Infection with *Helicobacter pylori* strains lacking *dupA* is associated with an increased risk of gastric ulcer and gastric cancer development

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Recently, *dupA* was reported as a new virulence factor in *Helicobacter pylori*, but its association with gastroduodenal disorders and its mode of action are still unclear. Here, an association of the *dupA* status with different disease groups was determined and a biological explanation for the observed associations was tested. In total, 216 *H. pylori* isolates were obtained from 232 presumed *H. pylori*-infected patients. A positive association was observed between the occurrence of duodenal ulcer (DU) and the presence of *dupA* [odds ratio (OR) 24.2; 95 % confidence interval (CI) 10.6–54.8]. In addition, an inverse association between the occurrence of gastric cancer (GC) [OR 0.16; 95 % CI 0.05–0.47] and gastric ulcer (GU) [OR 0.34; 95 % CI 0.16–0.68] with the presence of *dupA* was observed. A putative explanation for the observed associations might be a more corpus-located infection (pan-gastritis) by the *dupA*-positive strains due to their increased acid resistance. Indeed, a strong association between *dupA*-positive *H. pylori* isolated from gastritis patients and *in vitro* acid resistance was observed (*P*<0.05). The observed higher acid resistance of the *dupA*-positive strains suggests that these strains are adapted to a stomach with high gastric acid output. This may in part explain the observed associations, as an increased gastric acid output is thought to be typical for an antrum-predominant *H. pylori* infection and, whilst this is associated with an increased risk of DU formation, it also decreases the risk for the genesis of GUs and GC.

INTRODUCTION

*Helicobacter pylori* colonization in humans is considered to play a critical role in the genesis of a wide array of gastroduodenal diseases including gastritis, peptic ulcers and gastric cancer (GC) (Kusters et al., 2006). Although the clinical outcome of the infection is thought to be determined by host, bacterial and environmental factors (Mobley, 1997; Yamaoka, 2010), the exact mechanisms that determine the clinical outcome of the infection are still unknown. On the host side, immune status (Robinson et al., 2007) and host acid secretion seem to be critical determinants in this process (Sobala et al., 1991; Kuipers et al., 1995). On the bacterial side, virulence factors such as *babA*, *oipA*, *cagA* (a marker of the pathogenicity island) and *iceA* have been shown to affect the development of post-infection disorders (Covacci et al., 1999; Atherton, 2006). Recently, duodenal ulcer-promoting gene (*dupA*) has been proposed as a novel *H. pylori* virulence factor associated with an increased rate of occurrence of duodenal ulcer (DU) and a decreased risk for GC (Lu et al., 2005). The DupA protein is a homologue of the VirB4 ATPase (Lu et al., 2005; Gomes et al., 2008) and probably represents an outer-membrane protein of a type IV secretion system.

In addition to the *cagA* pathogenicity island-encoded VirB4 homologue (Hp0544), the total genomic sequence of *H. pylori* strain 26695 revealed the presence of three additional VirB4 homologues (HP0017, HP0441 and HP0459) (Tomb et al., 1997). The sequenced genome of strain J99 revealed the presence of an additional copy of a VirB4 homologue 027052 © 2012 SGM  Printed in Great Britain
(jhp0917/0918) that is not present in strain 26695. Whilst originally designated two separate genes (jhp0917 and jhp0918), they were subsequently shown to form one continuous ORF that is present in a substantial fraction of the tested H. pylori strains (Lu et al., 2005). Due to its putative association with DU formation, the gene was named dupA (Lu et al., 2005). The biological function of DupA has thus far not been identified, but in vitro studies indicate that DupA enhances survival rates at low pH and induces interleukin-8 production (Lu et al., 2005). Recently, the ability of dupA-positive strains to induce in vitro interleukin-8 production has been disputed (Schmidt et al., 2009). Whilst dupA is associated with disease outcome in various populations, this relationship is inconsistent among studies (Arachchi et al., 2007; Argent et al., 2007; Douraghi et al., 2008; Zhang et al., 2008; Nguyen et al., 2010). Nevertheless, a recent systematic review suggested that these inconsistencies might in part be due to the small numbers of isolates that were included in the individual studies, although alternatively they might be due to regional and ethnic differences (Hussein, 2010).

Iran is a location with a high prevalence of H. pylori infection (Massarrat et al., 1995; Talebi Bezmin Abadi et al., 2009), especially in the Mazandaran province (state of Sari) where the infection rate exceeds 90% (Talebi Bezmin Abadi et al., 2009, 2010). The high prevalence of infection allowed us to collect a large number of H. pylori strains from a narrow geographical region in order to perform a comprehensive study of the putative association between dupA status and the clinical outcome of H. pylori infection.

**METHODS**

**Patients.** All patients with gastroduodenal complaints who visited the medical centres at Sari, Mazandaran province, Iran, between May 2007 and March 2010 for an endoscopic evaluation of putative H. pylori infection were invited to participate in this study. The study was approved by the ethical review board of the Tarbiat Modares University, Tehran, Iran. Endoscopic findings and gastric histopathological examinations were used as criteria for the determination of DU, gastric ulcer (GU), gastritis only (G) or GC, as described previously (Dixon et al., 1996; Kusters et al., 2006). Patients who had received antibiotic treatment up to 4 months prior to this study or who lacked histopathological analysis, or where no antral biopsy sample was available for H. pylori culture, were excluded from the study. In total, 232 patients (median age 44 years, range 17–73, 58.7% male) were analysed (Fig. 1).

**Bacterial culture and identification.** Antral biopsy specimens were used for bacterial culture. Briefly, biopsies were collected in 1 ml...
sterile thioglycolate broth (Merck). Immediately after biopsy collection, samples were homogenized in the sample medium with a sterile syringe. The homogenate was subsequently plated onto Colombia agar (CA) plates (Merck) containing 7% defibrinated sheep blood (Jihad Daneshgahi), 7% fetal calf serum (Gibco) and antibiotics (ampicillin B, polymyxin and vancomycin; Mast). The CA plates were incubated for 7 days under microaerophilic conditions (5% O₂, 10% CO₂ and 85% N₂; Binder-USA) at 37°C and in a water-saturated atmosphere. Suspected colonies were identified as H. pylori based on their colony morphology, their shape as determined by Gram staining and positive biochemical tests for catalase, oxidase and urease activity. A single colony per patient was selected at random from the primary culture plate, multiplied by culture on CA plates and stored at −80°C in 20% glycerol for future testing.

DNA extraction and PCR analysis of glmM and dupA. Bacteria from the −80°C stocks were freshly grown on CA plates and chromosomal DNA was extracted using a commercially available DNA isolation kit (Roche). PCR assays for the glmM (encoding phosphoglucosamine mutase; Labigne et al., 1999) and dupA (Lu et al., 2005; Nguyen et al., 2010) genes were performed as described previously; the primers and PCR conditions are summarized in Table 1. All isolates were tested by the H. pylori-specific glmM PCR to validate the quality of the isolated DNA and to obtain independent confirmation of the H. pylori nature of the isolates. The correct size of the amplified product was tested by running the PCR product on a 1.5% agarose gel (Sinagene). To exclude the possibility that the absence of dupA amplicons was the result of inhibition of PCR rather than the absence of the gene, all dupA-negative PCRs were tested using an internal control, i.e. by the addition of the glmM primers to the dupA PCR mix. Only if there was a PCR product for the ubiquitously present glmM allele in the absence of the dupA product was the reaction scored as a true dupA-negative. In each PCR experiment, H. pylori strains J99 and 26695 were included as controls. Strains that gave no visible product for the jhp0917 and jhp0918 alleles were scored as dupA-negative, and strains that gave PCR products for both the jhp0917 and jhp0918 alleles were scored as dupA-positive. In this study, to obtain well-validated results on the presence of dupA, we repeated our experiments with an independent set of dupA primers (Table 1). To maximize the chance of including true dupA-positives, only the 202 strains that resulted in unambiguous PCR profiles for the jhp0917, jhp0918 and dupA gene primers (see Fig. 1) were included in our analysis.

Acid resistance testing. To evaluate the acid resistance of the isolated strains, we tested their ability to grow on a set of CA plates adjusted to different pH values in a range of pH 3.0–7.0 (in steps of 1 pH unit), as described previously (Bijlsma et al., 2000). Briefly, a random selection of dupA-negative (n=20) and all dupA-positive (n=12) H. pylori strains recovered from G patients were grown under normal conditions on regular CA plates for 5 days. Colonies were then suspended in 600 µl Brucella broth. Suspensions were adjusted to an OD₆₀₀ of 1. From this standardized inoculum, tenfold dilutions were prepared (10⁻¹, 10⁻² and 10⁻³) in Brucella broth and 100 µl of each dilution was plated on pH-adjusted CA plates. Successful growth resulted in ~10–10000 individual colonies on a single plate, depending on the dilution. Bacterial growth was assessed after 5 days. To avoid interference between closely spaced colonies, only dilutions that resulted in 20–200 colonies on the pH 7.0 plates were included in our analysis.

Statistical analysis. Depending on the dataset, Fisher’s exact test or Student’s t-test was used for analysis. A two-sided P value of <0.05 was considered statistically significant. The effect of the presence of dupA and cagA on the risk of developing specific gastric pathology was expressed as an odds ratio (OR) with a 95% confidence interval (CI). All statistical analyses were conducted using spss 15.0.

RESULTS

Association between the presence of dupA and gastroduodenal disorders

Of the 232 putative H. pylori-positive patients willing to participate in our study, 216 (mean age 44, range 17–73 years, 58.7% male) had a positive culture result for a single colony of H. pylori. In order to obtain a maximum likelihood of correct identification of strains with a functional dupA gene, we used three sets of PCR primers; thus, dupA genotyping was based both on the combined data of the PCR primer sets as described by Lu et al. (2005) and a third primer set designed by Nguyen et al. (2010). Twelve patients carried isolates that were positive for only a single PCR (jhp0917, jhp0918 or dupA) but not for all three alleles, and these were excluded from our association study (Fig. 1). Much to our surprise, only two discrepant results were observed when comparing the two dupA genotyping methods, i.e. two of the strains that were negative

Table 1. PCR primers for amplification of glmM, jhp0917, jhp0918, cagA and dupA sequences

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer sequence (5’→3’)</th>
<th>Product size (bp)</th>
<th>PCR conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>glmM</td>
<td>CAGTTTAGGGGTTAGGTGCTGCC</td>
<td>294</td>
<td>93°C, 1 min; 55°C, 1 min; 72°C, 1 min (32 cycles)</td>
<td>Labigne et al. (1991)</td>
</tr>
<tr>
<td>jhp0917</td>
<td>TGTTTCTACTGACAGCGC</td>
<td>307</td>
<td>94°C, 30 s; 59°C, 30 s; 72°C, 1 min (35 cycles)</td>
<td>Zhang et al. (2008)</td>
</tr>
<tr>
<td>jhp0918</td>
<td>AAGCTGACGCGTCGGTATAGC</td>
<td>276</td>
<td>94°C, 2 min; 58°C, 30 s; 72°C, 1 min (35 cycles)</td>
<td>Zhang et al. (2008)</td>
</tr>
<tr>
<td>dupA</td>
<td>TAAAGCTGATCATATAGGATTTGGAGCGCGCTG</td>
<td>350</td>
<td>92°C, 45 s; 56.5°C, 51 s; 72°C, 56 s (32 cycles)</td>
<td>Nguyen et al. (2010)</td>
</tr>
<tr>
<td>cagA</td>
<td>CCAAAGCGGCGCCATTCTATTTGGAGCGC</td>
<td>298</td>
<td>94°C, 60 s; 60°C, 60 s; 72°C, 55 s (35 cycles)</td>
<td>Hamlet et al. (1999)</td>
</tr>
</tbody>
</table>
isolated from G patients and acid resistance
resulted in a strong association between
grow on pH 4 and pH 3 plates, respectively (Fig. 2). This
however, at pH 4.0, only
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All 12
positive isolates were able to form normal-sized colonies.
study grew at pH 4.0 and 4/12 isolates (33 %) grew at
disease-specific pH conditions, only isolates from G
adaptation of the infecting strains to
grown bacteria directly onto plates adjusted to pH values
ranging from 7.0 to 3.0. In an attempt to reduce the risk
infection with this bacterium results in a wide range of
gastrointestinal disease symptoms (Salama et al., 2000).
CagA is probably the best-studied disease biomarker in H.
pylori. Many other disease-associated virulence factors have
now been identified, but unfortunately most seem linked
to the cagA status and hence probably do not represent
independent risk factors (Kusters et al., 2006). In this
study, cagA was found at a similar level in all of the
gastrointestinal disorders, which was somewhat unexpected
considering a recent report that failed to find a significant
link between cagA and H. pylori infection-associated
disease types among Iranian patients (Khodaii et al.,
2011). In the current study, cagA and dupA were not
colinked to a specific disease type and thus they were
independent predictors for the clinical outcome of H.
pylori infection, as has been suggested previously (Argent
et al., 2007; Zhang et al., 2008). The apparently unique
mode of action of dupA merits further studies into the
disease associations and biological functions of dupA. Here,
the association between the presence of the dupA gene in
the infecting strain and gastroduodenal disease outcome
was tested and a putative link with acid resistance of dupA-
positive strains was confirmed, thus providing a putative
biological explanation for a positive association with the
development of DU and a negative association with GC and
GU.

Lu et al. (2005) were the first to report the association of
the presence of the dupA gene with an increased risk of DU.
Subsequently, several other studies have been carried out
to test for a putative association between dupA status and
different digestive disorders in various geographical areas
(Argent et al., 2007; Hussein et al., 2008; Nguyen et al.,
2010).

In this study, the presence of dupA was observed in 56/65
(86.1 %) of the patients with DU and in 26.1, 20.3 and
12.5 % of the patients with G, GU and GC, respectively
(Tables 2 and 3). Thus, the relative risk of having DU when
infected with a dupA-positive strain compared with any
of the other three diseases was 4.22, with a sensitivity
of 83.6 % and a specificity of 79.6 %. Whilst the presence
of the dupA gene was associated with an increased risk of
developing DU (OR 24.2, 95 % CI 10.6–54.8), a strong
association for the inverse situation was seen in GC
patients where the infecting H. pylori strains seemed to be
preferentially dupA-negative (OR 0.16, 95 % CI 0.05–0.47).
A similar trend towards a protective effect was observed for
GU formation, as a significant relationship between GU
patients and dupA-negative strains was observed, but this
did not reach statistical significance (OR 0.34, 95 % CI
0.16–0.68). Thus, our data suggested that the absence
of dupA protects against GC and GU (Table 3). One has to
bear in mind that a study like this will probably generate
biased results, as it is based on patients who visit the

Acid resistance of G-derived H. pylori isolates

The presence of dupA has previously been associated with
an increased resistance to acid shock. As this might in part
explain the observed associations with gastric pathology,
we wanted to expand on this observation and compare the
ability of dupA-positive or -negative strains to grow at low
pH. To carry out this test, we plated dilutions of freshly
grown bacteria directly onto plates adjusted to pH values
ranging from 7.0 to 3.0. In an attempt to reduce the risk
of acid-resistance adaptations of the infecting strains to
disease-specific pH conditions, only isolates from G
patients were selected. At pH 7, 6 and 5, there were no
obvious growth differences between dupA-positive and
-negative strains (Fig. 2). However, at pH 4.0, only dupA-
positive isolates were able to form normal-sized colonies.
All 12 dupA-positive strains from the G patients in our
study grew at pH 4.0 and 4/12 isolates (33 %) grew at
pH 3.0. In contrast, only 8/20 (40 %) and 2/20 (10 %) of
the dupA-negative isolates from G patients were able to
grow on pH 4 and pH 3 plates, respectively (Fig. 2). This
resulted in a strong association between dupA-positive
H. pylori isolated from G patients and acid resistance
(P=0.003) (detailed data not shown).

DISCUSSION

H. pylori is highly adapted to the hostile environment of
the human stomach (Cover & Blaser, 2009), and chronic
infection with this bacterium results in a wide range of
gastrointestinal disease symptoms (Salama et al., 2000).

According to the criteria defined by Lu et al. (2005)
(negative for both the jhp0917 and jhp0918 alleles) were
positive using the primer set introduced by Nguyen et al.
(2010) (Table 1). In total, 14 of the 216 (6 %) strains
generated non-concordant results with the three primer
pairs, and these strains were excluded from our analysis
(Fig. 1). Detailed data relating to the histopathological
findings, age of patients, and the cagA and dupA status of
the remaining 202 patients are presented in Table 2. Whilst
the various age subgroups were too small to allow any
significant associations (data not shown), there was a clear
trend for GC and DU to present at a higher age. Also, a
trend for more severe atrophy was observed in the DU and
GC group, but again, due to the small subgroup sizes, these
differences did not reach statistical significance. When the
cagA status of the various patient groups was analysed, no
significant association between cagA and the G, GU or DU
group could be observed (Table 3). There was, however, a
weak association between cagA status and the GC group
(OR 2.4, 95 % CI 1.1–5.1; Table 3). This was in striking
contrast to dupA status where a significant statistical
correlation was observed for all gastroduodenal disorders
(Table 3). In our population, a strong positive correlation
(OR 24.2, 95 % CI 10.6–54.8) was found between the
occurrence of DU and the presence of a dupA-positive
genotype (Table 3). Interestingly, the dupA gene showed a
tendency to protect against the development of GU (OR
0.34, 95 % CI 0.16–0.68) and GC (OR 0.16, 95 % CI 0.05–
0.47) (Table 3). We did not observe a statistical association
between the cagA and dupA status, and a multivariate
analysis did not show a significant contribution of the cagA
status towards the observed associations with the dupA
status (data not shown).
### Table 2. Patient characteristics

<table>
<thead>
<tr>
<th>Disease type</th>
<th>Sample size</th>
<th>Median age (range)</th>
<th>Gender (% male)</th>
<th>cagA⁺ (%)</th>
<th>No. of dupA⁺ (%)</th>
<th>Histopathology</th>
<th>Distribution over age cohorts (no. of dupA⁺)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;20 years</td>
</tr>
<tr>
<td>Gastritis (G)</td>
<td>46</td>
<td>29 (20–58)</td>
<td>63</td>
<td>56.5</td>
<td>12 (26.1)</td>
<td>Mild</td>
<td>4/46 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
<td>37/46 (9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severe</td>
<td>5/46 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mild</td>
<td>14/59 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
<td>34/59 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severe</td>
<td>11/59 (7)</td>
</tr>
<tr>
<td>Gastric ulcer (GU)</td>
<td>59</td>
<td>24 (17–32)</td>
<td>64.4</td>
<td>42.6</td>
<td>12 (20.3)</td>
<td>Mild</td>
<td>14/59 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
<td>34/59 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severe</td>
<td>11/59 (7)</td>
</tr>
<tr>
<td>Duodenal ulcer (DU)</td>
<td>65</td>
<td>33 (22–71)</td>
<td>51.3</td>
<td>55.3</td>
<td>56 (86.1)</td>
<td>Mild</td>
<td>5/65 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
<td>49/65 (46)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Severe</td>
<td>11/65 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mild</td>
<td>3/32 (1)</td>
</tr>
<tr>
<td>Gastric cancer (GC)</td>
<td>32</td>
<td>34 (21–73)</td>
<td>62.5</td>
<td>43.7</td>
<td>4 (12.5)</td>
<td>Mild</td>
<td>3/32 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
<td>26/32 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severe</td>
<td>3/32 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
<td>44 (17–73)</td>
<td>60.3</td>
<td>50.0</td>
<td>84 (41.58)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Atrophy scores according to updated Sidney criteria (Dixon et al., 1996).
clinician with their first serious upper gastrointestinal symptoms. This might generate a strong selection bias, as dupA-positive-infected patients developing from G to DU are probably those developing complaints and presenting to a gastroenterologist with a desire to be treated. Thus, if there is a protective effect of the absence of dupA on GC, these patients will not develop complaints and thus are less likely to present themselves to a gastroenterologist, and this will result in a skewing of this patient group. To our knowledge, a putative protective effect of this will result in a skewing of this patient group. This might generate a strong selection bias, as these patients will not develop complaints and thus are less likely to present themselves to a gastroenterologist, and this will result in a skewing of this patient group. To our knowledge, a putative protective effect of dupA against GC has not been reported before. Possibly, this is due to the above-mentioned bias that selects against finding such an association, and/or the association may have been missed due to the limited number of GC patients included in other dupA studies (Lu et al., 2005; Arachchi et al., 2007; Argent et al., 2007; Douraghi et al., 2008; Zhang et al., 2008; Nguyen et al., 2010). This study included 59 GU and 32 GC patients (Table 2) and to our knowledge represents the largest population of GU and GC patients analysed for an association with the dupA gene. A trend for such an association has, however, been observed in a recent systematic review (Hussein, 2010) and in a meta-analysis by Shiota et al. (2010).

The lack of a functional DupA test prohibits defining true-positives and true-negatives, so the analytical sensitivity and specificity of the two PCR-based genotyping tests used cannot be calculated. Interestingly, there were no significant \((P<0.05)\) differences between the results obtained with the dupA primer sets, indicating that both tests performed equally well. Thus, in spite of a gold standard for determining true positives/negatives, these data suggest that the specificity of either test is \(>95\%\). Based on the common belief that non-functional genes are more prone to genetic drift resulting in a non-productive PCR due to primer mismatch, isolates with non-concordant dupA typing data were excluded from our analysis to maximize the chance of correct identification of the dupA status. Strictly adhering to this concordance rule resulted in the elimination of 14/216 (\(6\%\)) of the isolates (Fig. 1).

In the present study, the total rate of dupA-positive H. pylori strains was 41.6\% (84/202), a rate that was slightly less than that observed in a previously published study performed in Iran (Douraghi et al., 2008). Whilst the differences in dupA prevalence were only minor and can probably be explained by the sample size of the study populations, our result is more in line with most other reports (Lu et al., 2005; Hussein et al., 2008; Schmidt et al., 2009; Nguyen et al., 2010). Interestingly, the dupA frequency seems to vary widely among studies and ranges from 7.1\% in an Indian population (Schmidt et al., 2009) to 89.5\% in a Brazilian study (Gomes et al., 2008). Similarly, reports from Iran (49.7\%; Douraghi et al., 2008), India (31.3\%; Arachchi et al., 2007), China (27.6 and 65.3\%; Argent et al., 2007; Zhang et al., 2008), the USA (45.5\%; Argent et al., 2007) and Japan (21.3\% and 28.8\%; Lu et al., 2005; Nguyen et al., 2010) indicate a wide range for the prevalence of dupA. Collectively, it has been assumed that these differences in dupA prevalence probably reflect geographical differences (Hussein, 2010). Nevertheless, they may also be the result of bias in patient selection and/or the relatively small sample numbers present in most studies. The high prevalence of H. pylori infection in northern Iran allowed us to collect a large number of H. pylori strains from a narrow geographical region, thereby largely ruling out putative bias due to

### Table 3. Association of dupA and cagA genotypes with disease types in the 202 patients included in the study group

<table>
<thead>
<tr>
<th>Disease type</th>
<th>Sample size</th>
<th>cagA Positive (%)</th>
<th>OR (95 % CI)</th>
<th>dupA Positive (%)</th>
<th>OR (95 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>46</td>
<td>20 (43)</td>
<td>0.71 (0.36–1.4)</td>
<td>12 (26.1)</td>
<td>0.41 (0.19–0.85)</td>
</tr>
<tr>
<td>GU</td>
<td>59</td>
<td>25 (42)</td>
<td>1.5 (0.83–2.8)</td>
<td>14 (24.6)</td>
<td>0.45 (0.22–0.94)</td>
</tr>
<tr>
<td>DU</td>
<td>65</td>
<td>29 (43)</td>
<td>1.6 (0.85–3.0)</td>
<td>56 (86.1)</td>
<td>24.2 (10.6–54.8)</td>
</tr>
<tr>
<td>GC</td>
<td>32</td>
<td>18 (56)</td>
<td>2.4 (1.1–5.1)</td>
<td>4 (12.5)</td>
<td>0.16 (0.05–0.47)</td>
</tr>
</tbody>
</table>

**Fig. 2.** Comparison of the growth of dupA-positive versus -negative H. pylori isolates at various pH values. Dilutions of fresh H. pylori cultures were plated on CA plates with the pH ranging from 7 to 3. The plates were inspected after 5 days of incubation. Filled bars, dupA-positive isolates \((n=12)\); hatched bars, dupA-negative isolates \((n=15)\). Only when a significant difference between dupA-positive and dupA-negative isolates at a given pH was present are \(P\) values provided.
geographical distribution of the dupA gene. Whilst a recent study in Iran (Douragh et al., 2008) reported a dupA incidence of 49 % in DU patients, in the current study an incidence of 86.1 % was observed. This is probably due to differences among the study populations: Douragh et al. (2008) focused mainly on cases in Tehran (the densely populated capital of Iran), whilst the subjects in the current study were from the more rural northern areas of Iran where there is a different incidence of H. pylori-related symptoms (Malekzadeh et al., 2009). Also, when comparing the GC patients from our study with those of others, it was noted that the mean age of the GC patients in our study was relatively low (Table 2). Whilst we have no explanation for this, others have also noted that in this area there is a relative high incidence of H. pylori, and patients may already be suffering from H. pylori-induced GC at a relative young age (Malekzadeh et al., 2009; Talebi Bezmin Abadi et al., 2009).

In spite of the differences in dupA prevalence among individual reports, most studies confirm the association between the presence of dupA and an increased risk of DU and protection against GC as originally reported (Lu et al., 2005), or observe at least the same significant trend towards such an association. Two studies, both from Brazil, however, revealed no significant relationship between clinical outcomes including DU, GC, G and GU (Gomes et al., 2008; Pacheco et al., 2008). In addition to the reported observations, this study showed that there was a significant correlation between the absence of dupA and GU (OR 0.34, 95 % CI 0.16–0.68). This result suggests a strong protective effect of dupA against GU formation, a finding that to our knowledge has not been reported before.

Given that dupA-positive patients are more prone to developing DU (Table 3) and the common dogma that there is a cascade of events leading to H. pylori-induced GC (Atherton, 2006; Kusters et al., 2006), it is safe to assume that patients infected with a dupA-positive isolate are more likely to visit a clinician with clinical signs of an infection. Thus, dupA-positive isolates are more likely to be detected and treated, and hence the long-term infection effects of an H. pylori infection (i.e. GC) are more likely to be observed with dupA-negative isolates. If so, the negative association of dupA with GC might in part result from this selection bias. More probably, the negative association of dupA with GC can be explained by the higher acid shock tolerance of dupA-positive strains, as initially reported by Lu et al. (2005). Here, we extended their findings by analysing the ability of dupA-positive and -negative strains to grow at low pH (Fig. 2). Growth not only requires survival of the initial acid shock (as tested by Lu et al., 2005) but also the ability for long-term adaptation and replication at low pH. In order to minimize the possibility that the infecting strains had lost their acid resistance due to adaptation to a potentially less acidic environment resulting from atrophy of the gastric mucosa, only strains from G patients were selected for this test. A strong association (P=0.02) between the presence of dupA and the ability to grow at low pH was observed (Fig. 2). The ability of dupA-positive strains to grow at low pH might form an explanation for the positive association of these strains with DU, because, if correct, dupA-positive strains would be more prone to colonize the more acidic locations of the stomach (i.e. the antrum) than dupA-negative strains. The observed higher acid resistance of the dupA-positive strains suggests that these strains have been adapted to a high gastric acid output. Dixon et al. (1996) postulated that the type of gastric pathology that develops following an H. pylori infection is dependent on the location of chronic gastritis, and thus on the predominant colonization site of the infecting strain. A high gastric acid output is believed to be typical for an antrum-predominant H. pylori infection and, whilst associated with an increased risk of DU formation, it lowers the risk for the genesis of GU and GC. This is in line with our finding that dupA seems to protect against GC and perhaps also against GU. The trend for a negative association with both GU and GC is an indication that the selection bias hypothesis for GC as outlined above may not play a major role in the observed negative association between dupA and GC. Further analysis of the role of the H. pylori dupA gene in bacterial physiology and pathogenesis may lead to new options to prevent H. pylori-induced DU and GC.

**REFERENCES**


