dupA as a risk determinant in *Helicobacter pylori* infection

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The *Helicobacter pylori* duodenal ulcer promoting (*dupA*) gene has been previously described as a risk marker for duodenal ulcer (DU) development and a protective factor against gastric cancer (GC). Recent studies which have assessed the application of *dupA* in the prediction of clinical outcomes have been controversial. In the current study, the association of *dupA* with the clinical outcomes and histopathological changes following *H. pylori* infection was evaluated in Iranian patients. A total of 157 *H. pylori*-infected patients with DU ($n=30$), gastric ulcer ($n=23$), gastritis ($n=68$) or GC ($n=36$) were assessed. The presence of *jhp0917* and *jhp0918* genes was determined by gene specific PCR. Gastric histopathological changes were recorded according to the updated Sydney system. Seventy-eight (49.7%) and 71 (45.2%) of the 157 tested strains, respectively, were positive and negative for both genes. The remaining 8 (5.09%) of the 157 strains were *jhp0917*-positive/*jhp0918*-negative. Univariate analysis showed inverse associations between *dupA* and histological features including dysplasia as the penultimate stage of GC and lymphoid follicles as a consequence of relatively long-standing *H. pylori*-associated gastritis. The degrees of nucleotide sequence identity of Iranian strains to Colombian, Brazilian and Indian strains ranged from 86.1 to 100% for the aligned regions of *jhp0917*, from 88 to 98.8% for *jhp0918* and from 93.4 to 99.5% for the partial sequences of the *dupA* gene. Despite the fact that possession of the *dupA* gene showed no association with any disease category in our population as reported in several other countries, association of *dupA*-negative strains of *H. pylori* with pre-malignant lesions calls for additional studies to evaluate the role of this gene as a protective marker against GC.

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INTRODUCTION

Helicobacter pylori colonizes the gastric mucosa of humans, where it can establish a long-term infection resulting in active chronic gastritis, which may result in mutually exclusive outcomes of peptic ulcer disease versus gastric cancer (GC) (Blaser, 1997; Crabtree *et al.*, 1991; Weel *et al.*,

1996; Parsonnet *et al.*, 1997; Figueiredo *et al.*, 2001; Graham, 1989; Sipponen & Stolte, 1997; McColl *et al.*, 2000; Uemura *et al.*, 2001). Several virulence-associated genes of *H. pylori*, such as *cagA*, *vacA*, *babA2* and *oipA*, are believed to play a major role in the associated clinical picture. Numerous studies have provided new insights into the role of these putative virulence factors in gastroduodenal pathogenesis (Covacci *et al.*, 1993; Atherton *et al.*, 1995; Censini *et al.*, 1996; Gerhard *et al.*, 1999; Yamaoka *et al.*, 1999, 2000, 2002; Miehleke *et al.*, 2000; Prinz *et al.*, 2001; Yu *et al.*, 2002; Azuma *et al.*, 2002; Zambon *et al.*, 2003; Argent *et al.*, 2004). However, none of the mentioned virulence markers have demonstrated discriminating roles

Abbreviations: DU, duodenal ulcer; GC, gastric cancer; GU, gastric ulcer.

The GenBank/EMBL/DDBJ accession numbers for the target genes in this study are DQ865230, DQ865231, DQ865232, DQ865233, DQ865234, DQ865235, DQ865236, DQ865237, EF076755 and EF076756.

in the development of peptic ulcer disease versus GC. Genomic-sequence comparison of two unrelated isolates of *H. pylori* (26695 and J99) indicates the presence of several regions of exceptionally distinct G+C content. A large region of approximately 45 kb in J99 and 68 kb in 26695 with a G+C content lower than that of the rest of the genome has been termed the 'plasticity zone'. This region includes half of the strain-specific open reading frames (ORFs) and is composed of 48% and 46% unique genes in strains J99 and 26695, respectively. Among the 38 ORFs in the J99 plasticity zone (*jhp914-jhp951*), 33 are missing in strain 26695 (Tomb *et al.*, 1997; Alm *et al.*, 1999; Alm & Trust, 1999). The *jhp0917* and *jhp0918* genes, which form one continuous gene, were studied by Lu *et al.* (2005) and described as a risk marker for development of duodenal ulcer (DU) and a protective factor against GC in strains isolated from Japan, Korea and Colombia. This continuous gene was thus referred to as the duodenal ulcer promoting (*dupA*) gene, and was found to be more prevalent in DU patients and associated with a reduced risk for development of gastric atrophy and cancer in these populations. Conversely, *dupA* genotyping by Argent *et al.* (2007) of four other populations, from Belgium, South Africa, China and the United States, showed no association of this gene with DU, but suggested an association with GC. Gomes *et al.* (2007) also negated any association between *dupA* and either of the two disparate clinical outcomes in Brazilian patients. In contrast, a recent report from India demonstrated a significant discriminative role for *dupA* as a virulence marker of DUs (Arachchi *et al.*, 2007).

This study was thus undertaken to clarify the status of *H. pylori dupA* as a promoting marker for DU or a protective marker against GC, as well as the association of *dupA* with histopathological lesions in Iranian dyspeptic patients.

METHODS

Subjects and bacterial isolates. The study population consisted of 157 *H. pylori*-infected patients with upper gastrointestinal disorders who were referred for endoscopy or gastric resection and were composed of the following groups: (i) 30 DU patients; (ii) 23 gastric ulcer (GU) patients; (iii) 68 gastritis patients; and (iv) 36 GC patients (21 cardia and 13 non-cardia type plus 2 with total involvement of the stomach). Gastritis was defined as an inflammatory condition of the gastric mucosa with no peptic ulcer or GC. Informed consent was obtained from every patient prior to sample collection, which was performed according to standard protocols approved by the local ethical committee.

H. pylori strains were primarily isolated from two antral biopsy specimens. Tissue samples were homogenized and plated onto *H. pylori* Specific Peptone Agar medium. Incubation was performed at 37 °C in a microaerophilic atmosphere (10% CO₂, 5% O₂ and 85% N₂) for up to 7 days. Identification was based on morphology under Gram staining and biochemical tests including urease and catalase tests.

DNA extraction and PCR. Briefly, bacterial genomic DNA was extracted by incubating bacterial pellets in 50 mM NaOH at 100 °C for 20 min, followed by 10 min incubation in 1 M Tris/HCl (pH 7.5).

The supernatants containing genomic DNA were used as the template for PCR amplification. The *ureC* gene was primarily amplified to confirm the identity of the isolated *H. pylori* strains (Labigne *et al.*, 1991). This was followed by amplification of the *jhp0917* and *jhp0918* genes using specific primers (Lu *et al.*, 2005). Tested strains were considered *dupA*-positive or -negative if both genes were present or absent simultaneously. Every PCR was repeated to confirm the results. In order to confirm the base insertion downstream of nucleotide 1385, partial *dupA* genes of 10 randomly selected strains (*jhp0917/jhp0918*-positive) were amplified using JHP0917F and JHP0918R as the forward and reverse primers, respectively (described by Lu *et al.*, 2005), yielding a 1260 bp fragment. Amplification of the target genes was carried out in a total volume of 20 µl containing 2 µl 10× PCR buffer (Fermentas), 1.5 mM MgCl₂, 0.5 µl of each primer (25 pmol µl⁻¹), 0.2 mM deoxynucleotide and 0.5 U *Taq* polymerase. The cycling programmes were preceded by 4 min at 94 °C and consisted of 35 cycles of 94 °C for 50 s, 60 °C for 50 s and 72 °C for 50 s, followed by a final extension step at 72 °C for 4 min.

Sequence analysis. The amplified products of *jhp0917*, *jhp0918* and *dupA* were cut out of the agarose gel and purified using the standard phenol/chloroform procedure. The purified PCR products were sequenced using an automated sequencer. The obtained partial *jhp0917*, *jhp0918* and *dupA* gene sequences were deposited in GenBank and aligned with similar gene segments of other reported gene sequences (Table 1). Sequence analysis was performed with the BioEdit (version 5.0.6) and CLUSTAL_X (1.8) sequence alignment programs. The entire sequences of different strains were primarily aligned, followed by analysis of every sequence in comparison to that of the C142 strain (Table 1). Finally, the regions which were not common between the sequences of other strains were omitted and the sequence identity matrix for shorter fragments of various strains which matched those of strain C142 was generated.

Histopathological studies. Multiple gastric biopsies were fixed in 10% formalin and embedded in paraffin wax by routine methods. An expert pathologist blinded to the clinical diagnosis and characteristics of the infecting *H. pylori* strains examined the sections under haematoxylin and eosin and Giemsa staining for histopathological changes and the intensity of the colonizing *H. pylori*, respectively. The presence or absence of each histological feature, including chronic gastritis, neutrophil infiltration, lymphoid follicles, atrophy, intestinal metaplasia, dysplasia and colonization of *H. pylori*, were recorded.

Statistical analysis. Data were analysed using the SPSS (version 11.5) program. For univariate analysis, the χ^2 test and Fisher's exact test were used. The presence or absence of each pathological index was evaluated with respect to the possession of *dupA* by the infecting *H. pylori* strain, using the χ^2 test. A kappa test was used to determine the association and accordance between the presence of *dupA* and other virulence markers, including *cagA* and *vacA*. A univariate logistic regression analysis was performed to determine the risk of possession of *dupA* in relation to the clinical outcome. A *P* value of ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Distribution of *dupA*

Seventy-eight of the 157 tested strains (49.7%) were positive for both the *jhp0917* and *jhp0918* genes and thus considered *dupA*-positive. The prevalence of these genes in *H. pylori* strains in Iran compared to that in other studied populations is presented in Fig. 1.

Table 1. List of full and partial *dupA* sequences available in GenBank

Strain	Name	Amplified gene	Sequenced positions*	Aligned positions*	Country	Source	Genotype	GenBank accession no.
Iran-1	C003/L/U	<i>jhp0917</i>	417–711	423–712	Iran	Adult patient with GC	<i>cagA</i> ⁺ / <i>s1m1</i>	DQ865230
Iran-2	MJ003/A	<i>jhp0917</i>	417–711	423–712	Iran	Adult patient with gastritis	<i>cagA</i> ⁺ / <i>s1m2</i>	DQ865231
Iran-3	C020/L/U	<i>jhp0917</i>	410–711	423–712	Iran	Adult patient with GC	<i>cagA</i> ⁺ / <i>s2m2</i>	DQ865232
Iran-4	MV107/C	<i>jhp0917</i>	421–723	423–712	Iran	Adult patient with DU	<i>cagA</i> ⁺ / <i>s2m2</i>	DQ865233
Iran-1	C003/L/U	<i>jhp0918</i>	1450–1716	1457–1716	Iran	Adult patient with GC	<i>cagA</i> ⁺ / <i>s1m1</i>	DQ865234
Iran-2	MJ003/L/U	<i>jhp0918</i>	1452–1717	1457–1716	Iran	Adult patient with gastritis	<i>cagA</i> ⁺ / <i>s1m2</i>	DQ865235
Iran-3	C020/L/U	<i>jhp0918</i>	1452–1717	1457–1716	Iran	Adult patient with GC	<i>cagA</i> ⁺ / <i>s2m2</i>	DQ865236
Iran-4	MV107/C	<i>jhp0918</i>	1453–1716	1457–1716	Iran	Adult patient with DU	<i>cagA</i> ⁺ / <i>s2m2</i>	DQ865237
Iran-5	C048/R/U	<i>dupA</i>	428–1692	1277–1493	Iran	Adult patient with GC	<i>cagA</i> ⁺ / <i>s1m1</i>	EF076755
Iran-6	Hel200	<i>dupA</i>	421–1722	1277–1493	Iran	Adult patient with DU	<i>cagA</i> ⁺ / <i>s2m2</i>	EF076756
C142	C142	<i>dupA</i>	1–1839		Colombia	Patient with DU	–	AB196363
Brazil-1	LPB1326-95	<i>jhp0917</i>	401–707	423–712	Brazil	Adult patient with GC	–	DQ834302
Brazil-2	LPB647-95	<i>jhp0918</i>	1436–1711	1457–1716	Brazil	Adult patient with DU	–	DQ834303
Brazil-3	LPB119-99	<i>dupA</i>	1275–1488	1277–1493	Brazil	Adult patient with GC	–	DQ834309
Brazil-4	LPB166-02	<i>dupA</i>	1275–1488	1277–1493	Brazil	Adult patient with gastritis	–	DQ834306
Brazil-5	LPB232-03	<i>dupA</i>	1275–1488	1277–1493	Brazil	Paediatric patient with gastritis	–	DQ834311
Brazil-6	LPB312-03	<i>dupA</i>	1275–1488	1277–1493	Brazil	Paediatric patient with DU	–	DQ834310
Brazil-7	LPB374-96	<i>dupA</i>	1275–1488	1277–1493	Brazil	Adult patient with GC	–	DQ834305
Brazil-8	LPB573-02	<i>dupA</i>	1275–1488	1277–1493	Brazil	Adult patient with DU	–	DQ834308
Brazil-9	LPB588-95	<i>dupA</i>	1275–1488	1277–1493	Brazil	Adult patient with DU	–	DQ834304
Brazil-10	LPB595-96	<i>dupA</i>	1275–1489	1277–1493	Brazil	Adult patient with gastritis	–	DQ834307
India-1	HP TERI 3	<i>jhp0917</i>	401–747	423–712	India	Patient with DU	–	EU102285
India-2	TERI 3-DU	<i>jhp0917</i>	401–894	423–712	India	Patient with DU	–	EU109499
India-3	–	<i>jhp0918</i>	1347–1850	1457–1716	India	Patient with DU	–	EF591768
J99	J99	<i>jhp0917</i>	1–1429	423–712	USA	Adult patient with DU	<i>cagA</i> ⁺ / <i>s1m1</i>	AE001439
		<i>jhp0918</i>	1417–1839	1457–1716				
		<i>dupA</i>	1–1839	1277–1493				

*Positions were determined in comparison to strain C142.

Fig. 1. Prevalence of *dupA* in Iran and in other reported countries.

Linkage of *jhp0917* and *jhp0918*

The *jhp0917* and *jhp0918* genes were found in 86 (54.8 %) and 78 (49.7 %) of the *H. pylori* strains, respectively. Seventy-eight (49.7 %) strains were positive for both genes, whereas 71 (45.2 %) were negative. The presence of the *jhp0917* and *jhp0918* genes was strongly linked ($P < 0.001$) with a small number of strains (5.09 %) possessing the *jhp0917*-positive/*jhp0918*-negative genotype. A *jhp0917*-negative/*jhp0918*-positive genotype was not detected.

Association between *dupA* and clinical outcomes

The distribution of *H. pylori dupA*-positive and -negative strains in patients with different clinical diagnoses is listed in Table 2. The prevalence of *dupA* in DU patients (50 %) was slightly higher than that in non-cardia GC patients (46.2 %) but did not reach statistical significance. The distribution of *dupA* in relation to clinical outcomes in Iran compared to that in other countries is depicted in Table 3. A uniform distribution (approx. 50 %) of *dupA* was found in all Iranian patient categories as was seen in Brazil and South Africa. The prevalence of *dupA* in DU patients and non-cardia GC patients from different countries ranged from 18 to 92 % and from 6 to 100 %, respectively. Univariate logistic regression analysis indicated that possession of *dupA* was not a promoting or protective determinant for any of the disease categories in our subjects.

dupA gene status and gastric histology

We also evaluated whether the presence of *dupA* was associated with histopathology indices. Univariate analysis demonstrated that possession of *dupA* was inversely associated with dysplasia such that 83.3 % of dysplasia-positive patients were colonized with *dupA*-negative strains ($P < 0.05$) whereas only 30.2 % of dysplasia-negative patients harboured these strains (Table 4). The presence of lymphoid follicles was also inversely associated with *dupA*-positive strains ($P < 0.05$), taking into account that 81.3 % of patients with no lymphoid follicles possessed *dupA*-positive strains whereas only 52.1 % of patients with lymphoid follicles were infected with these strains. When histological indices were separately studied in each disease category, the same associations existed but with peritumoral dysplasia in GC patients ($P < 0.05$) and lymphoid follicles in gastritis patients ($P < 0.05$) (Table 4). In this analysis, 90 % of patients who lacked neutrophil infiltration carried *dupA*-positive strains in comparison to 56 % of patients with neutrophil infiltration. Nevertheless, this phenomenon did not reach statistical significance. The presence of the *dupA* gene was not, however, related to chronic gastritis, atrophy, intestinal metaplasia or the presence of *H. pylori* (Table 4).

Association of *dupA* and major virulence markers of *H. pylori*

Forty-nine per cent of *cagA*-positive strains and 57.9 % of *cagA*-negative strains were *dupA*-positive. The presence of

Table 2. Distribution of *dupA* in different clinical groups

Genotype	Clinical outcome [n (%)]						Total
	Gastritis	DU	GU	GC			
				Cardia	Non-cardia	Total	
<i>dupA</i> ⁺	34 (50)	15 (50)	9 (39.1)	12 (57.1)	6 (46.2)	2 (100)	78 (49.7)
<i>dupA</i> [−]	34 (50)	15 (50)	14 (60.9)	9 (42.9)	7 (53.8)	0 (0)	79 (50.3)
Total	68 (100)	30 (100)	23 (100)	21 (100)	13 (100)	2 (100)	157 (100)
				36 (100)			

Table 3. Distribution of *dupA* in relation to clinical outcome in Iran and other reported countries

Region	No. of <i>dupA</i> positive strains/no. of studied strains (%)			
	Gastritis	DU	GU	Non-cardia GC
Iran	34/68 (50)	15/30 (50)	9/23 (39.1)	6/13 (46.2)
Japan	7/50 (14)	11/30 (37)	13/50 (26)	3/30 (10)
Korea	2/30 (7)	24/65 (37)	5/30 (17)	3/50 (6)
China	3/12 (25)	2/11 (18.2)	2/5 (40)	1/1 (100)
Colombia	15/40 (39)	25/45 (55)	12/30 (40)	6/50 (12)
Brazil	133/144 (92.36)	110/126 (87.30)	—	71/81 (87.65)
South Africa	11/15 (73.3)	12/13 (92.3)	—	16/18 (88.9)
Belgium	29/76 (38.2)	20/40 (50)	—	10/19 (52.6)
USA	9/20 (45)	9/21 (42.9)	2/3 (66.7)	—
India	16/70 (22.86)	36/96 (37.5)	—	—

dupA was thus not associated with the presence of *cagA* in any patient category except for patients suffering from DUs ($P < 0.05$). Forty-eight per cent of *vacA* s1 strains, 60 % of *vacA* s2 strains, 56.6 % of *vacA* m1 strains and 47.4 % of *vacA* m2 strains were *dupA*-positive. Collectively, neither kappa agreement nor chi-square analysis showed any statistically significant association between *dupA* status and *cagA* status or *vacA* polymorphisms. The same held true when the status of *cagA* and *vacA* was independently evaluated.

Comparative analysis of *jhp0917*, *jhp0918* and *dupA* sequences

The nucleotide sequences of *jhp0917*, *jhp0918* and *dupA* of six Iranian strains were aligned with the sequences of the corresponding genes in J99, C142 and ten Brazilian and three Indian strains. In comparison with C142 and the Brazilian and Indian strains, the nucleotide sequence similarity of Iranian strains ranged from 86.1 to 100 % for *jhp0917*, from 88 to 98.8 % for *jhp0918* and from 93.4 to 99.5 % for *dupA*. The sequence similarities are presented in Tables 5, 6 and 7. This analysis revealed a significant similarity (>93 %) in partial sequences of *dupA* among different studied strains (Table 7). There was a 'C' insertion in a GC strain (Iran-5) and a 'T' insertion in a DU strain (Iran-6) downstream of nucleotide 1385. This is in accordance with every other studied strain except for J99, Brazil-4, Brazil-7 and Brazil-9 (Fig. 2).

Recent studies have proposed the possibility of using genetic markers in the plasticity zone as indicators of pathogenicity for *H. pylori* infection, in spite of a lack of credible knowledge regarding the functions of the putatively encoded proteins in this cluster. It seems that these determinants may play a key role in determining the virulence capacity of *H. pylori* strains either directly or by encoding factors that may lead to varying clinical outcomes. The association between some of the ORFs in the plasticity zone and various disease categories has been previously reported. For instance, Occhialini *et al.* (2000) found that two single ORFs (*jhp0940* and *jhp0947*) were more prevalent in strains isolated from patients with gastric adenocarcinoma in Costa Rica. However, Santos *et al.* (2003) showed the association between *jhp0947* and DU as well as GC in Brazilian patients. This was once more confirmed for *jhp0947* and *jhp0949* genes in DU patients from the Netherlands (de Jonge *et al.*, 2004). The implication that *jhp0917* and *jhp0918* promote DU and protect against GC was made by Lu *et al.* (2005) and the continuous gene formed by these was thus referred to as the duodenal ulcer promoting (*dupA*) gene. Herein, we have investigated this hypothesis in Iranian dyspeptic patients. Iran, located in South-West Asia, has a high prevalence of *H. pylori* infection with limited application of previously studied *H. pylori* virulence markers in patient screening (Talebkhan *et al.*, 2007).

Table 4. Association between possession of *dupA* and histological changes

<i>dupA</i>	Chronic gastritis		Neutrophil infiltration		Lymphoid follicles		Atrophy		Intestinal metaplasia		Dysplasia*		<i>H. pylori</i>	
	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence
% Positive	60.4	50	56	90	52.1	81.3	57.1	59.5	52.4	60.5	16.7	69.8	53.8	78.6
% Negative	39.6	50	44	10	47.9	18.8	42.9	40.5	47.6	39.5	83.3	30.2	46.2	21.4
<i>P</i> value	0.7		0.07		0.046		1		0.59		0.002		0.13	

*Dysplasia in non-tumoral tissue.

Table 5. Nucleotide similarity (%) of partial sequences of the *jhp0917* gene

The accession numbers and the aligned positions are as indicated in Table 1.

Isolate	Iran				USA	Colombia	Brazil	India	
	Iran-1	Iran-2	Iran-3	Iran-4	J99	C142	Brazil-1	India-1	India-2
Iran-1		100.0	90.8	87.2	90.1	90.5	90.8	90.8	89.5
Iran-2			90.8	87.2	90.1	90.5	90.8	90.8	89.5
Iran-3				92.2	92.3	91.6	91.2	91.6	90.6
Iran-4					87.7	87.0	86.7	87.7	86.1
J99						95.4	96.4	97.2	95.4
C142							97.5	95.1	94.4
Brazil-1								95.4	95.1
India-1									94.7
India-2									

Table 6. Nucleotide similarity (%) of partial sequences of the *jhp0918* gene

The accession numbers and the aligned positions are as indicated in Table 1.

Isolate	Iran				USA	Colombia	Brazil	India
	Iran-1	Iran-2	Iran-3	Iran-4	J99	C142	Brazil-2	India-3
Iran-1		94.8	93.3	94.0	89.4	90.2	89.1	88.0
Iran-2			97.6	97.2	88.7	89.4	88.7	88.0
Iran-3				96.8	88.7	89.4	88.7	88.0
Iran-4					90.6	90.2	90.2	89.6
J99						96.0	98.8	94.6
C142							95.7	95.3
Brazil-2								94.2
India-3								

Table 7. Nucleotide similarity (%) of partial sequences of the *dupA* gene

The accession numbers and the aligned positions are as indicated in Table 1.

Isolate	Iran		USA	Colombia	Brazil							
	Iran-5	Iran-6	J99	C142	Brazil-3	Brazil-4	Brazil-5	Brazil-6	Brazil-7	Brazil-8	Brazil-9	Brazil-10
Iran-5		94.9	96.2	96.2	97.2	94.8	95.8	96.7	96.2	97.2	96.2	95.8
Iran-6			95.3	95.8	95.3	93.4	95.3	95.3	95.3	95.8	95.3	94.4
J99				97.6	98.5	96.7	99.0	97.1	99.0	99.0	99.0	97.6
C142					97.6	95.7	97.6	96.7	97.6	98.1	97.6	96.7
Brazil-3						97.1	98.1	97.6	98.5	99.5	98.5	98.1
Brazil-4							97.1	96.2	96.7	97.6	97.6	98.1
Brazil-5								97.1	98.1	98.5	99.0	98.1
Brazil-6									97.1	97.6	97.1	97.2
Brazil-7										99.0	99.0	97.6
Brazil-8											99.0	98.6
Brazil-9												98.6
Brazil-10												

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AACAAACCTTTTTCTATTGATGAACTAAAGACTACATTATGCATCCTA-TAATGT Iran-5
AACAAACCTTTTTCTATTGATGAACTAAAGACTACATTATGCATCCTA-TAATGT Iran-6
AACAAACCTTTTTCTATTGATGAACTAAAGACTACATTATGCATCCTA-TAATGT C142
AACAAACCTTTTTCTATTGATGAACTAAAGACTACATTATGCATCCTA-TAA-- J99
*****C***** Brazil-3
*****T***** Brazil-4
*****T***** Brazil-5
*****T***** Brazil-6
*****G***** Brazil-7
*****C***** Brazil-8
*****C***** Brazil-9
*****C***** Brazil-10

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Fig. 2. Nucleotide sequence alignment of partial sequences of *dupA* from strains analysed in the current study. The asterisks refer to positions where nucleotide sequences match those of the consensus sequence and the hyphens represent deletions. The variant nucleotides are shown by shading.

Analysis of *dupA* in a limited group of patients revealed that it is present in approximately half of the collected strains regardless of the clinical status. This prevalence is higher than that reported for countries such as Colombia, the United States, Belgium, India and some East Asian countries, including Japan, Korea and China, but lower than that for South Africa and Brazil. The variations in the prevalence of *dupA* in different countries may be partly due to distinct dyspeptic populations and geographical heterogeneity of *H. pylori* isolates.

In accordance with the study by Lu *et al.* (2005), our study confirms the presence of *jhp0917* and *jhp0918*, linked to each other and constituting the complete *dupA* gene. This study also illustrated the lower distribution of non-functional strains in Iran (5.09%) than in India (11%) with regard to circulation of *jhp0917*-positive/*jhp0918*-negative isolates. The principal limitation of our study is the small number of DU cases, which impairs a definite conclusion. The prevalence of *dupA* in our limited number of DU patients was slightly higher than that in non-cardia GC, but this was not statistically significant. This is in accordance with the findings of Argent *et al.* (2007), who demonstrated a lack of association between the presence of *dupA* and DU in strains isolated from four different geographical regions, Belgium, South Africa, China and the United States. Furthermore, Gomes *et al.* (2007) confirmed the lack of association of *dupA* with DU as well as GC in both Brazilian adults and paediatric patients. In contrast to the mentioned studies, the report by Arachchi *et al.* (2007) confirmed the association of *dupA* with DU as an informative virulence marker. These discordant results may be explained by differences between the plasticity regions of *H. pylori* strains isolated from distinct geographical areas. On the other hand, Lu *et al.* (2005) reported that only a small proportion (6–12%) of GC patients was colonized with *dupA*-positive strains. This was fully negated by Argent *et al.* (2007), who suggested a direct association between the presence of *dupA* and GC (due to the high prevalence of *dupA* in GC patients), particularly in the combined populations of Belgian and South African patients. The current findings verify the need for analysis of larger numbers of dyspeptic patients in particular DU cases.

Having studied gastric histopathological features, Lu *et al.* (2005) demonstrated an inverse association between possession of the *dupA* gene and pathological precursors

of GC, including intestinal metaplasia and atrophy. Our study supports the hypothesis of this gene being a protective factor against GC, such that it was found to be inversely associated with pre-malignant histological features such as dysplasia. Despite the absence of a clear inverse association between possession of the *dupA* gene and GC, keeping in mind that gastric epithelial dysplasia represents the only true histological marker of GC and the increased prevalence of precursor lesions such as dysplasia as a main pre-malignant and precancerous lesion is associated with increased incidence of cancer, *dupA* gene may be applicable as a protective marker against GC development.

Another significant inverse relationship was found between lymphoid follicles and possession of *dupA*. Since lymphoid follicles represent a relatively common histological feature of chronic gastritis, which is believed to progress towards gastric malignancies, this may provide further support for *dupA* as a protective marker against GC.

In addition, sequencing of this gene from Iranian isolates and comparison with sequences from isolates from other geographical regions indicated more than 93% similarity and the presence of the continuous gene due to nucleotide insertion after position 1385. The presence of a continuous gene in these isolates may represent a functional protein, which requires further investigation. In summary, we conclude that the discrepant results obtained for this putative virulence marker may be a consequence of the plasticity of *H. pylori*, which contributes to its genetic diversity and requires additional studies for a firm conclusion.

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