Relevance of *Helicobacter pylori* dupA and OipA genotypes and development of gastric disease.

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**Abstract**

*Helicobacter pylori* infection is now accepted as the major cause of severe gastritis-associated diseases, including peptic ulcer and gastric cancer. In the current study, we decided to determine the *dupA* and OipA genotypes in *H. pylori* strains isolated from patients with gastrointestinal disorders and to evaluate whether these genes are associated with the severity of the gastroduodenal diseases. We obtained 100 *H. pylori* positive specimens from 296 patients by endoscopic biopsy. The prevalence of genotype was assessed by PCR and related to clinical histopathological parameters. The *dupA* and OipA positive strains had a prevalence of 39% and 57% respectively. In our cohort, there was no significant relationship between *dupA* genotype and peptic ulcer disease (P=0.98). However, there was a relationship between *dupA* strains and gastric cancer (P=0.019). We were unable to identify association between OipA genotype and clinical outcome (P=0.145). The relation between *dupA*, and OipA, positive genotype as a single genotype and the development of cases to gastric cancer was statistically significant (P<0.001). According to the results, the presence of *dupA* gene can be considered as a marker for the gastric cancer; however, the OipA gene cannot be regarded for prediction of peptic ulcer and gastric cancer.

**Keywords:** *Helicobacter pylori*, dupA, OipA, Gastric disease.

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**Introduction**

The bacterium *Helicobacter pylori* colonizes the gastric mucosa of approximately half the world’s population [1]. This infection is closely related to chronic gastritis, peptic ulcer disease; mucosa associated lymphoid tissue lymphoma and gastric cancer [2]. Since *H. pylori* is the only bacterium known to be a common cause of cancer in humans, the relationship between *H. pylori* and gastric cancer is of particular interest.

Several virulence-associated genes of *H. pylori* such as *cagA*, vacA and babA2 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 gastro duodenal pathogenesis [3]. However, none of the mentioned virulence markers have demonstrated discriminating roles in the development of peptic ulcer disease versus gastric cancer (GC).

Most recently, Lu et al. described a novel virulence factor, a virbB4 homologue, called duodenal ulcer (DU) promoting gene (*dupA*) [4]. It is located in the “plasticity region” of *H. pylori* genome and is composed of two genes, *jhp0917* and *jhp0918*, which form one continuous open reading frame by the insertion of a base T or C after the position 1385 in the *jhp0917* 3’ region [5,6]. Intriguingly, the presence of *jhp0917-jhp0918 (dupA) gene* was as a risk marker for development of duodenal ulcer disease and for protection against mucosal atrophy and gastric adenocarcinoma [4]. The function of *dupA* gene is not fully understood. It is possible that this gene acts in combination with other *vir* homologues in the plasticity region to form a type IV secretion system similar to the cagPAI [7]. Besides, it is associated with increased interleukin-8 production from the antral gastric mucosa in *vivo* as well as from the gastric epithelial cells in *vitro*. Some new studies have reported that the presence of *dupA* gene is not a specific marker for duodenal ulcer disease, and even in some of them, this gene is associated with development of gastric cancer [8]. However, the role of *dupA* has been questioned since a number of studies were unable to reproduce their observations in other populations.

The *H. pylori* outer inflammatory protein, OipA, which is an important virulence factor and one of the six genes encoding outer-membrane proteins, is associated with clinically important presentation of peptic ulcer such as enhanced interleukin-8 secretion and increased inflammation [9]. Expression of *OipA* is regulated by the slipped-strand repair mechanism based on the number of CT dinucleotide repeats in the 5′ region of the *OipA* gene (switch on=functional and switch off=non-functional) such that gene switch status may affect bacterial characteristics such as virulence. The isolates containing the cag pathogenicity island (*CagA* is a marker), too, typically have *OipA* with functional status “on” [10]. Furthermore, only a functional *OipA* was significantly
associated with high \textit{H. pylori} density, severe neutrophil infiltration, and high mucosal IL-8 levels.

Considering the high prevalence of \textit{H. pylori} infection in world population, there are several reports about common virulence markers such as \textit{vacA}, \textit{cagA} and \textit{iceA}; however, there are very limited documents about \textit{dupA} and \textit{OipA}. The aims of the present study are thus to undertake the prevalence of \textit{dupA} and \textit{OipA} genes in \textit{H. pylori} strains in our region and to clarify the presence of these genes as a promoting marker for Duodenal Ulcer (DU) or a protective marker against Gastric Cancer (GC), as well as to compare the results obtained between the two stages of the disease, trying to understand if the \textit{dupA} and \textit{OipA} genes could contribute to the development of one or the other.

**Materials and Methods**

This research was approved by the Medical Research Ethics Committee of Azad University of Medical Science on 25 Aug 2015 (No: 5/121742) and all the patients were in writing consent for this research. This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the Institution’s Human Research Committee.

**Patients**

One hundred clinical isolates of \textit{H. pylori} were obtained from gastric biopsies of patients with gastritis, peptic ulcer and gastric cancer, between February 2015 to November 2016 in Reza and Paediatric Hospital in Tabriz. At least, 1 week before endoscopy, the patients had not received antimicrobial agents.

**Rapid urease test and cultivation**

A piece of each specimen was examined by Rapid Urease Test (RUT) for detection of \textit{H. pylori}. Rapid urease test was performed with a Gastro urease kit (CLO test® Rapid Urease Test) according to manufacturer’s instruction. All positive samples in RUT were homogenized and cultured onto Brucella agar supplanted with 5% sheep blood and antibiotics (Vancomycin, Amphotericin B and Trimethoprim). Culture plates were incubated at microaerophilic condition, 37°C and high humidity for 5-7 d. Organisms were identified as \textit{H. pylori} based on colony morphology, gram staining and positive oxidase, catalase and urease tests.

**Extraction of Genomic DNA and \textit{H. pylori**}

**Detection:** Genomic DNA of total \textit{H. pylori} isolates was extracted using the QIAmp DNA mini kit (QIAGEN, Hilden, Germany) according to manufacturer’s protocol and stored at -20°C until it is used. In this study, PCR was used to detect the \textit{H. pylori} specific ureC (glmM) gene for confirmation of \textit{H. pylori} isolates.

**Detection of \textit{jhp0917}, \textit{jhp0918} and \textit{OipA} genes**

In this study, PCR was employed to establish presence or absence of \textit{jhp0917}, \textit{jhp0918} and \textit{OipA} genes. DNA from the reference strain J99 (GenBank accession number AE001439) and SS1 were used as positive controls. PCR reactions were performed in a volume of 50 µL containing 10 mmol/L Tris-HCl, 1.5 mmol/L MgCl₂, 0.2 mmol/L of each deoxynucleotide, and 25 pmol of each primer and 2.5 units of Taq polymerase (Geneone, Germany).

The thermal cycler program used consisted of the following steps: initial denaturation at 94°C for 3 min followed by 35 cycles of 30 s at 94°C (denaturation), 30 s at 58°C for glmM, 30 s at 53°C for \textit{jhp0917}, 30 s at 55°C for \textit{jhp0918}; 30 s at 53°C for \textit{OipA} all annealing steps, followed by 30 s at 72°C (extension step) and a final extension step was 3 min at 72°C. The amplified DNA fragments were separated via electrophoresis on 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. The primer information from each amplicon is summarized in Table 1.

**Statistics analysis**

Data were analyzed by SPSS version 16. The Fisher’s exact test or the Chi-square test was used for analysis of categorical data. A p-value of <0.05 was considered statistically significant.

### Table 1. List of primers used to detect \textit{H. pylori}, \textit{jhp0917}, \textit{jhp0918} and \textit{OipA}.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Primer sequence (5'-3')</th>
<th>Amplicon (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{jhp0917}</td>
<td>\textit{jhp0917-F}</td>
<td>TGTTTTCTACTGACAGAGGC</td>
<td>307</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>\textit{jhp0917-R}</td>
<td>AACACGTGACAGGACATCTCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{jhp0918}</td>
<td>\textit{jhp0918-F}</td>
<td>CCTATATCGCTAACGCGCGT</td>
<td>276</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>\textit{jhp0918-R}</td>
<td>AAGCTGAAAGGTTTGTAAACG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{OipA}</td>
<td>\textit{OipA-F}</td>
<td>GTTTTTGATGCAATGGGATTT</td>
<td>401</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>\textit{OipA-R}</td>
<td>GTGCCATCCTCTATGGCCTT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results

**Description of the patient cohort and isolation of study samples**

In this study, of the 100 patients infected with *H. pylori*, 76 patients were Non-Ulcer Dyspepsia (NUD), 18 patients were Peptic Ulcer Disease (PUD) and 6 patients were Gastric Cancer (GC).

Of the 100 patients, 46% were females and 54% were males (gender ratio F/M: 0.85). The mean age of the patients was 34 ± 19 y. Demographic information of study population is summarized in Table 2. There was no significant difference between the mean age and sex of patients with and without ulcers and cancer.

**Table 2. Demographic information of study population.**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number (Total=100)</th>
<th>%</th>
<th>Sex ratio F/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>54</td>
<td>54</td>
<td>0.85</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Age 15-34</td>
<td>34</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Age 34-53</td>
<td>66</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

**Distribution of dupA and OipA**

Thirty nine of the 100 tested strains (39%) were positive for both the *jhp0917* and *jhp0918* genes and thus considered *dupA*-positive; also the *OipA* genotype was detected in 56% isolates.

The *jhp0917* and *jhp0918* alleles were detected in 50 and 76 of the isolates, respectively. Thirty nine strains were positive for both genes, whereas 61 were negative. A *jhp0917*-negative/*jhp0918*-positive genotype was not detected.

**Association between dupA and OipA and clinical outcomes**

In our cohort, the distribution of *dupA* and clinical outcome was analyzed statistically and it was observed that the frequency of *dupA*-positive isolates in Non-Ulcer Dyspepsia (NUD), Peptic Ulcer Disease (PUD) and Gastric Cancer (GC) patients was 31 (40.79%) and 8 (44.44%) and 0 (0.00%), respectively. There was no significant relationship between *dupA* genotype and duodenal ulcer disease (P=0.98). However, there was a relationship between *dupA*-negative strains and gastric cancer, this apparent negative association was statistically significant (P=0.019). Also, the frequency of *OipA* -positive isolates obtained from patients with non-ulcer dyspepsia, peptic ulcer dyspepsia or gastric cancer patients was 47 (61.84%), 7 (38.88%) and 3 (50%), respectively, but this apparent positive association with more severe disease did not reach statistical significance (P=0.145) (Table 3).

**Table 3. Relationship between clinical outcome and status of dupA and OipA.**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number (%) of isolates</th>
<th>Total (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUD (n=76)</td>
<td>PUD (n=18)</td>
</tr>
<tr>
<td>dupA</td>
<td>31 (40.79%)</td>
<td>8 (44.44%)</td>
</tr>
<tr>
<td>OipA</td>
<td>47 (61.84%)</td>
<td>7 (38.88%)</td>
</tr>
</tbody>
</table>

**Combination of dupA, and OipA genotypes**

We examined different combinations based on analysis of *dupA* and *OipA* (positive and negative genotype) in patients with a single genotype (Table 4). We were able to identify an association between these genotypes and clinical outcome. The relation between *dupA* and *OipA* positive genotype as a single genotype and the development of cases to gastric cancer was statistically significant (P<0.001).

**Table 4. Combination of dupA and OipA genotypes and clinical outcome.**

<table>
<thead>
<tr>
<th>dupA</th>
<th>OipA</th>
<th>GC</th>
<th>NUD</th>
<th>PUD</th>
<th>Total</th>
<th>GC Pν</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>0</td>
<td>24</td>
<td>9</td>
<td>33</td>
<td>0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>0</td>
<td>13</td>
<td>5</td>
<td>18</td>
<td>0.114</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>3</td>
<td>22</td>
<td>2</td>
<td>27</td>
<td>0.175</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>4</td>
<td>16</td>
<td>2</td>
<td>22</td>
<td>0.194</td>
</tr>
</tbody>
</table>

Positive=Presence of a gene; Negative=Absence of a gene; GC Pν: P-value of GC; NUD: Non-Ulcer Dyspepsia; PUD: Peptic Ulcer Disease; GC: Gastric Cancer.

**Discussion**

Originally, infections with *dupA*-positive strains increased the risk for duodenal ulcer but were protective against gastric atrophy, intestinal metaplasia and gastric cancer in the Japanese, Korean and Columbian subjects [11]. Conversely, *dupA* genotyping by Argent et al. of four other populations, from Belgium, South Africa, China and the United States, showed no association of this gene with duodenal ulcer, but suggested an association with gastric cancer. Gomes et al. also negated any association between *dupA* and either of the two disparate clinical outcomes in Brazilian patients [12].

Our study showed no significant relationship between *dupA* genotype and duodenal ulcer disease. Moreover, the results demonstrated that there was a statistically significant association between the lack of this gene in strains and development of gastric cancer. These findings are supported by
previous studies (Nguyen et al.) [13,14]. Also the results obtained in the present study support the hypothesis of this gene being a protective factor against gastric cancer. These discordant results explained by the effect of the presence of the dupA gene on transcription factors showed that activation of transcription factors for tumor suppressor gene p5 decreased and activation of the YY1 increased using the dupA-deleted mutants [4]. In the dupA-deleted mutants, it is reported that there is a relation between cancer development and YY1 inhibiting activation of the p53 via binding competitively to the p53-binding site that contains the ACAT sequence. The activation of the Ets-1/PEA3 was also induced by the dupA gene. Ets-1/PEA3 is an oncogene and has been used as a marker of malignant potential in gastric cancer [15]. In accordance with the study by Lu et al., our study confirms the presence of jhp0917 and jhp0918, linked to each other and constituting the complete dupA gene.

OipA, a proinflammatory OMP (outer-membrane proteins), is called HopH. Initially, Yamaoka et al. discovered that it was correlated with mucosal IL-8 levels and that protein was present in 97.5% of patients with gastric or duodenal ulcer. Thereafter, Yamaoka et al. confirmed the proinflammatory role of OipA, considering that OipA isogenic mutants reduced the induction of IL-8 from gastric epithelial cell lines. The results of our study showed that OipA-positive isolates compared to OipA-negative isolates were more frequently isolated from non-ulcer dyspepsia patients while in peptic ulcer disease patients were the opposite. This finding was not statistically significant (p>0.05). We figured out 57% of isolated strains containing this gene which is in accordance with the other previous study showed the OipA prevalence varies from 33% to 71% in Iranian population based on different ethnic background [12]. In our cohort, the OipA allele was identified in 50% gastric cancer patients. This percentage is more than the results obtained (33.3%) by Douraghi et al. This finding was not statistically significant (p>0.145). These results are in agreement with previous study [12,16].

In the present study, there was a statistically significant relationship between the presence of dupA and OipA genes (dupA+/OipA+) in isolated strains. Our findings support previous studies Sallas et al. [17]. However, several studies have reported different results. Souod et al. reported a statistically significant association with the dupA+/OipA+ genotype [18,19]. Generally, the clinical outcomes resulting from the OipA genotype are still unclear.

Conclusion

According to the present study, there was a converse relationship between the dupA genotype and gastric cancer and there was a relationship between dupA+ and OipA+ genotype. However, it is not easy to conclude that dupA gene is considered as a protective factor in some area and associated with more severe diseases in other countries. Finally, further studies are required to determine the functions of dupA and OipA and their relationship with disease outcome.

Acknowledgement

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Conflict of Interest

With the submission of this manuscript I would like to undertake that the authors whose names are listed above certify that they report no conflicts of interest.

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