

Structure of *cag* pathogenicity island in Japanese *Helicobacter pylori* isolates

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Abstract

Background—*cag* pathogenicity island (PAI) is reported to be a major virulence factor of *Helicobacter pylori*.

Aim—To characterise *cagA* and the *cag* PAI in Japanese *H pylori* strains.

Methods—*H pylori* isolates from Japanese patients were evaluated for CagA by immunoblot, for *cagA* transcription by northern blot, and for *cagA* and 13 other *cag* PAI genes by Southern blot. *cagA* negative strains from Western countries were also studied. Induction of interleukin-8 secretion from gastric epithelial cells was also investigated.

Results—All Japanese strains retained *cagA*. Fifty nine of 63 (94%) strains had all the *cag* PAI genes. In the remaining four, *cag* PAI was partially deleted, lacking *cagA* transcripts and not producing CagA protein. Details of the PAI of these strains were checked; three lacked *cagB* to *cagQ* (*cagI*) and continuously *cagS* to *cag13* (*cagII*), and the remaining one lacked *cagB* to *cag8*. Western *cagA* negative strains completely lacked *cag* PAI including *cagA*. Nucleotide sequence analysis in one strain in which the *cag* PAI was partially deleted showed that the partial deletion contained 25 kb of *cag* PAI and the *cagA* promoter. Interleukin-8 induction was lower with the *cag* PAI partial deletion strains than with the intact ones. All Japanese *cag* PAI deleted strains were derived from patients with non-ulcer dyspepsia, whereas 41 of 59 (70%) CagA-producing strains were from patients with peptic ulcers or gastric cancer ($p < 0.05$).

Conclusions—Most Japanese *H pylori* strains had the intact *cag* PAI. However, some lacked most of the *cag* PAI in spite of the presence of *cagA*. Thus the presence of the *cagA* gene is not an invariable marker of *cag* PAI related virulence in Japanese strains.

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Keywords: *Helicobacter pylori*; pathogenicity island; Japanese

Helicobacter pylori infection is almost always associated with gastric mucosal inflammation, but more advanced diseases such as peptic ulcers and gastric cancer occur only in a subset of infected patients.¹⁻⁶ Although the pathogenesis of *H pylori* infection is not well understood, several factors have been proposed as possible virulence determinants.

The CagA surface protein is one of the most investigated putative virulence factors, en-

coded by the *cagA* gene.^{7,8} The gene is found in about 50-70% of *H pylori* isolates in Western countries, and the production of CagA protein is reported to be associated with advanced gastroduodenal diseases.⁹⁻¹⁶ CagA positive *H pylori* strains are known to induce interleukin-8 (IL-8) secretion from gastric epithelial cells.¹⁷⁻¹⁹ However, recent studies have indicated that it is not CagA itself but the products of other genes in the *cag* pathogenicity island (PAI) that are responsible for IL-8 induction.²⁰⁻²³ The *cag* PAI is an approximately 40 kb region in the *H pylori* genome, possibly of extraneous origin.^{20,21,24} It is divided into two parts: the upstream *cagII* region containing at least 14 genes, and the downstream *cagI* region containing 16 genes. The *cagA* gene is located in the most downstream portion of *cagI*. In some strains, the two parts of the *cag* PAI are reported to be interposed by a segment called insertion sequence 605 (IS605).²¹

Previous reports indicated that most *cagA* positive strains, most of which are capable of producing CagA protein,^{7,8} retain all the other genes of the *cag* PAI, whereas most *cagA* negative strains completely lack the *cag* PAI.²¹ This implies that the presence of the *cagA* gene may be used as a marker of enhanced virulence associated with the *cag* PAI. However, Censini *et al*²¹ reported partial deletion of the *cag* PAI. Thus the status of the *cagA* gene and the other *cag* PAI genes should be individually investigated.

By using recombinant CagA and antibodies raised against it, we have previously shown that the prevalence of CagA-non-producing *H pylori* strains is relatively low in Japan (about 10%).^{25,26} However, these strains had not been characterised, and we conducted the present study to elucidate the relation between *cagA* and the other *cag* PAI genes in Japanese isolates.

Materials and methods

PATIENTS

H pylori strains were isolated from gastric biopsy specimens of 63 *H pylori* infected patients who had undergone endoscopy in Tokyo University Hospital. The patient population consisted of 40 men and 23 women with a mean age of 52.5 (range 22-78). The endoscopic findings were as follows: gastric ulcer (13); duodenal ulcer (12); non-ulcer dyspepsia (22); gastric cancer (16).

Abbreviations used in this paper: IL, interleukin; IS605, insertion sequence 605; PAI, pathogenicity island; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis, SSC, standard saline citrate.

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Table 1 Oligonucleotide primers used in this study

Primer designation	Primer sequence	Location (reference)
cagA-F	5'-AGCTGCAGCATGACTAACGAAAACCTATTGACCAAC-3'	(1)
cagA-R	5'-AGCTGCAGCTTAAGATTTTTGGAAACCACCTTTT-3'	(1)
cagB-F	5'-ATGGAAAACAATCAATAGG-3'	(18495-18514*)
cagB-R	5'-CTACTTGTCCCAACCATT-3'	(18287-18268**)
cagC-F	5'-ATGAAATTTTTTACAAGAAT-3'	(18142-18123*)
cagC-R	5'-TTAGCTAGCTCCTCCACCCT-3'	(17795-17814*)
cagE-F	5'-TCTATAAAGAGAGGGGTGT-3'	(17170-17151*)
cagE-R	5'-GGCTAATCTTTGGTAATCAG-3'	(14451-14469*)
cagM-F	5'-ATGCTTGCAAAATTGTTT-3'	(7772-7791*)
cagM-R	5'-CTATTCAAAGGGATTATTCT-3'	(8902-8883*)
cagN-F	5'-GTCGCTCTTATTCTTTAGT-3'	(9837-9818*)
cagN-R	5'-CTATTTTTTCCATGAGCGA-3'	(8947-8966*)
cagQ-F	5'-CGTGTTTTAGTAGGAAGCAT-3'	(6570-6551*)
cagQ-R	5'-CTACAGTCTTACTTGAGAGA-3'	(6190-6209*)
cagT-F	5'-ATGAAAGTGAGAGCAAGTGT-3'	(344-363*)
cagT-R	5'-TCACTTACCCTGAGCAAAC-3'	(1186-1167*)
cag15-F	5'-TTATTATCTCTGACAAGAG-3'	(17463-17444**)
cag15-R	5'-ATGGGGCAGGCATTTTTTAA-3'	(17084-17105**)
cag13-F	5'-GCTAGAGAAAAGGCTGTTGC-3'	(12771-12752**)
cag13-R	5'-TGGCGTTAATAGTGGCAATA-3'	(12369-12388**)
cag12-F	5'-TTATTCCAAATTTAATTTA-3'	(11542-11523**)
cag12-R	5'-ATGGAACCTCGGTTTCAATGA-3'	(11103-11122**)
cag10-F	5'-ATGGAAGACTTTTTGTATAA-3'	(9498-9479**)
cag10-R	5'-TCACAGTTCGCTTGAACCCA-3'	(7271-7290**)
cag6-7-F	5'-ATGGCAACATGGAGATGGTT-3'	(4289-4308**)
cag6-7-R	5'-TTAGTTTCCTTTTTTTCAG-3'	(5174-5155**)
cagLEC-F	5'-ACATTTTGGCTAAATAAACGCTG-3'	(3920-3942**)
cagLEC-R	5'-ATCTTTAGTCTCTTTAGC-3'	(4732-4715**)
IS-F	5'-CGCCTTGATCGTTTCAGGATTAGC-3'	(4229-4253*)
IS-R	5'-CAACCAACCGAAGCAAGCATAATC-3'	(2596-2719*)

(1) Reference 25.
 *GenBank accession number U60176.
 **GenBank accession number AC000108.

H PYLORI CULTURE

Biopsy specimens were cultured on Columbia agar with 5% (v/v) horse blood and Dent antibiotic supplement (Oxoid, Basingstoke, Hants, UK) at 37°C for five days under microaerobic conditions (Campy-Pak Systems; BBL, Cockeysville, Maryland, USA). The organisms were identified as *H pylori* by Gram stain morphology, colony morphology, and positive urease, catalase, and oxidase activities. The isolates were kept at -80°C in Brucella broth with 5% (v/v) fetal bovine serum (FBS) containing 16% (v/v) glycerol.

DETECTION OF CagA PROTEINS IN *H PYLORI* ISOLATES

Western blot analysis was performed to detect CagA proteins produced by *H pylori* strains as previously described.²⁵ CagA protein was investigated using *H pylori* whole cell lysates. The

whole cell lysates were washed twice in normal saline, centrifuged, and boiled at 100°C for five minutes in Laemmli sample buffer²⁷ before use. The whole cell lysates were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% gel) and transferred to a nitrocellulose membrane (Schleicher & Schuell, Dassel Germany). The filter was blocked with buffer containing 5% (v/v) skimmed milk in 25 mM Tris/HCl, pH 7.4, containing 150 mM NaCl and 5 mM KCl. The membrane was incubated with a 1:100 dilution of rabbit antiserum against the recombinant CagA. After being washed, the membrane was incubated with ¹²⁵I-labelled goat anti-rabbit IgG, and then exposed to x ray film.

PREPARATION OF GENOMIC DNA

H pylori bacterial cells, suspended in 10 mM Tris/HCl, pH 7.5, containing 1 mM EDTA,

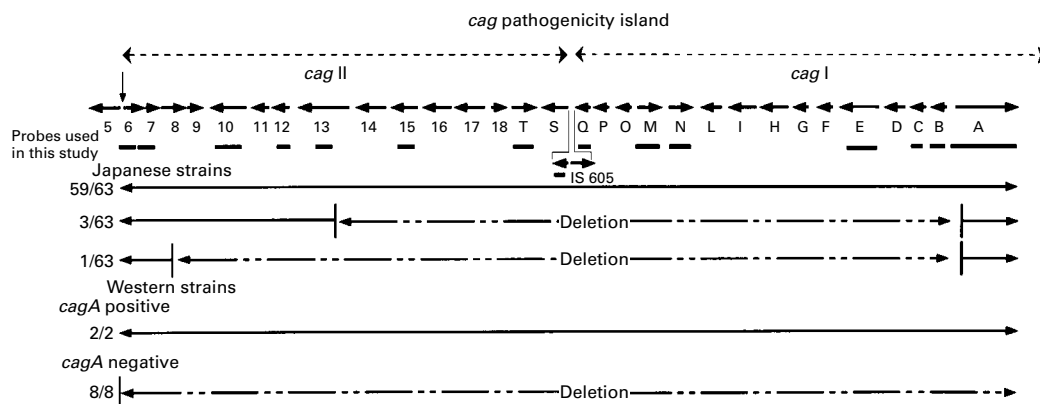


Figure 1 Map of the cag pathogenicity island. The names of the genes are from GenBank accession number AC000108 and U60176. Cross bars indicate probes used in this study. Fifty nine of 63 strains isolated in Japan all had cagI and cagII. Large deletions were revealed in the remaining four. cagA negative strains isolated in Western countries lacked whole cagI and cagII.

were treated with 0.3% SDS and 0.6 mg/ml proteinase K at 60°C overnight. DNA was extracted with phenol, phenol/chloroform and chloroform, precipitated with ethanol, and suspended in TE. The DNA was stored at -20°C until use.

CLONING OF THE GENES OF THE *CAG* REGION

DNA was extracted from *H. pylori* ATCC 43579 cells. Oligonucleotide primers were designed to amplify the genes of the *cag* region; *cagA*, *cagB*, *cagC*, *cagE* (*picB*), *cagM*, *cagN*, *cagQ*, *cagT*, *cag15*, *cag13*, *cag12*, *cag10*, *cag6-7*, IS605, and the left end of the *cag* region (*LEC*) which contained both inside and outside genes of the *cag* PAI according to the nucleotide sequences of the *cag* region previously reported (GenBank accession numbers AC000108 and U60176) (table 1, fig 1). PCR cycling conditions were 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for one to two minutes. The PCR products were cloned into pCRII using the original TA cloning kit (Invitrogen, San Diego, California, USA). The nucleotide sequence of the insert was determined by the dideoxy chain termination procedure.²⁸ Cloned plasmids were digested with *EcoRI* and *BstXI*, which were able to digest the full length of the PCR products, and the digests electrophoresed on 1% agarose gel. The appropriate fragments were then excised from the gels and the DNA extracted with the gene clean kit to be used as probes (Bio 101, La Jolla, California, USA).

SOUTHERN BLOT ANALYSIS

For each sample, 5 µg of the genomic DNA was digested with *HindIII* for 12 hours at 37°C, electrophoresed on 1% agarose gel, and transferred to a nylon membrane (Amersham International, Bucks, UK) as described by Sambrook *et al.*²⁹ The DNA samples were hybridised to previously prepared DNA probes, which were digested from cloned plasmids and labelled with [³²P]dCTP with the Ready To Go DNA labelling kit (Pharmacia Biotech, Uppsala, Sweden). The membrane was prehybridised with ExpressHyb solution (Clontech, Palo Alto, California, USA) at 60°C for one hour, and hybridisation was carried out at 60°C for two hours. The membrane was washed four times with 2 × standard saline citrate (SSC)/0.05% SDS for 10 minutes at room temperature, twice with 0.1 × SSC/0.1% SDS for 20 minutes at 50°C, and then autoradiography was performed using a BAS2000 image analyser (Fuji Photo Film Co, Ltd, Tokyo, Japan).

RNA EXTRACTION AND NORTHERN BLOT ANALYSIS OF *cagA* TRANSCRIPTS

H. pylori bacterial cells were washed with normal saline and suspended in TE. Total RNA was extracted using Rneasy Total RNA kit (Qiagen, Chatsworth, California, USA). For each sample, 10 µg total RNA was electrophoresed on formamide-agarose gel and transferred to a nylon membrane (Amersham International). The hybridisation was performed using the *cagA* gene as described above, except

that the membrane was kept at 68°C instead of 60°C, and washed four times with 2 × SSC/0.05% SDS for 10 minutes at room temperature, twice with 0.1 × SSC/0.1% SDS for 20 minutes at 50°C, and then exposed to x ray film.

SEQUENCE ANALYSIS OF *cagA* NEGATIVE STRAIN

The nucleotide sequence of *cagA* and the upstream transcript of *cagA* genes were analysed in one of the *cagA* negative strains, T-94. Preliminary analysis using 15 primers designed inside the *cagA* gene revealed that nucleotides from position 910 to the terminal codon were retained. Subsequently, a primer named *cagA*-20R (5'-TTGGTCTTTATAACCAACGG-3') corresponding to *cagA* position 1020-1001) was designed. PCR was performed using *cagA*-20R and 10 previously described random primers.³⁰ The amplicons were cloned into the pCRII vector using the original TA cloning kit (Invitrogen). The cloned samples were screened by colony hybridisation. The *Escherichia coli* colonies were transferred to a nylon membrane (Amersham International). The hybridisation was performed using the full length of the *cagA* gene as probe. Hybridisation and washing were performed as for the Southern blot described above. Plasmid DNA was extracted from positive colonies and the nucleotide sequence of the insert determined by the dideoxy chain termination procedure.²⁸

STIMULATION OF IL-8 SECRETION IN GASTRIC EPITHELIAL CELL LINES

Experiments were performed as previously described.^{17, 18} *H. pylori* strains were harvested in Brucella broth containing 7.5% FBS for 24 hours at 37°C. After centrifugation, they were resuspended at 8 × 10⁷ cells/ml in RPMI 1640 containing 10% FBS and used immediately.

MKN-28 cells were routinely maintained in RPMI 1640 supplemented with 10% FBS. Confluent monolayers of MKN-28 cells in 24 well plates were co-cultured with *H. pylori* strains for 16 hours in quadruplicate. Supernatants were then aspirated and stored at -70°C until assayed for IL-8 by enzyme immunoassay (PerSeptive Diagnostics, Framingham, Massachusetts, USA). Concentrations of IL-8 were determined from a standard curve of recombinant IL-8. Bacterial induced IL-8 secretion was expressed as ng/ml after subtraction of background unstimulated control culture values.

STATISTICAL ANALYSIS

Differences in categorised data were analysed with Fisher's exact probability test, and differences in mean values by analysis of variance. p<0.05 was considered significant.

Results

DETECTION OF *CAG*A PROTEIN

CagA protein was detected by western blot analysis as previously described. Overall, 57 of 63 (90%) strains were positive for CagA protein. The prevalence of CagA protein positive strains was 12/13 for patients with gastric

ulcer, 11/12 for those with duodenal ulcer, 18/22 for patients with non-ulcer dyspepsia, and 16/16 for gastric cancer patients.

SOUTHERN BLOT ANALYSIS OF *cagA* GENE

Southern blot analysis of chromosomal DNA derived from 63 *H pylori* strains cleaved with *Hind*III revealed that all 63 (100%) strains isolated in Japan were positive for the *cagA* gene. Six strains that were negative for CagA protein, as determined by western blot analysis, also had the *cagA* gene. Some restriction fragment length polymorphism patterns were observed, but no relation was found between gastroduodenal disease and these patterns. We also analysed two *cagA* positive and eight *cagA* negative strains isolated in the Western countries as controls. Negative strains did not contain *cagA* gene, as expected.

NORTHERN BLOT ANALYSIS OF *cagA* TRANSCRIPTS

To determine whether CagA protein expression is inhibited at the transcriptional level, we analysed 17 CagA protein positive strains and six CagA protein negative strains isolated in Japan. All 17 positive strains had *cagA* transcripts. In the negative strains, two of six had *cagA* transcripts, but the remaining four did not. ATCC 43526 and 43579 were used as positive controls, and Tx30a was used as a negative control (fig 2).

SOUTHERN BLOT ANALYSIS OF *cagI*

The *H pylori* isolates were analysed for the *cagB*, *cagC*, *cagE* (*picB*), *cagM*, *cagN*, and *cagQ* genes of the *cagI* region. Overall, 59/63 (94%) strains had all the *cagI* genes, but the remaining four (6%) lacked all six *cagI* genes in spite of the presence of the *cagA* gene. Surprisingly,

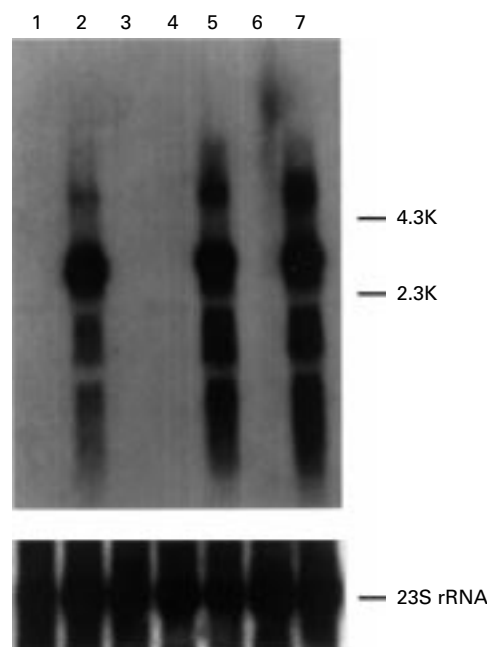


Figure 2 Northern blot analysis of *cagA* transcripts from seven strains. *cagA* transcripts were present in T-57 (lane 2), T-1 (lane 5), and ATCC 43526 (lane 7), and absent from T-94 (lane 1), T-85, T-25 (lanes 3 and 4), and Tx30a (lane 6). The membrane was also hybridised to 23S rRNA to monitor the amount of RNA loaded.

Table 2 Relation between IS605 and *cag* region of *H pylori*

	IS605 +	IS605 -	Total
All PAI +	18	41	59
Partial PAI +	2	2	4
All PAI -*	3	5	8
Total	23	48	71

PAI, pathogenicity island.

*The strains lacking all PAI were isolated in Western countries. These strains were kindly provided by Dr John Atherton (Nottingham, UK).

four strains that lacked *cagI* did not have *cagA* transcripts and CagA protein. In the strains derived from patients with non-ulcer dyspepsia, 4/22 (18%) strains did not have *cagI*, whereas all 41 strains from patients with peptic ulcer and gastric cancer had *cagI*. This difference was statistically significant ($p < 0.05$; Fisher's exact probability test). ATCC 43526 and 43579 had all the genes of *cagI*, and eight CagA negative strains including Tx30a lacked *cagI*.

SOUTHERN BLOT ANALYSIS OF *cagII*

We analysed *cagT*, *cag15*, *cag13*, *cag12*, *cag10*, and *cag6-7* of *cagII*, and *LEC*. All isolates with all *cagI* genes (59 strains) were positive for all six *cagII* genes. All clinical isolates (63 strains) hybridised with *cag6-7* irrespective of the presence of *cagI*. In the analysis of *cagT* and *cag15*, strains that had *cagI* all had these genes, but four strains without *cagI* did not have *cagT* and *cag15* genes. In the analysis of *cag13*, *cag12*, and *cag10* genes, strains that had *cagI* all had *cag13*, *cag12*, and *cag10* genes, in four strains without *cagI*, three strains had *cag13*, *cag12*, and *cag10* genes, and one strain did not have these genes. ATCC 43526 and 43579 had all the *cagII* genes and eight CagA negative Western strains including Tx30a lacked all the *cagII* genes (fig 1). All 73 strains used in this study hybridised with the *LEC*.

SOUTHERN BLOT ANALYSIS OF IS 605

Overall, 20/63 (31%) Japanese strains had IS605. The existence of IS605 was not related to the existence of *cag* PAI nor to gastroduodenal status in the host. We also investigated IS605 in eight PAI negative strains isolated in Western countries, and three of the eight (38%) were positive (table 2).

JUNCTIONAL SEQUENCES OF THE *cag* PAI DELETED REGION

Sequence analysis of the T-94 strain revealed that the *cagA* gene was truncated at position 500, and the *cag13* sequence was found directly upstream. About 25 kb of *cag* PAI including the *cagA* promoter region was deleted, and no interstitial sequences were found in the junctional region (fig 3).

H PYLORI STIMULATION OF IL-8 SECRETION IN GASTRIC EPITHELIAL CELL LINES

We analysed IL-8 stimulation by 16 strains: 10 clinical isolates and ATCC 43579, which were CagA protein and PAI positive; four clinical isolates (T-25, T-68, T-85, T-94), which were characterised as CagA protein negative with


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T-94      ATAAGCCTTAACACTCTTCTTAACTTCTGCTCTAAAAGTTTTTCGCTTCAGGGGTGAG
          ***** ** *****
NCTC 11638 ATAAGCTTTAACGCTCTCTTTGGCTTCTTCTAAAAGTTTTTCGCTTCAGGGGTGAG
146411)
          deletion
          |-----|
T-94      TAATTTCTCGCATTCTTTTTCT                                     ITGCAGGAA
          *****
NCTC 11638 CAATTTCTCGCATTCTTTTTCTCAGCTTCGGT---//---AAACAATCTTTGCAGGAA
          cag13                                     19462) cagA
          ←-----→
T-94      TTATCATAGGGAACCAATCCGATCGGATGAAAATTCATGGCGTGTGTTGATGAATCTT
          * * *****
NCTC 11638 TCATTATAGGGAATCAATCCGACGGATCAAAGTTCATGGCGTGTGTTGATGATCTT

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Figure 3 Functional sequences of T-94, which has *cag* PAI deleted. *cagII* was present down to position 14 723 (GenBank accession number AC000108) in the middle of *cag13*. The *cagA* gene was present up to position 19 462 (GenBank accession number U60176). This position is equal to position 500 of the *cagA* gene. An approximately 25 kb segment of *cag* PAI was deleted. 1) indicates the position of GenBank accession number AC000108. 2) indicates the *cagA* position of GenBank accession number U60176. Asterisks denote identity.

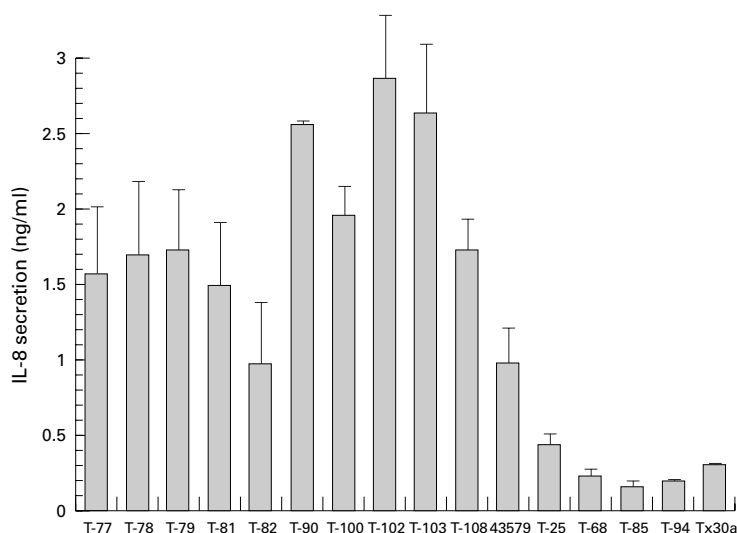


Figure 4 Secretion of interleukin-8 (IL-8) from MKN-28 cells over the 16 hours after stimulation. T-77, T-78, T-79, T-81, T-82, T-90, T-100, T-102, T-103, T-108 strains were clinical isolates with an intact *cag* pathogenicity island (PAI). 43579 indicates ATCC 43579. Tx30a is a strain with *cag* PAI completely deleted. In T-25, T-68, T-85, and T-94, *cag* PAI was partially deleted. Results are expressed as the mean and SD from four to six experiments.

PAI deleted; and the *cag* PAI negative Tx30a. As shown in fig 4, IL-8 secretion induced by the PAI deleted strains and Tx30a was significantly lower than that induced by ATCC 43579 and the other *cag* PAI positive isolates ($p < 0.05$; analysis of variance). These results indicate that intact *cag* PAI is necessary for the stimulation of IL-8 secretion, and the *cagA* gene is not an appropriate marker of IL-8 secretion.

Discussion

Previous reports have used *cagA* status to divide *H. pylori* strains into two main categories: CagA-producing strains which possess the *cagA* gene, and non-producers which lack the gene. However, a small number of strains possess the gene but do not produce the protein.³¹ This study investigated Japanese isolates belonging to the third category, but not only *cagA* was examined but also whole *cag* PAI.

Japanese strains with the *cag* PAI partially deleted were further characterised by the absence of all the genes of the *cagI* region except for *cagA* itself and most of the genes in the *cagII* region. Whereas genes *cag6* and *cag7*, located at the most upstream portion of the *cagII*, were retained in all of them, *cag10*, about 4 kb downstream of *cag7*, was deleted in one of four, and *cag15*, about 6 kb further downstream, was not detected in any. *cagB*, located next to *cagA* in the downstream portion of *cagI*, and all the genes in the *cag* PAI from *cag15* to *cagB* were deleted. Thus the nucleotide deletion in Japanese *cag* PAI deleted strains covers most but not all of the *cag* PAI, with a slight variation in the upstream border. Censini *et al*¹ reported that about 5–10% of strains had a partial deletion in the *cag* PAI. The prevalence of *cag* PAI deleted strains in Japan is the same as in Western countries, but it is not known whether the Western strains had a similar deletion to the Japanese strains.

Most Japanese CagA-non-producing strains lacked *cagA* transcripts in spite of the presence of the *cagA* gene, indicating that CagA expression was inhibited at the transcriptional level in these strains. This may be related to the deletion of the *cagA* promoter region, which is located between *cagB* and *cagA*. We determined the sequences of the exact downstream border of the deletion in one strain and found that the promoter and the head of the *cagA* gene were deleted.

All strains with *cag* PAI partially deleted were derived from patients with non-ulcer dyspepsia (4/4), whereas only 18/59 strains with *cag* PAI intact were from such patients and the balance from patients with peptic ulcers or gastric cancer ($p < 0.05$; Fisher's exact probability test). Thus the presence of intact *cag* PAI appears to be a necessary condition for advanced gastro-duodenal disease, and this is consistent with the in vitro finding that stimulation of IL-8 secretion from MKN-28 cells was significantly lower with *cag* PAI deleted strains than with intact ones. Indeed, previous studies using mutagenesis analysis have shown that many genes in the *cagI* and *cagII* regions, but not *cagA* itself, are essential for the induction of IL-8 secretion from epithelial cells.^{20 21 23}

According to previous reports, the *cagA* gene appears to be of use as a marker of whole *cag* PAI genes and PAI related virulence.²¹ However, this study shows that the presence of the *cagA* gene does not necessarily indicate the presence of intact *cag* PAI in Japanese *H. pylori* isolates. It remains to be investigated whether CagA-non-producing strains of this type are found outside of Japan.

Recent studies on the *cagA* gene in Japan, Korea, and China, where the incidence of gastric cancer is high, have shown that the prevalence of *cagA* positive strains is very high with no relation to gastro-duodenal disease.^{32–34} For example, Pan *et al*³² reported that 47 of 48 strains and 35 of 35 strains from Chinese patients with peptic ulcers and gastritis respectively were positive for the *cagA* gene. Infection with *cagA* positive strains is very common.

However, our results indicate that genes of the PAI other than *cagA* should be also evaluated.

This study reveals another characteristic of Japanese *H pylori* strains. IS605 is located between *cagI* and *cagII* in the prototype *H pylori*, and reported to be closely associated with the presence of *cag* PAI.²¹ However, IS605 status in Japanese isolates was not related to *cag* PAI status: IS605 was detected in 18 of 59 (31%) strains with *cag* PAI intact and in two of four (50%) with *cag* PAI partially deleted.

In conclusion, this study shows genetic diversity between Western and Japanese *H pylori* strains. The non-virulent Japanese strains that do not produce CagA nevertheless retain *cagA* and a few other genes in the *cag* PAI. However, they do not induce IL-8 secretion from MKN-28 cells. Thus the presence of the *cagA* gene does not necessarily indicate the presence of intact *cag* PAI, and cannot be used invariably as a marker of *cag* PAI related virulence in Japanese strains.

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