was originally identified as an activity isolated from T lymphocytes which was capable of inhibiting the random migration of macrophages (21). MIF contributes toward an excessive inflammatory response both directly and via an induction of proinflammatory cytokine secretion (22). Polymorphisms with potential functional relevance have been identified in the MIF gene promoter; an SNP at position -173 (G to C) (23) and a tetranucleotide CATT repeat beginning at nucleotide position -794 (24) were found to be associated with altered levels of MIF gene transcription *in vitro*. Important roles of both IL-17 and MIF in the inflammatory response to *H.* pylori infection have been demonstrated (25,26). Recently, we have also shown the potential roles of IL-17A, -17F and MIF polymorphisms in the gastric inflammation (27,28) and carcinogenesis (29,30).

Consequently, this study was performed to clarify whether the polymorphisms of IL-17A (-197G>A, rs2275913), -17F (7488T>C, rs763780) and MIF (-173C>G and -794 tetranucleotide repeats) influence the methylation related carcinogenesis. In this study, we investigated the prevalence of CIHM of 4 candidate genes: p14, p16, CDH1 and DAP-kinase, they have been thought to be most susceptible for methylation in the stomach (7,11-15) among non-cancerous gastric mucosa and its relation to IL-17A, IL-17F and MIF polymorphisms.

Materials and methods

Tissue samples and DNA extraction. The study population comprised 121 non-cancer subjects, attending the Endoscopy Center of Fujita Health University Hospital from January 2005 to May 2008. All the subjects underwent upper gastroscopy as part of a health check, as a secondary procedure following barium X-ray examination for suspected stomach cancer, or for the investigation of abdominal discomfort. This cohort was partly recruited from recent study investigating the association between promoter CIHM and severity of chronic gastritis (12), host genetic factors (31). Patients who had severe systemic disease, or malignancy in the stomach or other organ, who had a history of gastric surgery were excluded from the study. Patients who had a history of continuous or occasional use of non-steroidal anti-inflammatory drugs and who had ungone *H. pylori* eradication were also excluded from this study. Biopsy specimens were taken from the antrum along the greater curvature, from grossly non-pathological mucosa in all the subjects. The specimens were cut into two pieces. One of the pieces was fixed in 10% buffered formalin and embedded in paraffin for microscopic histological examination and the other part was immediately frozen and stored at -80˚ until use. Histological analysis of all the selected biopsy samples also showed that these samples contained more than 70% of epithelial cells. Genomic DNA was extracted directly from the frozen specimens using a standard phenol/chloroform method. *H. pylori* infection status was assessed by serological, histological analysis or urea breath test. Patients were diagnosed as infected when at least one of the diagnostic tests was positive. The Ethics Committee of the Fujita Health University School of Medicine approved the protocol and prior, written informed consent was obtained from all participating subjects.

Bisulfite modification and methylation-specifc PCR (MSP). To detect CIHM, we chose four candidate promoter CpG islands, which were thought to be most susceptible for methylation in the stomach (7,11-15): p14, p16, DAP-kinase and CDH1. For the examination of DNA methylation, genomic DNA was treated with sodium bisulfite using BislFast DNA modification kit for methylated DNA detection (Toyobo, Co., Ltd., Osaka, Japan). MSP were carried out with primers as follows: p14 methylated forward (p14 MF); 5'-gtgttaaagggcggcgtagc-3', p14 methylated reverse (p14 MR); 5'-aaaaccctcactcgcgacga-3', which amplify 122-bp product, p14 un-methylated forward (p14 UF); 5'-tttttggtgttaaagggtg gtgtagt-3', p14 un-methylated reverse (p14 UR); 5'-cacaaaaac cctcactcacaacaa-3' which amplify 132-bp product (32), p16 methylated forward (p16 MF); 5'-ttattagagggtggggcggatcgc-3', p16 methylated reverse (p16 MR); 5'-gaccccgaaccgcgaccg taa-3', which amplify 150-bp product, p16 un-methylated forward (p16 UF); 5'-ttattagagggtggggtggattgt-3', p16 unmethylated reverse (p16 UR); 5'-caaccccaaaccacaaccataa-3', which amplify 151-bp product (33), CDH1 methylated forward (CDH1 MF); 5'-ttaggttagagggttatcgcgt-3', CDH1 methylated reverse (CDH1 MR); 5'-taactaaaaattcacctaccgac-3', which amplify 115-bp product, CDH1 un-methylated forward (CDH1 UF); 5'-taattttaggttagagggttattgt-3', CDH1 unmethylated reverse (CDH1 UR); 5'-cacaaccaatcaacaacaca-3', which amplify 97-bp product (33), DAP-kinase methylated forward (DAP-kinase MF); 5'-ggatagtcggatcgagttaacgtc-3', DAP-kinase methylated reverse (DAP-kinase MR); 5'-ccc tcccaaa cgccga-3', which amplify 98-bp product, DAP-kinase unmethylated forward (DAP-kinase UF); 5'-ggaggatagttggat tgagttaatgtt-3', DAP-kinase un-methylated reverse (DAP-kinase UR); 5'-caaatccctcccaaacaccaa-3', which amplify 106-bp

(34). An annealing temperature and times were ed using DNA from peripheral blood of a young individual without *H. pylori* infection and DNA methylated with SssI methylase (New England BioLabs Inc., Beverly, MA). The MSP was carried out in a volume of 20 μl containing 0.1 μg of bislufite-modificated DNA. The DNA was denatured at 95˚C for 5 min, followed by 33-35 cycles at 95˚C for 30 sec and 57-69˚C according to primers for 1 min, and 72˚C for 1 min with a final extension at 72˚C for 5 min. MSP reactions were done using EX Taq HS (Takara Bio Inc., Shiga, Japan). PCR products (10 μl) were separated by electrophoresis in 2.5% agarose gels, and visualized by UV illumination using an ethdium bromide staining. CIHM was defined as the presence of positive methylation band, showing signals approximately equivalent to or greater than that of size marker (10 ng/μl: 100 bp DNA Ladder, Takara Bio Inc.), irrespective of the presence of un-methylated bands. Samples giving faint positive signals were analyzed a further two times and only those samples with consistent positive methylation band were considered as CIHM. In addition, we measured the fluorescence intensities of methylated bands for randomly selected 50 CHIM samples using digital densitometer (Lane Analyzer, ATTO, Tokyo, Japan), and confirmed that the fluorescence intensities of all 50 methylated bands were approximately equivalent to or greater than that of size marker (data not shown). CIHM high was also defined as three or more CpG islands methylated.

Genotyping of polymorphisms. Polymorphism was genotyped by PCR-SSCP method as previously described (27-30). To detect the IL-17A and -17F polymorphisms, using the primer pairs (IL-17AF, 5'-aacaagtaagaatgaaaagagg acatggt-3'; IL-17AR, 5'-cccccaatgaggtcatagaagaatc-3'; IL-17FF, 5'-gtg taggaacttgggctgcatcaat-3'; and IL-17FR: 5'-agtggatat gcacctcttactgcaca-3', respectively), one-tube multiplex PCR was carried out in a volume of 20 μl containing 0.1 μg of genomic DNA. The DNA was denatured at 96˚C for 90 sec, followed by 35 cycles at 96˚C for 15 sec, 58˚C for 30 sec and 72˚C for 45 sec, with a final extension at 72˚C for 3 min. Thereafter, 2 μl of the PCR product was denatured with 10 μl of formamide (Sigma-Aldrich Co., St. Louis, USA) at 90˚C for 5 min. SSCP was carried out at 6 or 12˚C using a GenePhor DNA separation system with GeneGel Excel 12.5/24 (Amersham Biosciences Corp., USA), after which the denatured singlestrand DNA bands were detected using a DNA Silver Staining kit (Amersham Biosciences Corp.).

To detect MIF-794 CATT repeats and the G-173C polymorphism, using the primer pairs (MIFTRF, 5'-tgatccagt tgctgccttgtc-3'; MIFTRR, 5'-tccactaatggtaaactcggggac-3'; MIF173F, 5'-tctagccgccaagtggagaaca-3'; and MIF173R, 5'-act gtggtcccgccttttgtga-3', respectively), PCR was carried out in a volume of 20 μl containing 0.1 μg of genomic DNA. The DNA was denatured at 95˚C for 3 min, followed by 35 cycles at 95˚C for 30 sec, 60 or 62˚C for 40 sec and 72˚C for 45 sec, with a final extension at 72˚C for 5 min. Thereafter, SSCP was carried out in a similar manner as described above.

Statistical analysis. Statistical analysis was done with χ^2 test for the comparison of CIHM in different gender and, *H. pylori* infection status. Student's t-test was used for the association between CIHM and age. Logistic regression analysis with adjustment for sex, age and *H. pylori* infection status were used for the association between CIHM and two groups of different genotypes. A probability value of <0.05 was considered statistically significant.

Table I. Characteristics of the 121 subjects.

Variables	
Age (mean SD)	62.7±12.6
Sex (Male/female)	69/52
H. pylori infection positive ratio (%)	57
Active ulcer	14

Results

Characteristics of subjects. A total of 121 cancer free subjects participated in this study. The characteristics of subjects are shown in Table I. After gastroscopy, 14 subjects were diagnosed as having active peptic ulcers.

Association between CIHM and age, gender and H. pylori infection status. All 121 gastric mucosa samples were available for MSP analysis. In all subjects, CIHM was found in 45 (37.2%) for p14, 37 (30.6%) for p16, 54 subjects (44.6%) for CDH1 and 62 (51.2%) for DAP-kinase. CIHM high was also found in 34 subjects (28.1%). CIHM of DAP-kinase was significantly associated with aging, while CIHM of p16, CDH1 and CIHM high were significantly associated with *H. pylori* infection status. Weak association was also found between CIHM of p14 and age (Table II).

Multivariate logistic regression analysis for relationship between CHIM and IL-17A, IL-17F and MIF polymorphisms. IL-17A (-197G>A) and IL-17F (7488T>C) polymorphisms were successfully genotyped for 120 subjects, while MIF (-173G>C) and MIF (-794-CATT repeat) polymorphisms were successfully genotyped for 115 subjects. The prevalence of IL-17A, IL-17F and MIF genotypes are shown in Table III. We did not find significant association between CIHM status and L-17A (-197G>A), IL-17F (7488T>C) and MIF (-173G>C) polymorphisms. However, concerning the MIF (-794-CATT repeat) polymorphism, we found that 5-CATT repeat carrier (5/5+5/6+5/7) held a significantly higher risk of CIHM of DAP-kinase (OR=2.33, 95% CI=1.07-5.09, p=0.03) and CIHM high (OR=3.63, 95% CI=1.31-10.08, p=0.01). Weak association was also found between the same genotype and increased risk of CIHM of p16 (OR=2.45, 95% CI=0.90-6.68, p=0.08) and CDH1 (OR=2.23, 95% CI=0.94-5.32, p=0.07). On the other hand, 6-CATT repeat carrier (5/6+6/6+6/7) was significantly associated with reduced risk of CIHM of p16 (OR=0.31, 95% CI=0.11-0.90, p=0.03), CDH1 (OR=0.40, 95% CI=0.17-0.98, p=0.045), DAP-kinase (OR=0.37, 95% CI=0.17-0.83, p=0.02) and CIHM high (OR=0.25, 95% CI=0.09-0.74, p=0.01). We also found that -7-CATT repeat carrier (6/7+7/7) was weakly associated with reduced risk of CIHM of p16 (OR=0.34, 95% CI=0.10-1.13, p=0.08),

Table II. CIHM status of 4 promoter CpG islands in gastric mucosa, in relation to gender, age and *H. pylori* infection status.

Variables (n)	Age[a] (mean ± SD)	Gender		*H. pylori* infection status[b]	
		Male	Female	*H. pylori* (-)	*H. pylori* (+)
p14					
Un-methylated (76)	**61.0±12.8**	41	35	35	41
Methylated (45)	**65.5±11.8**	28	17	17	28
p16					
Un-methylated (84)	63.3±13.4	48	36	**49**	**35**
Methylated (37)	61.4±10.6	21	16	**3**	**34**
CDH1					
Un-methylated (67)	63.7±13.2	39	28	**41**	**26**
Methylated (54)	61.5±11.7	30	24	**11**	**43**
DAP kinase					
Un-methylated (59)	**60.8±12.3**	31	28	27	32
Methylated (62)	**64.5±12.7**	38	24	25	37
CIMH high					
CIMH high(-) (87)	64.4±9.9	50	37	**47**	**40**
CIMH high(+) (34)	62.1±13.5	19	15	**5**	**29**

CIHM high was defined as three or more CpG islands were methylated. [a]p14; p=0.06, DAP-kinase; p=0.10: Student's t-test. [b]p16, CDH1; p<0.0001, CIHM high; p=0.0003: χ^2.

DAP-kinase (OR=0.43, 95% CI=0.17-1.06, p=0.07) and CIHM high (OR=0.38, 95% CI=0.12-1.20, p=0.098). No association was found between CHIM status and homozygote genotypes of each repeats (-794-CATT 5/5, 6/6, and 7/7) (data not shown).

Discussion

Our present result showed that CIHM of DAP-kinase was significantly associated with aging, while CIHM of p16, CDH1 and CIHM high were significantly associated with *H. pylori* infection status. It has been reported that CIHM is observed in non-neoplastic gastrointestinal tissues as age related phenomenon (1,2,35). There have also been reports showing that *H. pylori* infection is the most predisposing factor for gene methylation in the stomach (7,11,12). Thus, our present result is in line with other studies and seems reasonable.

Although, the mechanisms of CIHM are unknown, several factors may contribute to this methylation, such as exogenous carcinogens, generated reactive oxygen and host genetic differences (31). One of the most important factors causing CIHM in the stomach is *H. pylori* infection (7,11,12), which induces chronic inflammation, causing oxidative stress to the gastric epithelium (16,17). Indeed, the methylation of certain genes in non-neoplastic gastric mucosa correlate with *H. pylori* related histological or serological severity of gastritis (12) and gastric cancer occurrence (13-15). Thus, this epigenetic change is thought to be an early step in gastric carcinogenesis. However, not all patients with *H. pylori* infection show CIHM and develop gastric cancer. This difference may be attributed to some genetic factors.

In our study, MIF 5-CATT repeat carrier was significantly associated with higher risk of CIHM of DAP-kinase and CIHM high. While, the 6-CATT repeat carrier was significantly associated with reduced risk of CIHM of p16, CDH1, DAP-kinase and CIHM high. Weak association was also found between the MIF-794-CATT 5 repeat carrier and increased risk of CIHM of p16 and CDH1, -794-CATT 7 repeat carrier and reduced risk of CIHM of p16, DAP-kinase and CIHM high.

MIF was originally identified as an activity isolated from T lymphocytes that was capable of inhibiting the random migration of macrophages (36,37). Many studies have shown MIF to be a key modulator of many chronic and disabling human disorders, such as rheumatoid arthritis (38), sepsis (39), acute respiratory syndrome (40), and atopic diseases (41,42). Several studies have also shown significant associations of MIF gene promoter functional polymorphisms with cystic fibrosis (43), psoriasis (44) and atopic disorders (45). An important role of MIF in gastric disorders has also been shown in gastric inflammation (26), ulcer (46) and carcinogenesis (47). It is well known that a polarized T helper 1 immune response occurs in *H. pylori* infection (29). MIF directly activates or promotes cytokine expression [TNF (48), IL-2 (49), IL-8 (50) and INF-γ (51)]. Furthermore, Xia *et al* have reported that *H. pylori* stimulates MIF release in monocytes and expression of MIF in gastric mucosa (26). We have recently shown the potential association between MIF gene promoter functional polymorphisms and gastric cancer

Table III. Association between IL-17A, IL-17F, MIF polymorphisms and methylation status of 4 promoter CpG islands in gastric mucosa.

	IL-17A (-197G>A)			*IL-17F (7488T>C)*			*MIF (-173G>C)*			*MIF (-794-CATT repeat)*[a]					
	Genotype (n)			Genotype (n)			Genotype (n)			Genotype (n)					
Variables (n)	GG	GA	AA	TT	TC	CC	GG	GC	CC	5/5	5/6	5/7	6/6	6/7	7/7
p14															
Un-methylated (76)	33	41	9	70	13	0	47	31	3	13	25	7	9	13	3
Methylated (45)	10	23	4	33	3	1	20	13	1	8	14	3	9	11	0
p16															
Un-methylated (84)	30	40	6	65	10	1	40	26	4	15	24	6	14	21	1
Methylated (37)	13	24	7	38	6	0	27	18	0	6	15	4	4	3	2
CDH1															
Un-methylated (67)	22	35	9	56	10	0	39	22	3	14	16	4	15	14	1
Methylated (54)	21	29	4	47	6	1	28	22	1	7	23	6	3	10	2
DAP kinase															
Un-methylated (59)	22	29	7	50	8	0	30	24	1	11	13	4	10	16	1
Methylated (62)	21	35	6	53	8	1	37	20	3	10	26	6	8	8	2
CIMH high															
CIMH high(-) (87)	32	44	10	74	12	0	46	33	3	16	32	6	16	20	2
CIMH high(+) (34)	11	20	3	29	4	1	21	11	1	5	17	4	2	4	1

[a]*5 repeat carrier (5/5+5/6+5/7) vs. others;* p16 methylated: OR (95% CI) p=2.45 (0.90-6.68)0.08, CDH1 methylated: OR (95% CI) p=2.23 (0.94-5.32) 0.07, DAP-kinase methylated: OR (95% CI) p=2.33(1.07-5.39)0.03, CIMH high: OR (95% CI) p=3.63(1.31-10.08)0.01.
6 repeat carrier (5/6+6/6+6/7) vs. others; p16 methylated: OR (95% CI) p=0.31 (0.11-0.90)0.03, CDH1 methylated: OR (95% CI) p=0.40 (0.17-0.98)0.45, DAP-kinase methylated: OR (95% CI) p=0.37(0.17-0.83)0.02, CIMH high: OR (95% CI) p=0.25 (0.09-0.74)0.01.
7 repeat carrier (6/7+7/7) vs. others; p16 methylated: OR (95% CI) p=0.34(0.10-1.13)0.08, DAP-kinase methylated: OR (95% CI) p=0.43(0.17-1.06)0.07, CIMH high: OR (95% CI) p=0.38(0.12-1.20) 0.98.
All data were adjusted for age, sex and *H. pylori* infection status. CIHM high was defined as three or more CpG islands were methylated. IL-17A and IL-17F polymorphisms could not be genotyped for one subjects and MIF polymorphisms could not be genotyped for six subjects.

(29) and *H. pylori* related chronic gastritis (27). Since the important roles that MIF plays with respect to gastric inflammation and carcinogenesis, it seems reasonable to expect that MIF gene promoter functional polymorphisms is also associated with CIHM status in the gastric mucosa.

From the functional perspective, promoter sequence analysis indicated that the -173C allele creates a potential activator protein 4 transcription factor binding site (23), and levels of MIF expression significantly differed among -173G>C genotypes in a cell type manner. Regarding CATT repeats, the 5-CATT allele was shown to be associated with lower basal and stimulated MIF promoter activity *in vitro* than 6-, 7- and 8-CATT alleles (24). Donn *et al* showed that increasing CATT repeats with the -173C allele significantly increased the promoter activity in a T lymphoblast cell line (23). Thus, the -173C allele and 7-CATT seemed to promote the production of MIF, although there is no clear relationship between these polymorphisms and the transcriptional regulation of the MIF gene. Baugh *et al* reported the correlation of the 5/5-CATT repeat with low disease severity in rheumatoid arthritis patients (53), and Hizawa *et al* also reported an increased risk of non-5-CATT carriers for atopy (45). Donn *et al* demonstrated that the -173C·7-CATT haplotype is of importance in the susceptibility to psoriasis (44). We have recently shown that both the 7/7-CATT repeat and the -173 C/C genotypes, as well as *H. pylori* infection and elder age, were significantly associated with the development of gastric mucosal atrophy. In addition, we have also shown that the 5-CATT carriers had a reduced risk of gastric cancer, especially the diffuse type cancer, and -173C carriers and the number of 7-CATT alleles were also positively correlated with the risk of gastric cancer in older subjects and intestinal type histopathology.

However, our present result showed that 5-CATT repeat carrier, which is associated with the lower basal and stimulated MIF promoter activity, increased, but not decreased risk of CIHM of DAP-kinase and CIHM high. While, the 6-CATT repeat carrier reduced risk of CIHM of p16, CDH1, DAP-kinase and CIHM high. As for -173G>C genotypes, we did not find significant association with CIHM. Although our observation indicates that the MIF-794-CATT repeat polymorphism, rather than the -173G>C polymorphism influences the risk of CIHM in the human gastric mucosa, why the 5-CATT repeat carrier increases the risk of CIHM needs to be explained.

Concerning the IL-17A and IL-17Fa polymorphisms, we did not find any significant association between IL-17A (-197G>A) and IL-17F (7488T>C) polymorphisms and CIHM status. Although IL-17 have a significant role in the inflammatory response to *H. pylori* infection (25,28,30), IL-17A and IL-17Fa polymorphisms may not influence the risk of methylation related carcinogenesis.

In conclusion, we have shown that, MIF-5-CATT repeat carrier is associated with higher risk of CIHM especially for DAP-kinase and CIHM high. Furthermore, the 6-CATT repeat carrier is associated with reduced risk of CIHM especially for p16, CDH1, DAP-kinase and CIHM high. The present results provided the first evidence that the genetic polymorphisms of MIF may be involved in DNA methylation in human gastric mucosa. However, our data did not provide the detailed mechanisms of MIF-794-CATT repeat polymorphism in CIHM. In addition, why the interplay between MIF-794-CATT repeat polymorphism and CIHM is different in different genes is still unexplained. Only a more extensive understanding of the regulation of methylation in relation to gene expression and carcinogenesis will allow us to fully interpret our findings.

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