BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Medical Microbiology

Helicobacter pylori with East Asian-type *cag*PAI genes is more virulent than strains with Western-type in some *cag*PAI genes

Xiao-yan Yuan, Jin-Jun Yan, Ya-chao Yang, Chun-mei Wu, Yan Hu, Jian-li Geng *

Weihai Municipal Hospital affiliated to Dalian Medical University, Department of Clinical Lab, Weihai, Shandong, PR China

ARTICLE INFO

Article history:
Received 14 December 2015
Accepted 7 July 2016
Available online 22 December 2016
Associate Editor: Elizabeth de Andrade Marques

Keywords:
Helicobacter pylori
*cag*PAI
Virulence
IL-8

ABSTRACT

The severity of *Helicobacter pylori*-related disease is correlated with the presence and integrity of a *cag* pathogenicity island (*cag*PAI). *cag*PAI genotype may have a modifying effect on the pathogenic potential of the infecting strain. After analyzing the sequences of *cag*PAI genes, some strains with the East Asian-type *cag*PAI genes were selected for further analysis to examine the association between the diversity of the *cag*PAI genes and the virulence of *H. pylori*. The results showed that gastric mucosal inflammatory cell infiltration was significantly higher in patients with East Asian-type *cag*PAI genes *H. pylori* strain compared with mosaicism *cag*PAI genes *H. pylori* strain ($p < 0.05$). *H. pylori* strains with the East Asian-type *cag*PAI genes were closely associated with IL-8 secretion *in vitro* and *in vivo* compared with *H. pylori* strains with the mosaicism *cag*PAI genes ($p < 0.01$). *H. pylori* strains with East Asian-type *cag*PAI genes are able to strongly translocate CagA to host cells. These results suggest that *H. pylori* strains with East Asian-type *cag*PAI genes are more virulent than the strains of *cag*PAI gene/genes that are Western type.

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium and is the main pathogenic factor of gastroduodenal diseases. *H. pylori* contains many virulence factors, including the *cag* pathogenicity island (*cag*PAI), that are involved in the pathogenesis of several diseases.[1,2] *H. pylori cag*PAI consists of approximately 30 genes encoding for proteins of the type IV secretion system (T4SS), which transfers CagA and peptidoglycan into host epithelial cells, resulting in increased cellular release of interleukin 8 (IL-8).[3,4] Studies have also demonstrated that *H. pylori* strains with a functional T4SS are more frequently associated with the etiology of *H. pylori*.[5,6]

H. pylori is a highly genetically diverse species that is attributed to its high rate of DNA recombination.[7–9] It has been reported in Japan that the genetic diversity within the *cag*PAI may have modifying effects on the pathogenic potential of infecting strains.[10] The similarity coefficient revealed that all *cag*PAI genes could be placed into two major groups, East Asian-type or Western-type. Most of *cag*PAI genes belong to the East Asian-type and is found associated with high risk

* *Corresponding author.*
E-mail: gengjianli1962@outlook.com (J. Geng).
http://dx.doi.org/10.1016/j.bjm.2016.12.004

of developing gastric cancer in some regions. Noticeably, in some regions, some strains of the Western-type of several *cag*PAI gene/genes are of East Asian-type of other *cag*PAI genes, potentially due to the mosaicism of *cag*PAI genes in *H. pylori* and genetic recombination.[11]

Much work has been reported on the genetic diversity of *cag*PAI, particularly on the genotype of *cag*A.[12,13] Minimal research has been conducted in applying *cag*PAI genotype analysis to evaluate the virulence of *H. pylori*. In some *H. pylori* strains, the two parts of the *cag*PAI genes are interposed by a segment called insertion sequence 605 (IS605): the upstream as *cag*II region, and the downstream as *cag* I region. In this study, some *cag* PAI genes in *cag* II (*cagT*, *cagX*) and *cag*I region (*cagI*, *cagL*, *cagM*) were selected for sequencing. The virulence of the strains with East Asian-type *cag*PAI genes was evaluated by comparing strains with mosaic *cag*PAI genes.

Materials and methods

Patients and sampling

Patients with abdominal symptoms were clinically examined, and biopsy samples were taken for *H. pylori* isolation and histological analysis in Weihai Municipal Hospital (affiliated with Dalian Medical University) from June 2010 to June 2014. None of the patients underwent antimicrobial therapy, or have taken proton pump inhibitors (PPIs) or non-steroidal anti-inflammatory drugs a month before their inclusion in the study. Written and informed consent was obtained from all patients, and the study was conducted upon approval by the *ad hoc* Ethical Committee of Weihai Municipal Hospital.

H. pylori cultivation and identification

Biopsy specimens were collected in brain heart infusion broth (Oxoid, United Kingdom), and dispersed by using the tissue homogenizer. Every homogenate was inoculated onto Campylobacter agar (Oxoid, United Kingdom) with 8% sheep blood and *H. pylori* selective supplement (Oxoid, United Kingdom) under microaerophilic condition (5% O_2, 10% CO_2 and 85% N_2) at 37 °C for 72 h. Small dew drop colonies of *H. pylori* were selected for further phenotypic analysis by PCR of the 16SrRNA gene sequence as previously described.[11]

Sequence analysis of cagPAI genes

The presence of five *cag*PAI genes, which spread over the *cag* I (*cagI*, *cagL*, *cagM*) and *cag* II regions (*cagT*, *cagX*) in *H. pylori* strains, was analyzed by PCR using five sets of primers listed in Table 1. The amplicons were selected for sequence analysis by Life Technologies Corporation. After the full-length amino acid sequences of each gene were translated from the nucleotide sequences by Primer 5.0, the phylogenetic trees of CagPAI proteins were constructed by the neighbor-joining method of Saitou and Nei by using a program called MEGA. All *cag*PAI genes in the isolates could be placed into two major groups. Most *H. pylori* strains from the region are either East Asian-type or Western-type. Three previously reported Western strains have been identified so far: 26695 (UK), J99 (USA), and NCTC11637 (Australia).

In the present study, the strains were classified based on the genotypes of *cag*PAI genes: East Asian-type *cag*PAI genes group (all five *cag*PAI genes), and mosaicism *cag*PAI genes group (less than five *cag*PAI genes identified as East Asian-type, and containing more than one Western-type *cag*PAI gene except *cagA*).

Analysis of cagA status and 3′ variable region of cagA

PCR analyses were performed to analyze *cagA* status and *cagA* 3′ variable region using specific primers (Table 1). After PCR, the amplified PCR products were electrophoresed in 2% agarose gels and examined under UV illumination. The amplicons of *cagA* 3′ variable region were sequenced by Life Technologies Corporation.

Histological analysis

Stomach biopsy specimens from each patient were examined by an experienced pathologist. For each biopsy specimen, the grades of inflammatory cell infiltration, gastric mucosal atrophy, and *H. pylori* density were scored on the basis of

Table 1 – Polymerase chain reaction (PCR) primer pairs used for *cag*PAI genes in *Helicobacter pylori* isolates.

Region	Primer	Sequence	Amplicon size (bp)
cagI	f:	ATAGAATTCACAGAAGTAGTAATAACGCTTGAAC	1086
	r:	AGACTCGAGTTTGACAATAACTTTAGAGCTAG	
cagL	f:	GATGGATCCGAAGATATAACAAGCGGTTT	654
	r:	GCCCTCGAGTTTAACAATGATCTTACTTGA	
cagM	f:	TAGGGATCCGAGCAGTTTGGTTCATTT	1149
	r:	CGCCTCGAGCTATTCAAAGGGATTATTC	
cagT	f:	TCCGGATCCATGAAAGTGAGAGCAAGTGT	843
	r:	GCCAAGCTTTCACTTACCACTGAGCAAAC	
cagX	f:	GGAATTCATGGGGCAGGCATTTTTTA	1569
	r:	GGGTCGACTTATTTATCTCTGACAAGAGGGAG	
cagA conservative region	f:	GATAGGGATAACAGGCAAGC	297
	r:	GGGGGTTGTATGATATTTTC	
cagA 3′ variable region	cag2	GGAACCCTAGTCGGTAATG	Uncertain

Table 2 – Histological differences among the two *cag*PAI groups.

Parameter	East Asian-type *cag*PAI genes group ($n=42$)	Mosaicism *cag*PAI genes group ($n=26$)
H. pylori density	1.52 ± 0.41	1.63 ± 0.52
Inflammatory cell infiltration	1.71 ± 0.68[a]	1.03 ± 0.42
Atrophy	0.51 ± 0.17	0.47 ± 0.16

[a] $p<0.05$.

the updated Sydney System (0, none; 1, mild; 2, moderate; 3, severe).[14]

Mucosal IL-8 level analysis in biopsy samples

IL-8 levels in the biopsy specimens were measured after homogenization using an enzyme-linked immunosorbent assay (ELISA) (Invitrogen). Briefly, the supernatants from homogenized specimens were obtained by centrifugation (10,000 × *g* for 15 min), and the total proteins of the supernatants were measured by using the Bradford assay (Bio-Rad, Richmond, CA). IL-8 levels in samples were expressed as pg/mg of protein.

Determination of IL-8 secretion from GES-1 cells co-cultured with H. pylori strains

The strains were selected and co-cultured with GES-1 cells for analysis of IL-8 secretion. Briefly, GES-1 cells were seeded at a density of 8×10^4 cells/well in 96-well plates. *H. pylori* was harvested from agar dishes and washed twice with PBS before being added to culture wells at a MOI of 100. The cell culture media were collected at 24 h. IL-8 levels were detected by ELISA (Invitrogen) according to the manufacturer's protocol. The experiments were performed twice independently.

CagA Translocation Assay

GES-1 cells infected with *H. pylori* (1:100) as described above were washed with PBS until no *H. pylori* were found adhered to cells as observed under a microscope. Cells were then scraped and centrifuged at 4000 rpm for 30 min. Cells were suspended in RIPA Lysis Buffer on ice for 30 min. After centrifuging at 13,500 rpm for 30 min, the supernatant was collected to analyze CagA by Western blotting. Colony-forming units (CFU) assay was performed to confirm that no *H. pylori* were detected on scraped cells.

Statistical analysis

Differences between the Asian-type strains group and the mosaic-type strains group were analyzed using Student's *t* test and Chi-square test. *p* values less than 0.05 was considered significant.

Results

After sequence analysis of *cag*PAI genes (Fig. 1), 76 strains with East Asian-type *cag*PAI genes were isolated from gastroduodenal diseases, including chronic gastritis (CG, $n=29$), gastric ulcer (GU, $n=21$), duodenal ulcer (DU, $n=19$), and gastric cancer (GC, $n=7$). Twenty-six strains with mosaicism *cag*PAI genes were isolated from chronic gastritis (CG, $n=14$), gastric ulcer (GU, $n=6$), duodenal ulcer (DU, $n=4$), and gastric cancer (GC, $n=2$). Isolates with East Asian-type *cag*PAI genes were found at lower frequency in CG (38.2%, 29/76) than those with mosaicism of *cag*PAI genes in CG (53.8%, 14/26, $p<0.01$).

Detection of cagA status and sequencing of the cagA 3′ variable region

PCR products were obtained from all isolates of *H. pylori*. After nucleotide analysis, *cagA* 3′ variable region were grouped as East Asian-type or Western-type. The East Asian-type (521 bp) possessed three **EPIYA** motifs, while the Western-type (502 bp) possessed two **EPIYA** motifs and one **EPIYT** (Fig. 2). All 76 strains with East Asian-type *cag*PAI genes harbored East Asian-type, and only 2 strains possessed Western-type in 26 strains with mosaicism *cag*PAI genes.

Histological analysis of H. pylori density, inflammatory cell infiltration, and atrophy in the biopsy specimens

Further histopathologic evaluations of biopsy specimens were performed on 42 patients with East Asian-type *cag*PAI genes *H. pylori* strains and 26 patients with mosaicism *cag*PAI genes *H. pylori* strains. The biopsy specimens of East Asian-type *cag*PAI genes group were selected randomly for histological analysis.

Gastric mucosal inflammatory cell infiltration was significantly higher in East Asian-type *cag*PAI genes group than mosaicism *cag*PAI genes group ($p<0.05$) (Fig. 3). However, there were no significant differences in the grade of *H. pylori* density and gastric mucosal atrophy according to the diversity of *cag*PAI genes (Table 2). This suggested that the status of *cag*PAI genotype is associated with progression of gastric mucosal inflammatory cell infiltration.

IL-8 production in gastric mucosa (in vivo) and from GES-1 cells (in vitro)

We randomly selected 36 patients with East Asian-type *cag*PAI genes *H. pylori* strains and 26 patients with mosaicism *cag*PAI genes *H. pylori* strains to measure gastric mucosal IL-8 levels. The gastric mucosal IL-8 level in patients with East Asian-type *cag*PAI genes *H. pylori* strains was significantly higher (112.7 ± 33.1 pg/mg) than those containing mosaicism *cag*PAI genes *H. pylori* strains (75.8 ± 24.7 pg/mg, $p<0.01$).

IL-8 production in GES-1 cells that were co-cultured with 20 *H. pylori* strains was also examined. These

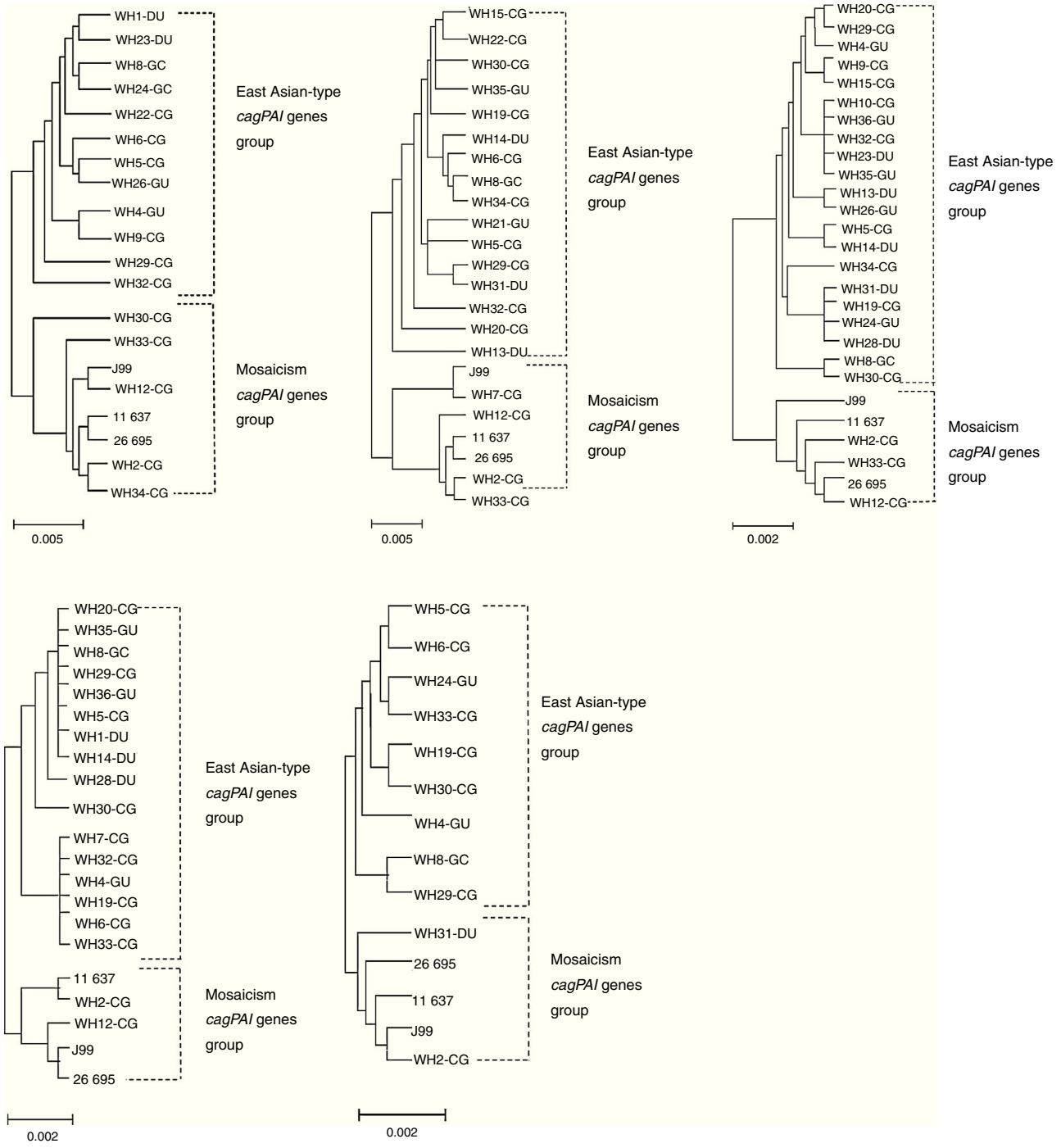

Fig. 1 – Phylogenetic tree of the complete amino acid sequences of *cagI*, *cagL*, *cagT*, *cagM* and cag*X* in some strains. Scale bars represent calculated distances. All three proteins were divided into two major groups: East Asian-group or Western-group.

included 10 strains with East Asian-type *cag*PAI genes and 10 strains with mosaicism *cag*PAI genes. IL-8 production (1423.9 ± 195.3 pg/mL) was significantly higher in strains with East Asian-type *cag*PAI genes than in those with mosaicism *cag*PAI genes (1113.9 ± 103.6 pg/mL, $p < 0.01$) (Table 3). These results suggested that the *cag*PAI genotype of *H. pylori* strain is important for inducing IL-8 production *in vivo* and *in vitro*.

CagA delivery to host cells

Ten *H. pylori* strains with East Asian-type *cag*PAI genes and 10 strains with mosaicism *cag*PAI genes were randomly selected for further analysis of CagA translocation. After infection of GES-1 cells by *H. pylori*, cells with translocated CagA were found more increased in East Asian-type *cag*PAI genes group than mosaicism *cag*PAI genes group. These results indicated

```
11637        FSDIKKELNEKFKNFNNNNNNGLEN----EPIYAKVNKKKTGQVASPEEPIYA
WH2 (CG)     FSDIKKELNEKFKNFNNNNNNGLKN----EPIYAKVNKKKTGQVASLEEPIYT
WH8 (GC)     FSDIRKELNEKFKNFNNNNNNGLKN----EPIYAKVNKKKTGQVASLEEPIYT
WH1 (CG)     FSDIRKELNEKLFGNSNNNNNGLKNNTEPIYAQVNKKKTGQVASPEEPIYA
WH4 (GU)     FSDIRKELNEKLFGNSNNNNNGLKNNTEPIYAQVNKKKVGQATSPEEPIYA
WH13 (DU)    FSDIRKELNEKLFGNSNNNNNGLKNNTEPIYAQVNKKKAGQATSPEEPIYA

11637        QVAKKVNAKIDRLNQAASGLGGVGQAGFPLKRHDKVDDLSKVGRSVSPEPIYA
WH2 (CG)     QVAKKVNAKIDRLNQIASGLGDVGQAAGFPLKRHDKVDDLSKVGLSASPEPIYA
WH8 (GC)     QVAKKVKAKIDRLDQIASGLGGVGQAAGFPLKRHDKVDDLSKVVLSAGPEPIYA
WH1 (CG)     QVAKKVSAKIDQLNEATSAINRKIDRIN----KIASAGKGVGGFSGAGRSASPEPIYA
WH4 (GU)     QVAKKVSAKIDQLNEATSAINRKIDRIN----KIASAGKGVGGFSGVGRSASPEPIYA
WH13 (DU)    QVAKKVSAKIDQLNEATSAINRKIDRIN----KIASAGKGVGGFSGVGRSASPEPIYA

11637        TIDDLGGPFPLKRHDKVDDLSKVGRSVSPEPIYATIDDLGGPFPLKRHDKVDDLSKV
WH2 (CG)     TIDDLGGPFPLKRHDKVDDLSKVGLSREQELKQKIDNLNQAVSEAK
WH8 (GC)     TIDELGGPFPLKRHDKVDDLSKVGLSREQELKQKIDNLNQAVSEAK
WH1 (CG)     TIDFDEANQAGFPLRRSAAVNDLSKVGLSREQELTRRIGDLNQAVSEAK
WH4 (GU)     TIDFDEANQAGFPLRRSAAVNDLSKVGLSREQELTCRIGDLNQAVSEAK
WH13 (DU)    TIDFDEANQAGFPLRRSAAVNDLSKVGLSREQELTRRIGDLNQAVSEAK

11637        GRSVSPEPIYATIDDLGGPFPLKRHDKVDDLSKV
```

Fig. 2 – Deduced amino acid sequence of *cag*A 3′ variable region from some strains.

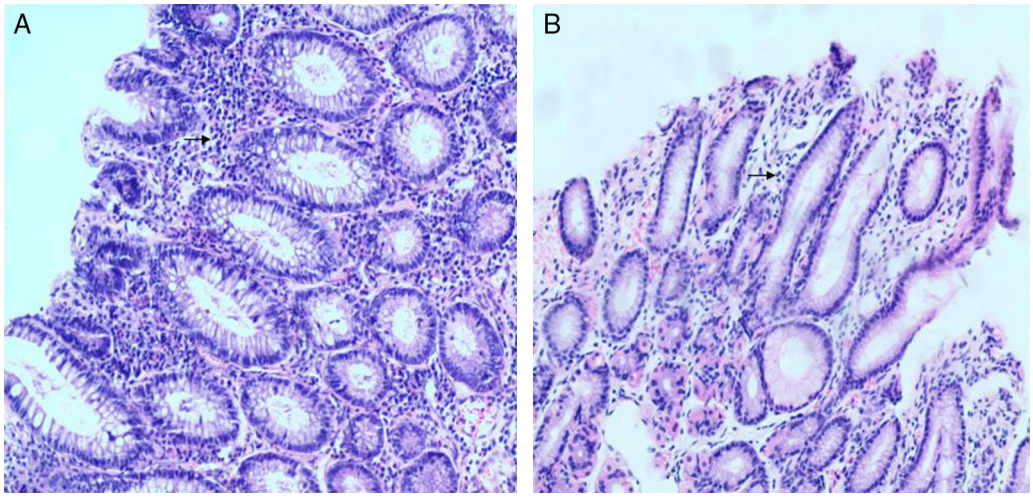

Fig. 3 – Histopathologic evolutions of biopsy specimens in two groups according to H&E stain (100×). (A) East Asian-type *cag*PAI genes group; (B) mosaicism *cag*PAI genes. Infiltration of inflammatory cells (*arrows*).

Table 3 – IL-8 levels in gastric mucosa (*in vivo*) and GES-1 cells (*in vitro*).

	East Asian-type *cag*PAI genes group	Mosaicism *cag*PAI genes group
Gastric mucosa (pg/mg)	112.7 ± 33.1 (n = 36)[a]	75.8 ± 24.7 (n = 26)
GES-1 cells (pg/mL)	1423.9 ± 195.3 (n = 10)[a]	1113.9 ± 103.6 (n = 10)

[a] $p < 0.01$.

that the translocation of CagA to host cells was less effective in strains with mosaicism *cag*PAI genes (Fig. 4).

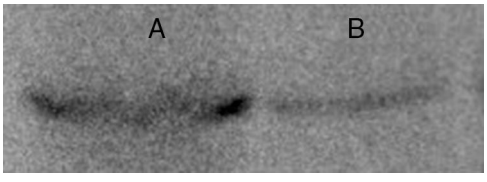

Fig. 4 – CagA translocation between two groups. (A) East Asian-type *cag*PAI genes group; (B) mosaicism *cag*PAI genes group.

Discussion

*H. pylori cag*PAI is a major virulence determinant in *H. pylori*-related diseases. In comparison with *cag*PAI-negative strains, infection with *cag*PAI-positive *H. pylori* strains significantly increases the risk of developing severe gastric mucosal inflammation, duodenal ulcers, and gastric cancers.[15,16] The *cagA*

gene is part of *cag*PAI, and CagA is the primary virulence factor of *H. pylori* that sufficiently induces tumorigenesis in cell and animal models. CagA has also been reported to be epidemiologically associated with higher risk of developing gastric cancer.[17,18] Based on the sequence of the 3′ region, *cagA* was classified into 2 types: East Asian-type and Western-type. Intriguingly, both *in vitro* and *in vivo* studies have clearly showed that East Asian-type CagA protein is more carcinogenic than Western-type CagA protein.[19] In this region, most strains possess East Asian-type of *cagA* with three **EPIYA** motifs.

In a littoral region of Northeast China, nearly 100% of the strains possess *cagA*, and most isolates of *cagA* were included in East Asian-type.[20] Noticeably, a high incidence of atrophic gastritis and gastric cancer has also been reported in this region.[21] Early studies showed that the distinct distribution of *cag* PAI diversity in this region may be involved in the development of atrophic gastritis, potentially increasing the risk of worsened outcome in different diseases based on phylogenetic analysis of *cag*PAI genes.[11] We hypothesize that the T4SS encoded by Eastern-type *cag*PAI genes in the region results in the ability of *H. pylori* to increase the risk of developing gastric cancer.

In the study, isolates with East Asian-type *cag*PAI genes were found at lower frequency in CG, compared to strains with mosaicism *cag*PAI genes in CG. Two *cag*PAI genotypes of *H. pylori* strains were selected based on the phylogenetic analysis of full-length CagPAI proteins, including East Asian-type *cag*PAI and mosaicism *cag*PAI genes strains. Some strains were selected to study their virulence, and to examine whether there is an association between the diversity of the *cag*PAI genes and the virulence of *H. pylori*. We found that gastric mucosal inflammatory cell infiltration was significantly higher in patients with East Asian-type *cag*PAI genes *H. pylori* than others with mosaicism *cag*PAI genes strains. These observations suggest that *cag*PAI genotype is associated with progression of gastric mucosal inflammatory cell infiltration. Meanwhile, we also found that *H. pylori* strains with the East Asian-type *cag*PAI genes are closely associated with IL-8 secretion compared with *H. pylori* strains with the mosaicism *cag*PAI genes. Intriguingly, *H. pylori* strains with the East Asian-type *cag*PAI genes can easily translocate CagA to host cells.

H. pylori exhibits extensive genetic diversity and rapid allelic diversification attributed to its high mutation rate and frequent recombination in different diseases.[22–24] We believe that the mosaicism in *cag*PAI genes is best explained by genetic recombination. Thus, the genetic recombination in *cag*PAI between the west strain and East strain could increase the diversification and virulence of *H. pylori*.

In conclusion, *H. pylori* with the East Asian-type *cag*PAI genes is associated with gastric mucosal inflammatory cell infiltration and IL-8 secretion, which seems to be more virulent compared with other strains.

Conflicts of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

This work was supported by Shandong Provincial Natural Science Foundation, China (No. ZR2015HM072) and Jiangsu Key Laboratory of Medical Science and Laboratory Medicine (No. JSKLM-2014-014).

REFERENCES

1. Sánchez-Zauco NA, Torres J, Pérez-Figueroa GE, et al. Impact of *cag*PAI and T4SS on the inflammatory response of human neutrophils to *Helicobacter pylori* infection. *PLOS ONE*. 2013;8(6):e64623.
2. Nguyen LT, Uchida T, Tsukamoto Y, et al. Clinical relevance of *cag*PAI intactness in *Helicobacter pylori* isolates from Vietnam. *Eur J Clin Microbiol Infect Dis*. 2010;29:651–660.
3. Odenbreit S, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science*. 2000;287:1497–1500.
4. Viala J, Chaput C, Boneca IG, et al. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori cag* pathogenicity island. *Nat Immunol*. 2004;5:1166–1174.
5. Tegtmeyer N, Wessler S, Backert S. Role of the *cag*-pathogenicity island encoded type IV secretion system in *Helicobacter pylori* pathogenesis. *FEBS J*. 2011;278:1190–1202.
6. Vannini A, Roncarati D, Spinsanti M, Scarlato V, Danielli A. In depth analysis of the *Helicobacter pylori* cag pathogenicity island transcriptional responses. *PLOS ONE*. 2014;9(6):e98416.
7. Censini S, Lange C, Xiang Z, et al. cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci U S A*. 1996;93:14648–14653.
8. Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R. *Helicobacter pylori* virulence and genetic geography. *Science*. 1999;284:1328–1333.
9. Alm RA, Ling LS, Moir DT, et al. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature*. 1999;397:176–180.
10. Azuma T, Yamakawa A, Yamazaki S, et al. Distinct diversity of the *cag* pathogenicity island among *Helicobacter pylori* strains in Japan. *J Clin Microbiol*. 2004;42:2508–2517.
11. Wang MY, Lin J, Sun SB, Yuan XY. Identification of *Granulicatella elegans* from a case of infective endocarditis, based on the phenotypic characteristics and 16SrRNA gene sequence. *J Cardiovasc Med (Hagerstown)*. 2015;16(suppl 2):S138–S140.
12. Bustamante-Rengifo JA, Matta AJ, Pazos A, Bravo LE. In vitro effect of amoxicillin and clarithromycin on the 3′ region of *cag*A gene in *Helicobacter pylori* isolates. *World J Gastroenterol*. 2013;19(36):6044–6054.
13. Matos JI, de Sousa HA, Marcos-Pinto R, Dinis-Ribeiro M. *Helicobacter pylori* CagA and VacA genotypes and gastric phenotype: a meta-analysis. *Eur J Gastroenterol Hepatol*. 2013;25(12):1431–1441.
14. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol*. 1996;20:1161–1181.
15. Hatakeyama M. *Helicobacter pylori* and gastric carcinogenesis. *J Gastroenterol*. 2009;44:239–248.
16. Kumar S, Kumar A, Dixit VK. Evidences showing association of interleukin-1B polymorphisms with increased risk of gastric cancer in an Indian population. *Biochem Biophys Res Commun*. 2009;387:456–460.

17. Ohnishi N, Yuasa H, Tanaka S, et al. Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. *Proc Natl Acad Sci U S A*. 2008;105(3):1003–1008.
18. Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. *Nat Rev Cancer*. 2004;4(9): 688–694.
19. Miura M, Ohnishi N, Tanaka S, Yanagiya K, Hatakeyama M. Differential oncogenic potential of geographically distinct *Helicobacter pylori* CagA isoforms in mice. *Int J Cancer*. 2009;125(11):2497–2504.
20. Wang MY, Chen C, Gao XZ, et al. Distribution of *Helicobacter pylori* virulence markers in patients with gastroduodenal diseases in a region at high risk of gastric cancer. *Microb Pathog*. 2013;59–60:13–18.
21. Gao XZ, Chu YL, Qiao XL, et al. Narrow band imaging endoscopy for diagnosis of malignant and premalignant gastric lesions. *Chin J Dig*. 2009;29(5):289–292.
22. Xiang Z, Censini S, Bayeli PF, et al. Analysis of expression of CagA and VacA virulence factors in 43 strains of *Helicobacter pylori* reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin. *Infect Immun*. 1995;63(1):94–98.
23. Krebes J, Didelot X, Kennemann L, Suerbaum S. Bidirectional genomic exchange between *Helicobacter pylori* strains from a family in Coventry, United Kingdom. *Int J Med Microbiol*. 2014;304(8):1135–1146.
24. Falush D, Wirth T, Linz B, et al. Traces of human migrations in *Helicobacter pylori* populations. *Science*. 2003;299(5612):1582–1585.