September 2009

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British Journal of Biomedical Science

ISSN: 0967-4845 (Print) (Online) Journal homepage: http://www.tandfonline.com/loi/tbbs20

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To cite this article: J. Yakoob, W. Jafri, Z. Abbas, S. Abid, R. Khan, N. Jafri & Z. Ahmad (2009) Low prevalence of the intact *cag* pathogenicity island in clinical isolates of *Helicobacter pylori* in Karachi, Pakistan, British Journal of Biomedical Science, 66:3, 137-142, DOI: 10.1080/09674845.2009.11730260

To link to this article: https://doi.org/10.1080/09674845.2009.11730260

Low prevalence of the intact *cag* pathogenicity island in clinical isolates of *Helicobacter pylori* in Karachi, Pakistan

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Accepted: 29 May 2009

ABSTRACT

Clinical diseases that follow *Helicobacter pylori* infection are associated with expression of the *cag*A gene, a part of cytotoxin-associated gene pathogenicity island (*cag*-PAI). This study aims to determined whether or not the presence of *cag*A is associated with the presence of complete *cag*-PAI and to evaluate inflammatory changes associated with the five loci in the *cag*-PAI of *H. pylori* comprising *cag*A, *cag*A promoter region (*cag*AP), *cag*E, *cag*T and the left end of the *cag*A gene (LEC). *H. pylori* isolates were obtained from patients with dyspeptic symptoms. Clinical strains of *H. pylori* were screened by the polymerase chain reaction (PCR) for respective genes of the *cag*-PAI. Of 115 *H. pylori* isolates, 31 (28%) were positive for the five *cag*-PAI loci. *H. pylori* isolates with intact *cag*-PAI were associated with gastric carcinoma (GC; n=9[60%]) and gastric ulcer (GU; n=5[45%]) compared to non-ulcer dyspepsia (NUD; n=14[18%]) (P=0.001 and P=0.049, respectively). In patients with intact *cag*-PAI, acute on chronic inflammation was present in 25 (81%) and was more common than chronic inflammation (P=0.013). The *cag*E and *cag*AP had deletions in 25 (37%) and 23 (35%) cases, respectively. The *cag*AP region was significantly associated with GC (n=12[80%], P<0.001) and GU (n=9[82%], P=0.001) compared to NUD (n=24[30%] and with significant acute on chronic inflammation (n=40[80%], P=0.007). The distribution of *vac*As1a with intact *cag*-PAI in GC was 9(60%) and in NUD was 10(13%) (P<0.001). The presence of the *cag*A gene does not signify presence of an intact *cag*-PAI. Most of the *H. pylori* isolates studied had partial *cag*-PAI with missing *cag*E and *cag*A promoter regions.

KEY WORDS: Duodenal ulcer.
Genes.
Genomic islands.
Helicobacter pylori.
Non-ulcer dyspepsia.

Introduction

Helicobacter pylori is a Gram-negative microaerophilic bacterium that infects human gastric mucosa and is recognised as a major cause of chronic active gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma.[1,2] It also has a relationships with gastro-oesophageal reflux disease and adenocarcinoma of the gastric cardia.[3] It is a class I carcinogen that colonises the human stomach of more than 50% of the world's population. However, only a small subset of infected people experience *H. pylori*-associated illnesses.

Although the pathogenesis of *H. pylori* infection is not well understood, there are several virulence factors that may contribute to mucosal damage. The *cag*A gene is conventionally used as a marker of pathogenic strains. However, the literature suggests that the *cag*A gene alone is not a suitable marker of *H. pylori* virulence. A strong predictor of severe disease outcome is infection with a bacterial strain harbouring the *cag* pathogenicity island (*cag*-PAI), a 40 kb segment of DNA.[4]

H. pylori cag-PAI is horizontally acquired and follows free recombinations within the species. The chromosomal integrity of this island, or the lack thereof, is thought to play an important role in the progress of the gastroduodenal pathology caused by *H. pylori*. These genes encode a type IV secretion system that forms a syringe-like structure to translocate the immunodominant cagA protein into the gastric epithelial cells.[5] *cag*-PAI has also been implicated in the induction of interleukin-8 (IL-8) in cultured gastric cells.[6]

At least 17 out of 27 genes were found to be essential for translocation of *cag*A into host cells (syringe-like function), and 14 were required to fully induce transcription of IL-8.[6] The *cag*-PAI and vacuolating toxin A (*vac*A) gene are located far apart on the chromosome, but there is a statistical linkage between presence of the *cag*-PAI and the s1 genotype of *vac*A.[7] Strains with this genotype are called type I strains and are generally more virulent than type II strains that lack the *cag*-PAI and harbour the less virulent *vac*A s2 genotype.[8]

The aim of this study is to determine the association of *cag*A with intact *cag*-PAI to evaluate inflammatory changes associated with intact *cag*-PAI and determine any association between *cag*-PAI and the *vac*A allele s1. The completeness of *cag*-PAI is determined using specific flanking polymerase chain reaction (PCR) primers from five loci in the *H. pylori cag*-PAI comprising *cag*A, *cag*A promoter region (*cag*AP),

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*cag*E, *cag*T and the left end of the *cag*-PAI (LEC) in clinical isolates of *H. pylori*. The absence of *cag*-PAI is detected by the PCR for the *cag* empty site flanking the left and right ends of the *cag*-PAI indicating that they truly lacked the *cag*A gene.[9]

Materials and methods

Patients and H. pylori *strains*

A total of 115 *H. pylori* isolates were obtained from patients undergoing gastroscopy and gastric biopsy for dyspeptic symptoms attending the endoscopy suite of the gastroenterology department at the Aga Khan University from July 2005 to December 2007 (Table 1). Mean age was 40±14 years (range: 18–78 years). Informed consent was obtained for oesophagogastroduodenoscopy (EGD) and biopsy from the antrum and body of the stomach. The study was approved by the institution's ethics review committee.

Sterilised biopsy forceps were used to obtain gastric biopsy specimens from an area of inflammation for rapid urease test, histopathology, *cag*-PAI-associated virulence genes, *cag*A empty site and *vac*A PCR.

After primary isolation and identification of the gastric organisms as *H. pylori*, the strains were frozen at –70°C until their use in the study. Subsequently, bacteria were cultured on Columbia blood agar (Oxoid) plates containing 7% sheep blood for 3–5 days at 37°C under microaerobic conditions using Campygen strips in anaerobic jars (Oxoid).

Several small, rounded colonies were picked from different parts of the culture plate and subcultured twice to obtain a pure culture. Gram's stain, urease and catalase tests were used to confirm identification and to exclude the possibility of contamination.

H. pylori cells were harvested from plates by suspension in 2-mL sterile phosphate-buffered saline (PBS) and were pelleted by centrifugation at 3000 x*g* for 20 min. The clinical symptoms at the time of presentation, diagnosis, drug treatment dosage and duration were noted, together with endoscopy findings.

Rapid urease test

Pronto Dry, a commercial rapid urease test (Medical Instrument Corp., France), results were read in 30 min after sampling, in accordance with the manufacturer's instructions. Colour change from yellow to pink was indicative of a positive reaction.[10]

Histopathology

Gastric antral biopsy specimens stained with haematoxylin and eosin (H&E) were examined by a pathologist unaware of the patient's clinical diagnosis. In doubtful cases, Giemsa staining was performed to ascertain the presence of *H. pylori*. Degree of gastritis on H&E stain was scored in accordance with the Sydney system[11] for the level of inflammation and neutrophil infiltration (0=none, 1=mild, 2=moderate, 3=severe).

Extraction of genomic DNA

DNA was extracted from confluent cultures with fewer than three *in vitro* passages.[12] The bacterial cells from a chocolate agar plate were washed (x2) with PBS and centrifuged at 3000 x*g* for 20 min. *H. pylori* DNA was extracted by a modified phenol/chloroform method.[13] Briefly, a bacterial pellet was resuspended in Tris-HCl buffer containing ethylenediaminetetraacetate (TE, pH 8.0) and lysozyme (30 mg/mL) and was incubated at 37°C for 30 min. The suspension was treated with sodium dodecyl sulphate (SDS, 10%), proteinase K (2.5 mg/mL) and RNase A (40 mg/mL). DNA was extracted with phenol/chloroform/isoamyl alcohol, precipitated by sodium acetate and ice-cold absolute alcohol, and washed with ice-cold alcohol (70%). The DNA pellet was finally resuspended in TE buffer. DNA content and purity were determined by measuring absorbance at 260 nm and 280 nm using a spectrophotometer (Beckman DU-600, USA).

Table 1. Demographic and clinical findings of patients (n=115).

Gender	Male: Female	77:38
Age	Range	18–78 years
	Mean age±SD	40±14 years
Symptoms	Abdominal pain	92 (82%)
	Vomiting	13 (10%)
	Weight loss	10 (8%)
Diagnosis	Non-ulcer dyspepsia	79 (69%)
	Gastric ulcer	11 (9%)
	Gastric carcinoma	15 (13%)
	Duodenal ulcer	10 (9%)
Histopathology	Acute inflammation	10 (9%)
	Chronic inflammation	19 (16%)
	Acute on chronic inflammation	86 (75%)

cag-*PAI PCR analysis*

The PCR analyses were carried out with oligonucleotide pairs (Table 2A), as described previously,[14] to amplify five different loci spread over the *cag* I and *cag* II regions. *cag*A, the *cag*A promoter region and *cag*E represented *cag*I, while the left end of *cag* (LEC) and *cag*T were selected to represent *cag*II.

Pairs of oligonucleotide primers were used to detect the presence of the *cag*-PAI genes (*cag*A, the *cag*AP region, *cag*E, *cag*T and the LEC, containing both inside and outside genes of *cag*-PAI). These primer pairs were designed on the basis of published sequences.[4,9,15] To test for the absence of the *cag*-PAI, PCR for the *cag* empty-site was used with primers that flanked the left and right ends of the *cag*-PAI.[9]

H. pylori strains ATCC 43526 and ATCC 43504, which have been determined to have the entire *cag*-PAI, were used as positive controls, and ATCC 51932 was used as a negative control as it lacks *cag*A. Amplification specificity for the different loci was assessed by testing *H. pylori* strains ATCC 43526, ATCC 43504 and ATCC 51932 with each experiment. Amplification by PCR was performed in 25-µL volumes containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5–2.5 mmol/L $MgCl_2$, 200 µmol/L deoxynucleoside triphosphates, 2 units *Thermus aquaticus* (*Taq*) DNA polymerase (Promega) and 25 ρmol both forward and reverse primers (synthesised using an MWG automatic synthesiser). The total volume was made up with double-distilled water (DDW).

Amplification was performed in a Perkin Elmer 9700 thermal cycler (Applied Biosystems). The cycles comprised

Table 2A. Sequences and locations of oligonucleotide primers.

Primer	Primer sequence	Amplicon (bp)	Location[a]
*cag*A-F1	5'-AACAGGACAAGTAGCTAGCC-3'	349	2700–2719*
*cag*A-R1	5'-TATTAATGCGTGTGTGGCTG-3'		3400–3381*
*cag*A-F2	5'-GATAACAGGCAAGCTTTTGA-3'	701	157–176*
*cag*A-R2	5'-CTGCAAAAGATTGTTTGGCAGA-3'		505–484*
*cag*E-F1	5'-GCGATTGTTATTGTGCTTGTAG-3'	329	16,891–16,870†
*cag*E-R1	5'-GAAGTGGTTAAAAAATCAATGCCCC-3'		16,563–16,587†
*cag*T-F1	5'-CCATGTTTATACGCCTGTGT-3'	301	442–461†
*cag*T-R1	5'-CATCACCACACCCTTTTGAT-3'		723–742†
LEC-F1	5'-ACATTTTGGCTAAATAAACGCTG-3'	384	3920–3942‡
LEC-R1	5'-TCTCCATGTTGCCATTATGCT-3'		4303–4283‡
LEC-F2	5'-ATAGCGTTTTGTGCATAGAA-3'	877	3856–3875‡
LEC-R2	5'-ATCTTTAGTCTCTTTAGCTT-3'		4732–4713‡
*cag*AP-F1	5'-GTGGGTAAAAATGTGAATCG-3'	730	18,738–18,757†
*cag*AP-R1	5'-CTGCAAAAGATTGTTTGGCAGA-3'		505–484*
*cag*AP-F2	5'-CTACTTGTCCCAACCATTTT-3'	1181	18,495–18,514†
*cag*AP-R2	5'-CTGCAAAAGATTGTTTGGCAGA-3'		505–484*
*cag*A empty site§		360	
2	5'-ACATTTTGGCTAAATAAACGCTG-3'		
25	5'-TCATGCGAGCGGCGATGTG-3'		

[a]GenBank Accession No: *AF001357; †U60176; ‡AC000108; §regions flanking the left and right ends of the *cag*-PAI insert location for NCTC 12455.

an initial denaturation of target DNA at 94°C for 5 min and then denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min and extension at 72°C for 1 min. The final cycle included an extension step for 7 min at 72°C to ensure full extension of the product. Samples were amplified through 35 consecutive cycles.

The amplified products were resolved on 2% agarose gel in 1x TAE buffer (40 mmol/L Tris-acetate, 1 mmol/L EDTA [pH 8.0]) containing ethidium bromide (0.5 µg/mL) and then visualised under ultraviolet (UV) light.

vac*A PCR analysis*

Amplification of *vac*A alleles by PCR was performed in a 25 µL volume containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5–2.5 mmol/L $MgCl_2$, 200 µmol/L deoxynucleoside triphosphates, 2 units *Taq* DNA polymerase (Promega) and 25 µmol forward and reverse primers (synthesised by an MWG automatic synthesiser) (Table 2B).[16]

The amplification cycle for *vac*A alleles consisted of an initial denaturation of target DNA at 95°C for 5 min and than denaturation at 95°C for 1 min, primer annealing at 52°C for 1 min and an extension at 72°C for 1 min. The final cycle included an extension step for 7 min at 72°C to ensure full extension of the product. Samples were amplified through 35 consecutive cycles.

Positive and negative reagent controls were performed with each batch of amplifications. DNA from *H. pylori* strains ATCC 43504 (*vac*As1am1, *cag*A-positive), ATCC 51932 (*vac*A s2m2, *cag*A-negative) and ATCC 43526 (*vac*As1bm1, *cag*A-positive) was used to define the accuracy of the *vac*A PCR.

Table 2B. Oligonucleotide primers used in typing of *H. pylori vac*A alleles.

Region amplified	Primer	Primer sequence	Size and location of PCR product
m1	VA3-F	5'-GGTCAAAATGCGGTCATGG-3'	290 bp (2741–3030)
	VA3-R	5'-CCATTGGTACCTGTAGAAAC-3'	
m2	VA4-F	5'-GGAGCCCCAGGAAACATTG-3'	352 bp (976–1327)
	VA4-R	5'-CATAACTAGCGCCTTGCAC-3'	
S1a	SS1-F	5'-GTCAGCATCACACCGCAAC-3'	190 bp (866–1055)
	VA1-R	5'-CTGCTTGAATGCGCCAAAC-3'	
S1b	SS3-F	5'-AGCGCCATACCGCAAGAG-3'	187 bp (869–1055)
	VA1-R	5'-CTGCTTGAATGCGCCAAAC-3'	

a. Location in published *cag*A sequence (ref. 14).
b. Location in published *vac*A sequence (ref. 16).

Statistical assessment

The Statistical Package for Social Science SPSS (Release 11.5, standard version, SPSS; 1989–99) was used for data analysis. Descriptive analysis was performed for demographic and clinical features. Results were presented as mean±SD for quantitative variables and number (%) for qualitative variables. Differences in proportion were assessed using Pearson's χ^2 test, Fisher's exact or likelihood ratio test, where appropriate. $P<0.05$ was considered significant.

Results

The clinical features and diagnosis of patients included in the study are given in Table 1. Partial deletions involved *cag* I and *cag* II regions of the *cag*-PAI equally. In isolates from NUD, deletions of cag-PAI genes were more frequent (Table 3A). These deletions predominantly involved *cag*E and *cag*A promoter region of the *cag*-PAI (Table 3B). The product corresponding to the *cag*A empty site was obtained from five patients with partially intact *cag*A PAI, indicating that they had mixed infections (i.e., a mixture of *H. pylori* strains with and without the *cag*A gene).

Presence of* cag*-PAI genes

The *cag*A gene was positive in 66 (57%), *cag*A LEC in 63 (55%), *cag*AT in 63 (55%), *cag*AP region in 50 (43%) and *cag*AE in 48 (42%) *H. pylori* isolates. In 31 (28%) isolates *cag*-PAI was intact, it had partial deletions with some genes missing in 42 (36%), and was completely deleted in 42 (36%). Deletions were present in *cag*E ($n=23$ [37%], $P<0.001$) and in *cag*AP (23 [35%], $P<0.001$) (Figs. 1 and 2).

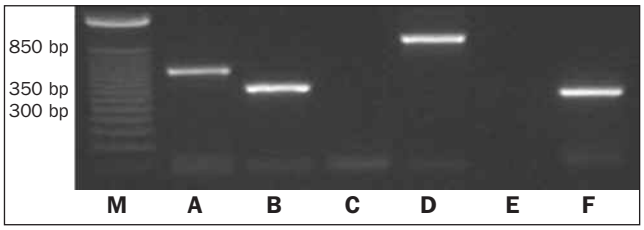

Fig. 1. Distribution of *cag*-PAI gene amplified products in patients with non-ulcer dyspepsia. Lane M: molecular weight marker 50 bp (Promega); Lane A and B: *cag*A gene present; Lane C: *cag*A promoter absent; Lane D: LEC present; Lane E: *cag*E absent; Lane F: *cag*T present.

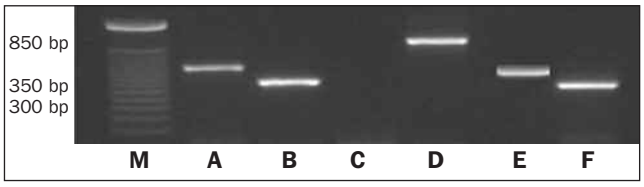

Fig. 2. Distribution of *cag*-PAI gene amplified products in patients with duodenal ulcer. Lane M: molecular weight marker 50 bp (Promega); Lane A and B: *cag*A gene present; Lane C: *cag*A promoter absent; Lane D: LEC present; Lane E: *cag*E present; Lane F: *cag*T present.

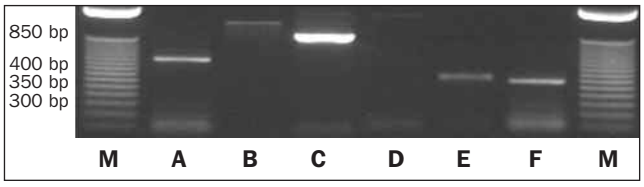

Fig. 3. Distribution of *cag*-PAI gene amplified products in patients with gastric carcinoma. Lane M: molecular weight marker 50 bp (Promega); Lane A: *cag*A gene; Lane B: *cag*A promoter; Lane C: LEC; Lane D: negative control; Lane E: *cag*E; Lane F: *cag*AT.

Table 3A. Correlation of *cag*PAI with diagnosis and histopathology.

	*cag*PAI		
	Intact *n*(%) 31(28)	Partial *n*(%) 42(36)	Deleted *n*(%) 42(36)
Diagnosis			
Non-ulcer dyspepsia	14 (18)	28(35)	37(47)
Gastric ulcer	5(45)	6(55)	0
Gastric carcinoma	9 (60)	5(33)	1(7)
Duodenal ulcer	3 (30)	3(30)	4(40)
Histopathology			
Inflammation			
Acute inflammation	5(50)	3(30)	2(20)
Chronic inflammation	1(5)	12(63)	6(32)
Acute on chronic inflammation	25(29)	27(31)	34(39)
Lymphoid aggregates			
Positive	26(31)	26(34)	30(35)
Negative	5(17)	13(43)	12(40)
Neutrophil infiltration			
Mild	1(7)	7(50)	6(43)
Moderate	25(30)	27(33)	30(37)
Severe	5(26)	8(42)	6(32)

Associations of* cag*-PAI genes with gastroduodenal disorders

Association of *H. pylori* isolates with intact *cag*-PAI was seen in GC ($n=9$[60%]), GU ($n=5$[45%]) and NUD ($n=14$[18%]) (Table 3A, Fig. 3). The *cag*A gene was associated with GU ($n=9$[82%]) and GC ($n=14$[93%]), compared to NUD ($n=38$[48%]) ($P=0.049$ and $P=0.001$, respectively). The *cag*AP region was significantly associated with GC ($n=14$[93%], $P<0.001$), GU ($n=9$[82%], $P=0.001$) and DU ($n=7$[70%], $P=0.016$) compared to NUD ($n=24$[30%]). *cag*A LEC was associated with GU ($n=11$[100%]) and GC ($n=13$[87%]) compared to NUD ($n=40$[51%]) ($P<0.001$ and $P=0.010$, respectively). *cag*A LEC was also associated with GU ($n=11$[100%]) compared to DU ($n=5$[50%]) ($P=0.002$) and GC ($n=13$[87%]) compared to DU ($n=5$[50%]) ($P=0.045$). *cag*E was associated with GU ($n=7$[64%], $P=0.043$) and GC ($n=13$[87%], $P<0.001$) compared to NUD ($n=25$[32%]). *cag*T was associated with GU ($n=10$[91%], $P=0.006$) and GC ($n=14$[93%], $P=0.002$) compared to NUD ($n=40$[51%]).

Associations of* cag*-PAI genes with histology

In patients infected with *H. pylori* with intact *cag*-PAI, acute on chronic inflammation was present in 25 (81%) ($P=0.013$, Table 3A). In these patients, acute on chronic inflammation was more common than chronic inflammation ($P=0.014$), and *cag*AP ($n=40$ [47%], $P=0.007$) was associated with acute on chronic inflammation. Intestinal metaplasia was present in four (57%) isolates with intact *cag*-PAI ($P=0.082$).

Table 3B. Distribution of *cag*-PAI genes in the clinical isolates.

		Intact *n*(%) 31(28)	Partial *n*(%) 42(36)	Deleted *n*(%) 42(36)
*cag*A	Positive	31(46)	35(52)	0(0)
	Negative	0(0)	7(14)	40(86)
*cag*AP	Positive	31(62)	19(38)	0(0)
	Negative	0(0)	23(35)	42(65)
*cag*LEC	Positive	31(48)	32(49)	0(0)
	Negative	0(0)	10(19)	42(81)
*cag*E	Positive	31(65)	17(35)	0(0)
	Negative	0(0)	23(37)	42(63)
*cag*T	Positive	31(49)	32(51)	0(0)
	Negative	0(0)	10(19)	42(81)
*cag*A empty site	Positive	0(0)	5(11)	41(89)
	Negative	31(45)	37(54)	1(1)

*cag*AP: *cag*A promoter region; LEC: left end of *cag*-PAI.

Associations of* cag-*PAI genes with* vac*As1a and m1

The presence of *vac*As1a was seen in 80(70%) isolates and that of *vac*Am1 was seen in 64(56%). Distribution of *vac*As1a with intact *cag*-PAI was GC (n=11[73%]), NUD (n=10[13%], P<0.001), GU (n=4[36%], P=0.05) and DU (n=3[30%] P=0.03). It was associated with acute inflammation in three (30%) cases and acute on chronic inflammation in 20 (23%) (P=0.164). Distribution of *vac*Am1 with intact *cag*-PAI was GC (n=8[53%]), DU (n=3[30%]) and GU (n=3[27%]) compared to NUD (n=5[6%]) (P<0.001, P=0.038, P=0.050, respectively). Acute on chronic inflammation was more frequent (n=16[19%]) than chronic inflammation (P=0.008).

Discussion

The epidemiology of *H. pylori* infection remains important in public health because of its high prevalence and its association with gastric cancer and lymphoma. The seroprevalence of *H. pylori* in the adult population of Pakistan is reported as 58%.[18] A recent study revealed early colonisation/infection of infants with *H. pylori* and a prevalence of 67% at nine months of age in a peri-urban community in Karachi, Pakistan.[19]

In the present study, *H. pylori* isolates from patients with NUD constituted the majority, which reflects the high numbers in this group attending the gastroenterology clinic and having endoscopy. *H. pylori* isolates with intact *cag*-PAI were associated with GC, GU and DU and on histopathology with acute and acute on chronic inflammation. *cag*A gene, *cag*AP region, *cag*LEC, *cag*E and *cag*T were all associated with GC, GU and DU compared to NUD.

H. pylori isolates from NUD lacked the *cag*AP region and *cag*E (Fig. 1) while they were intact in those from GC (Fig. 2). There was a significant difference in inflammatory infiltration of the gastric mucosa between patients with intact *cag*-PAI and those with partial *cag*-PAI type strains (Table 3A). These findings suggest that the strains with partial *cag*-PAI have less ability to cause inflammation than those with intact *cag*-PAI.

The implications of the present study are that *cag*-PAI is partially intact in the majority of our clinical isolates. This is consistent with a previous study in which *cag*-PAI appeared

Table 4. Relationship between intact *cag*-PAI and *vac*A subtypes in *H. pylori* strains.

	cag-PAI s1a			*cag*-PAI m1		
	Positive *n*(%)	Negative *n*(%)	*P* value	Positive *n*(%)	Negative *n*(%)	*P* value
Diagnosis						
Non-ulcer dyspepsia	10(13)	69(87)	0.002	5(6)	74(94)	<0.001
Gastric ulcer	3(27)	8(73)		3(27)	8(73)	
Duodenal ulcer	2(20)	8(80)		3(30)	7(70)	
Gastric carcinoma	9(60)	6(40)		8(53)	7(47)	
Histopathology						
Inflammation						
Acute	3(30)	7(70)	0.164	3(30)	7(70)	0.016
Chronic	1(5)	18(95)		0(0)	19(100)	
Acute on chronic	20(23)	66(77)		16(19)	70(81)	
Lymphoid aggregates						
Positive	20(23)	65(77)	0.237	3(10)	27(90)	0.243
Negative	4(13)	26(87)		16(19)	69(81)	
Neutrophil infiltration						
Mild	1(7)	13(93)	0.302	0(0)	14(100)	0.011
Moderate	18(22)	64(78)		18(22)	64(78)	
Severe	5(26)	14(74)		1(5)	18(95)	

cag-PAI: *cag* pathogenicity island.

to be partial in the majority of clinical isolates around the world.[19] Only 12% of the Indian isolates carried an intact *cag*-PAI. Deletion frequencies of *cag*A, *cag*E, and *cag*T genes were higher in benign cases than in isolates from severe ulcers and gastric cancer.[19]

In contrast, another study from Calcutta, India, showed that 80–90% of Calcutta strains carried *cag*-PAI and the potentially toxigenic *vac*As1 alleles of *vac*A, independently of disease status.[20] In the present study, the clinical outcome of *H. pylori* infection was predicted by analysing genes of the *cag*-PAI, with the *cag*AP region and *cag*E more commonly associated with GC and GU compared to NUD, which is consistent with a previous study.[21]

Jenks *et al*. described the presence of *cag*E and correlated it with that of *cag*A.[22] However, in the present study it was associated with the *cag*A promoter region. Also, the majority of partial deletions of *cag*-PAI were observed in *cag*E, followed by the *cag*AP region.

The *cag*A gene promoter region appeared to be a better marker of *H. pylori* virulence; however, it is known for the diversity of its sequences in isolates. The *cag*E gene, which is consistently retained near the *cag*AP region, appeared to be a better substitute for the *cag*AP region. Also, *cag*E gene PCR can be used to screen the *cag*-PAI structure, as suggested previously.[22]

An association was noted between *vac*As1a and *vac*Am1, with intact *cag*-PAI. In the present study, *vac*As1 with intact *cag*-PAI was associated with GC, GU and DU. This is in keeping with a previous study that showed *vac*As1 occurs significantly more often in isolates from patients with peptic ulceration or gastric adenocarcinoma, while *vac*As2 invariably occurs in patients with gastritis alone.[23]

A shortcoming of the present study is the small number of patients in the different groups. The study, however, is ongoing but the results presented here indicate that the presence of the *cag*A gene does not signify the presence of an intact *cag*-PAI, as most of the *H. pylori* isolates had partial *cag*-PAI with missing *cag*E and *cag*AP regions. □

*This work was supported by an Aga Khan University Research Grant SM 100 327*11 431 to JY. The authors are grateful to staff at the Juma Research Building, The Aga Khan University, for their assistance during this work.*

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