MINIREVIEWS

Novel virulence factor dupA of Helicobacter pylori as an important risk determinant for disease manifestation: An overview

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Abstract

Helicobacter pylori (H. pylori) is a microaerophilic, Gram-negative, human gastric pathogen found usually in the mucous lining of stomach. It infects more than 50% of the world’s population and leads to gastroduodenal diseases. The outcome of disease depends on mainly three factors: Host genetics, environment and bacterial factors. Among these, bacterial virulence factors such as cagA, vacA are well known for their role in disease outcomes. However, based on the global epidemiological results, none of the bacterial virulence (gene) factors was found to be associated with particular diseases like duodenal ulcer (DU) in all populations. Hence, substantial importance has been provided for research in strain-specific genes outside the cag pathogenicity island, especially genes located within the plasticity regions. dupA found within the plasticity regions was first demonstrated in 2005 and was proposed for duodenal ulcer development and reduced risk of gastric cancer in certain geographical regions. Due to the discrepancies in report from different parts of the world in DU development related to H. pylori virulence factor, dupA became an interesting area of research in elucidating the role of this gene in the disease progression. In this review, we shed light on the detailed information available on the polymorphisms in dupA and their clinical relevance. We have critically appraised several pertinent studies on dupA and discussed their merits and shortcomings. This review also highlights dupA gene as an important biomarker for DU in certain populations.

Key words: Helicobacter pylori; Plasticity region; Duodenal ulcer; Gastric cancer; dupA gene

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Helicobacter pylori (H. pylori) is a curved rod-shaped, Gram-negative, microaerophilic bacterium found usually in the mucous lining of the stomach. H. pylori infects more than 50% of the world’s population and 70%-80% of the Indian population[1,2]. H. pylori is acquired during childhood and remains in the stomach throughout the life if not treated effectively[3]. Infection with H. pylori causes duodenal ulcer (DU), gastric ulcer (GU), gastric cancer (GC) and gastric mucosa-associated lymphoid tissue lymphoma[4,5]. Considering its clinical importance, the World Health Organization has declared H. pylori as a class I carcinogen and enlisted GC as the fifth most common cancer and the third most common cause of cancer-related death[6,7]. Infection of H. pylori is comparatively more prevalent in developing countries than Western countries due to socioeconomic and sanitary conditions[8]. The mode of transmission of H. pylori is not clearly understood. However, most of the studies suggest that H. pylori is transmitted from person to person via oral-oral and fecal-oral route and also through contaminated food and water[9-11].

The enigma of H. pylori research is that the majority of infected patients remain asymptomatic, whereas around 15%-20% of infected individuals develop symptoms of peptic ulcer (duodenal or gastric) as a long-term consequence of infection. It is not clear what governs the manifestation of H. pylori infection in some people. This apparent puzzle prompted the proposal that the sheer presence of H. pylori in the stomach is inadequate to develop acute gastric disease and that other conditions are required. However, it is assumed that the responsible factors in H. pylori-associated diseases are due to its virulence factors, host genetics, immunity and environmental influences. Host factors like polymorphism in the genes (pro-inflammatory cytokine genes) increase the risk of the specific clinical outcome[12]. None of the H. pylori virulence factors such as cagA, vacA, the blood group antigen babA and oipA have been linked with specific diseases like DU or GC uniformly in all populations[13-15].

Analysis of the full genome sequences of different H. pylori strains reported specific genetic locus whose G+C content was lower than that of the rest of the H. pylori genome. This indicates the possibility of horizontal deoxyribonucleic acid (DNA) transfer from other species. H. pylori carry an open pan-genome, which maintain a discrete group of strain-specific genes. These strain-specific genes mostly reside in genomic regions that had earlier been coined as plasticity zones. This term was previously used to describe a specific genetic segment with high variation between the H. pylori genome sequences[16,17]. The complete genome sequence of H. pylori reveals that part of the plasticity zone is normally arranged as genomic islands that may be integrated in the genetic loci. About 50% of the strain-specific genes of H. pylori are located in the plasticity region, Here, our focus is on the gene dupA, which is located within the plasticity region. This gene was first reported in 2005 as an important biomarker for DU[18]. During subsequent years, several investigations were carried out on dupA, and this has become an interesting area of research, as shown in Table 1.

**Core tip:** A novel virulence factor dupA located in the plasticity region of Helicobacter pylori genome was found to be associated with duodenal ulcer development in certain geographical regions. Well-known bacterial virulence factors in this pathogen like cagA, vacA are not found to be associated with duodenal ulcer in Asia. Studies focused on the epidemiology and clinical relevance of dupA around the world exhibit significant variations. Hence, we focused on the variations in dupA and the plausible role of such variation in disease etiology with the goal of bringing attention to this topic to the scientific community and eventually opening up avenues for further research.

**INTRODUCTION**
Table 1 Important finding on dupA of Helicobacter pylori in chronological order

| Year | Observation and conclusion | Sample location | Sample size | Techniques used in the study | Proposed name | Ref. |
|------|---------------------------|-----------------|------------|------------------------------|---------------|-----|
| 2005 | dupA was novel marker associated with increased risk for DU and reduced risk for gastric cancer in East Asia and South America | Japan, Korea, Colombia | 500 | PCR, southern blot | dupA | Lu et al[1][3] |
| 2007 | Significant association of dupA gene with DU | North India | 166 | PCR, Dot-blot hybridization, partial sequencing | dupA | Arachchi et al[4][5] |
| 2007 | Presence of dupA significantly associated with GC than DU | Belgium, South Africa, China, North America | 258 | PCR | dupA | Argent et al[6][7] |
| 2008 | dupA gene was not associated with any diseases outcome | Iran | 157 | PCR, partial sequencing | dupA | Douraghi et al[8][9] |
| 2008 | dupA was not associated with H. pylori associated diseases in children and adults | Brazil | 482 | PCR, partial sequencing | dupA | Gomes et al[10][11] |
| 2008 | dupA was associated with peptic ulcer in Iraqi population but not with Iranian population | Iraq and Iran | 108 | PCR | dupA | Hussein et al[12][13] |
| 2008 | There was no association between the occurrence of dupA and DU | Brazil (Sao Paulo) | 79 | PCR | dupA | Pacheco et al[14][15] |
| 2008 | The prevalence of dupA was significantly higher in DU patients than in gastric cancer | China | 360 | PCR | dupA | Zhang et al[16][17] |
| 2009 | There was no consistent association between dupA and DU or GC development | Sweden, Australia, Malaysia (ethnic groups Indian, Malay) | 243 | PCR, partial sequencing | dupA | Schmidt et al[18][19] |
| 2010 | dupA was not associated with gastroduodenal diseases or IL-8 production | Japan | 244 | PCR, partial sequencing RT-PCR, IL-8 assay | dupA | Nguyen et al[20][21] |
| 2010 | dupA is not association with DU in patients from Turkey | Turkey | 91 | PCR | dupA | Tuncel et al[22][23] |
| 2010 | Meta-analysis of case control studies confirmed the presence of dupA gene for DU | Asian and western countries | 2466 | - | dupA | Shiota et al[24][25] |
| 2010 | Meta-analysis of previous report showed dupA gene promotes DU formation some population and GU and GC in others | Around the world | 2358 | - | dupA | Hussein et al[26][27] |
| 2010 | In Taiwanese female population, MMP-3 promoter polymorphism is correlated with DU rather than dupA gene | Taiwan female | 181 | PCR | dupA | Yeh et al[28][29] |
| 2010 | dupA and gastric cancer is negatively associated with GC in Japanese population | Japan | 136 | PCR | dupA | Imagawa et al[30][31] |
| 2010 | Proposed two alleles of dupA [dupA1 (intact), dupA2 (truncated)]. dupA1 (not dupA2) increased IL-12p40 and IL-12p70 production from CD14+ mononuclear cell | United Kingdom, United States, Belgium, South Africa, China | 34 | PCR, full Sequencing, Cytokine ELISA, real tome PCR, flow cytometry | dupA1 | Hussein et al[32][33] |
| 2011 | Presence of mutation on dupA at 1311 and 1426 leads to stop codon called truncated dupA | Brazil | 252 | PCR, full sequencing | dupA | Queiroz et al[34][35] |
| 2011 | Intact dupA (dupA1) without stop codon was associated with decreases rate of gastric carcinoma in Brazilian population | Brazil | 6 | Full sequencing | dupA1 | Queiroz et al[36][37] |
| 2012 | Found a positive association between presence of dupA and DU [OR 24.2; 95% CI: 10.6-54.8] and inverse association | Iran | 216 | PCR | dupA | Abadi et al[38][39] |
between presence of dupA and GU [OR 0.34; 95% CI: 0.16-0.68] and GC [OR 0.16; 95% CI: 0.05-0.47]

| Year   | Study Details                                                                 | Country | Sample Size | Test Method(s) | Gene   | Authors     |
|--------|-------------------------------------------------------------------------------|---------|-------------|----------------|--------|-------------|
| 2012   | Prevalence of dupA was higher in the eradication failure group than in the success group (36.3% vs 21.9%) | Japan   | 142         | PCR, Drug sensitivity test | dupA   | Shiotia et al. [60] |
| 2012   | The logistic analysis report in Brazilian population showed the presence of intact dupA independently associated with duodenal ulcer (OR = 5.06; 95% CI: 1.22-20.96, P = 0.02) | Brazil  | 75          | Sequencing      | Intact dupA | Moura et al. [61] |
| 2012   | dupA gene was found to be significantly associated with DU than in NUD in south east Indian population | India   | 140         | PCR, partial sequencing, real time PCR, | dupA   | Alam et al. [62] |
| 2012   | Found a significant association between dupA1 and DU (P < 0.01) along with a significant higher level of gastric mucosa IL-8 in dupA1 than in dupA2 or dupA negative Iraqi strain | Iran    | 68          | PCR, full sequencing, IL-8 ELISA | dupA1  | Hussein et al. [63] |
| 2012   | classified dupA into two types (long types and short types) depend on the presence of 615 bp at the N-terminal of dupA. Found high prevalence of intact long type dupA (24.5%) than short type dupA (6.6%) and significantly associated with GU and GC than gastritis (P = 0.001 and P = 0.019) in Japanese population | Japan   | 319         | PCR, full sequencing      | Long type and short type | Takahashi et al. [35] |
| 2012   | Complete dupA cluster (dupA with six vrb homologues) was associated with DU rather than dupA gene only in United States population | United States | 245       | PCR and cytokine ELISA | dupA cluster | Jung et al. [70] |
| 2013   | Prevalence of long type dupA (2499 bp) was significantly higher in GU, GC and DU (40.3%) than from gastritis (20.4%) (P = 0.02) in China | China   | 116         | PCR, Full sequencing | dupA cluster | Wang et al. [69] |
| 2013   | PUD was significantly associated with cagA (P ≤ 0.01; OR 0.4; 95% CI: 0.18-0.85) rather than dupA | Iraq    | 154         | PCR            | dupA   | Salih et al. [64] |
| 2014   | dupA was found to play an important role in the development of DU, BGU and dysplasia in South Korean population | South Korea | 401       | PCR            | dupA   | Kim et al. [19] |
| 2014   | dupA was associated with cagA and vacAs1m1 genotypes | Brazil  | 205         | PCR            | dupA   | Pereira et al. [89] |
| 2014   | The prevalence of dupA and cagA were more in MTZ, CLR and AML resistance strain as compared to other virulence factor in Pakistan | Pakistan | 46          | PCR            | dupA   | Rasheed et al. [61] |
| 2015   | cagA, complete dupA cluster and smoking were significantly associated with increased level of IL-8 production from gastric mucosa of Iraqi population | Iraq    | 81          | PCR, IL-8 ELISA | dupA   | Hussein et al. [60] |
| 2015   | Prevalence of dupA1 was significantly higher in DU than NUD (P = 0.02) in Indian strains and dupA1 positive strains were similar to East Asian strains and distinct from western strains. | India   | 170         | PCR, sequencing, IL-8 ELISA | dupA1  | Alam et al. [80] |
| 2015   | Significant association of complete dupA cluster with IL-8 production (P < 0.01) in north East of China | China   | 262         | PCR, western blotting, IL-8 ELISA | dupA cluster | Wang et al. [61] |
| 2015   | DupA protein have ATPase activity and play a role in apoptosis of gastric cancerous cells through mitochondrial pathway but neither adhere nor translocate to host cell | China   | 1 (WH21)    | PCR, western blotting, ATPase, Adhesion, translocation and cytotoxic assay | Long type dupA | Wang et al. [81] |
| 2015   | dupA1 have a significant association with A2147G clarithromycin resistance strain but not with IL-8 production from gastric mucosa | Iraq    | 74          | PCR, IL-8 ELISA, antibiotic susceptibility test | dupA1  | Hussein et al. [60] |
| 2015   | Significant association between the presence of dupA and DU diseases (P = 0.03 OR 3.14, 95% CI: 1.47-7.8). | Iran    | 128         | PCR            | dupA   | Haddadi et al. [63] |
| 2015   | There was no significant relationship between dupA status and duodenal ulcer disease (P = 0.25) but, there was a converse relationship between dupA negative strains and gastric cancer disease (P = 0.02) | Iran    | 123         | PCR            | dupA   | Souod et al. [65] |
| 2015   | There was no association of dupA gene with the ethnic group (Indian, Chinese, Malay) of Malaysia | Malaysia | 105        | PCR            | dupA   | Osman et al. [62] |

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**METHODOLOGY**

To review the importance of *dupA*, we have searched the “NCBI-PubMed” using the keywords: "*dupA*", "*H. pylori*" and a total of 80 articles were found, of which 76 were published in English till January 2020. Out of 76, 13 are published as review articles and two as meta-analysis of previous data. The remaining 61 documented as research articles. The research on *dupA* has spanned 15 years with contradictory findings. In this review, we summarize the result of relevant studies and discuss the pathogenesis of *dupA* since its early stage to recent advancements. Finally, this review highlights the significance of *dupA* gene of *H. pylori* as a virulence factor (virulence marker) and its role in pathogenesis including the progression of DU.

**DISCREPANCIES OF DUPA WITH CLINICAL OUTCOMES**

Studies conducted with *H. pylori* strains from East Asia and South America identified a novel *H. pylori* virulence factor encoded in the *dupA* that was associated with increased risk of DU and decreased risk of GC. However, this perception seems to be region specific. This *dupA* was homologous to *virB4* gene, located in the plasticity zone of *H. pylori*. *dupA* contained two open reading frames (ORFs), *jhp0917* and *jhp0918*, with an overlap of twelve bases and an insertion of either base thymine (T) or cytosine (C) after the position 1385 of the *jhp0917* that leads to continuous gene of 1839 bp. Since 2005, several studies have been conducted from different geographical areas to check the

| Year | Study Design | Country | Sample Size | Methodology | Results |
|------|--------------|---------|-------------|-------------|---------|
| 2017 | Significant association of *dupA* gene with non-severe clinical outcome (*P* = 0.0032, OR 0.25, 95%CI: 0.09-0.65) and play a role in protecting against gastric cancer in Chile | Chile | 132 | PCR | *dupA* association with non-severe clinical outcome |
| 2017 | A complete tfs plasticity zone cluster including *dupA* is a virulence factor that may be important for the colonization of *H. pylori* and to the development of severe outcomes of the infection with *cagA*-positive strains | Portugal | 18 | PCR, whole genome sequencing, cytokine assay | *dupA* association with severe clinical outcomes |
| 2019 | *dupA* was significantly associated with decreased risk of duodenal ulcer (*P* = 0.024) | Costa Rica | 151 | PCR | *dupA* association with decreased risk of duodenal ulcer |
| 2019 | Significant relationship was observed between the occurrence of DU and the presence of the 112 bp segment (*P* = 0.002; OR 6.98; 95%CI: 1.94-25.00) | Iran | 143 | PCR | *dupA* association with DU occurrence |
| 2019 | The prevalence of *dupA* was 53.4% in South African population, but it was not associated with duodenal ulcer | South Africa | 234 | PCR | *dupA* prevalence in South Africa |
| 2019 | The prevalence of *dupA* was higher (30.4%) in peptic ulcer (mild diseases) than gastric cancer (severe diseases) 18.2% | Northern Spain | 102 | PCR | *dupA* prevalence in peptic ulcer and gastric cancer |
| 2019 | *dupA* was present in 10/41 (24.4%) of population, and it was not associated with severe gastritis | Switzerland | 41 | Whole genome sequence | *dupA* association with severe gastritis |
| 2019 | Significant association was found between metronidazole resistance and *dupA* genotypes (*P* = 0.0001) | Iran | 68 | PCR | *dupA* association with metronidazole resistance |

CI: Confidence interval; DU: Duodenal ulcer; GC: Gastric cancer; GU: Gastric ulcer; IL-8: Interleukin-8; MMP-3: Matrix metalloproteinase -3; NUD: Non-ulcer dyspepsia; OR: Odds ratio; PCR: Polymerase chain reaction.
association of dupA with disease outcome considering the dupA has two ORFs (jhp0917/jhp0918) with the insertion of one base (T/C) at position 1385 of jhp0917. Studies performed in North India during 2007 support the finding of Lu et al\textsuperscript{[28]}. However, studies conducted in different countries (Belgium, South Africa, China, North America and Brazil) found that dupA is not associated with DU in the respective population\textsuperscript{[29-31]}.

Investigations made in Sao Paulo, Brazil showed that dupA was detected in H. pylori strains of 41.5% patients, which was less from a previous study made by Gomes et al\textsuperscript{[32]} (2008), in which dupA was present in 89.5% patients\textsuperscript{[33]}. This study showed an association of dupA with cagA and vacA s1m1 genotypes but without any link to disease outcome. The difference in the results of these two studies from Brazil could be explained by variation in geographic regions, a re-arrangement in the plasticity zone distribution in H. pylori and various methods used for the analysis.

The distribution of dupA in H. pylori was similar in Iraqi and Iranian population, but there was an association between peptic ulcer and dupA only in the Iraqi population\textsuperscript{[34,35]}. An independent study by Pouraghi et al\textsuperscript{[36]} (2008) reported a non-significant higher distribution of dupA in DU than non-cardia GC patients in the Iranian population\textsuperscript{[36]}. Another study by Talebi Bezmin Abadi et al\textsuperscript{[37]} (2012) found a positive association between the presence of dupA and DU along with an inverse association between dupA and GU in Iranian population\textsuperscript{[37]}. The discrepancy in the finding of Pouraghi et al\textsuperscript{[36]} (2008) and Talebi Bezmin Abadi et al\textsuperscript{[37]} (2012) may be due to differences in the study populations. Pouraghi et al\textsuperscript{[36]} (2008) focused mainly in Tehran (the densely populated capital of Iran), whereas Talebi Bezmin Abadi et al\textsuperscript{[37]} (2012) collected samples from the extremely rural northern areas of Iran. Recently, Fatahi et al\textsuperscript{[38]} (2019) tested a highly conserved region of dupA in the Iranian population\textsuperscript{[38]}. Another group from Iran studied the relationship between antibiotic resistance pattern and virulence genotype among 68 H. pylori strains and found that metronidazole resistance was significantly associated with the strains harboring cagA, saba and dupA\textsuperscript{[39]}. One study from Kurdistan region of Northern Iraq reported that cagA gene was significantly associated with peptic ulcer disease rather than dupA, which contradict the result of Hussein et al\textsuperscript{[40]} (2008). This might be due to the differences in sample size and also in the geographical location of Iraq\textsuperscript{[40]}. In the Shiraz area of Iran, a significant relationship was found between strains with dupA, CagA motif (ABC types) and DU disease, which supports the previous finding in this region\textsuperscript{[41]}. Another study from Western Iran indicated that presence of dupA gene could be considered as a marker for the onset of severe gastroduodenal diseases\textsuperscript{[41]}. However, there was no association of dupA with DU in the results obtained from the Turkish population\textsuperscript{[42]}.

In China and South Korea, presence of dupA in clinical H. pylori isolates is significantly associated with DU and peptic ulcer (DU, benign GU, dysplasia), respectively\textsuperscript{[43-45]}. In the Taiwanese female population, the host factor matrix metalloproteinase-3/tissue inhibitor matrix metalloproteinase-1 genotypes rather than dupA was found to increase the risk of DU in H. pylori infected cases\textsuperscript{[46]}. A case control study conducted in Sweden, Australia, Malaysia and Singapore showed that there was significant variation in the prevalence of dupA in different locations and among different ethnic groups (Chinese, Indian and Malaya) within a country\textsuperscript{[47]}. Another study in ethnic groups (Indian, Chinese and Malaya) of Malaysia reported that the prevalence of dupA was 22.9% in patients, which was in line with previous data (21.3%) conducted in Malaysia by Schmidt et al\textsuperscript{[28]} (2009). In the later study, there was no association between dupA and clinical outcome\textsuperscript{[28]}.

Two independent systematic review and meta-analyses showed that dupA is more associated with DU in some Asian populations than in Western populations\textsuperscript{[48-49]}. Between 2005 and 2009, almost all the studies used polymerase chain reaction (PCR) of two ORFs jhp0917, jhp0918 and sequencing to identify the dupA. Functional analysis of dupA in the Japanese population showed no association with DU but another study from different parts of Japan showed that dupA is inversely related to GC\textsuperscript{[50,51]}. Results from a study using different molecular methods [PCR, dot-blot hybridization, sequencing and reverse transcription PCR (RT-PCR)] indicated that dupA gene was prevalent more than six times in DU than in non-ulcer dyspepsia patients, indicating its significant association in India\textsuperscript{[52]}. This result also corroborated the finding of Arachchi et al\textsuperscript{[33]} (2007) from North India. The RT-PCR analysis of South and East Indian population revealed that all PCR positive strains were not able to produce dupA transcripts, which was inconsistent with the finding of Nguyen et al\textsuperscript{[31]} (2009) where all the dupA positive strains showed the expression of the gene\textsuperscript{[31]}. Further, the real-time PCR analysis revealed that the expression level of the dupA
transcripts varied from strain to strain in this study. Studies conducted in Chile supported a significant association of \textit{dupA} gene with non-severe clinical outcome like DU and also played a role in protecting severe diseases like GC\textsuperscript{39}. The Costa Rica study with 151 dyspeptic patients showed that presence of \textit{dupA} was significantly associated with decreased risk of DU\textsuperscript{39}.

Some of the above-mentioned studies verified the finding of Lu \textit{et al}\textsuperscript{55,56} (2005), but others could not find an association between \textit{dupA} and disease outcome in their study populations. The differences in the results could be explained due to variation in the distribution of plasticity region genes and differences in the study population and techniques chosen for detection of \textit{dupA} gene. Several studies on \textit{dupA} were restricted to PCR of \textit{jhp0917} and \textit{jhp0918} along with sequencing of only the 3' region of \textit{jhp0917} to find the insertion of T/C at 1385 position of \textit{jhp0917}. Numerous studies have shown the presence of frame shift mutation within \textit{dupA} gene leading to the formation of truncated non-functional DupA. These findings provide evidence that only PCR based analysis of \textit{dupA} may yield erroneous interpretation. Studies conducted by Queiroz \textit{et al}\textsuperscript{57} (2011) and Moura \textit{et al}\textsuperscript{58} (2012) from Brazil showed the presence of a mutation in \textit{dupA} that results in a stop codon, making the gene truncated or non-functional. In addition, these studies revealed the importance of sequence analysis of \textit{dupA} amplicons\textsuperscript{59,60}. Truncated \textit{dupA} might not be involved in the pathogenesis of \textit{H. pylori}.

Hussein \textit{et al}\textsuperscript{61} (2010) coined the term “\textit{dupA1}”. The \textit{dupA} positive \textit{H. pylori} strains were categorized into two alleles based on the sequence; \textit{dupA1} (intact 1884 bp) and \textit{dupA2} (truncated). It was shown that the intact \textit{dupA1} positive strains induced the production of interleukin (IL)-12 subunit p40 (IL-12p40) and IL-12p70 from CD4\textsuperscript{+} mononuclear cells and IL-8 expression in the human stomach, respectively\textsuperscript{62}. Takahashi \textit{et al}\textsuperscript{63} (2012) first reported the presence of an additional 615 bp in the 5' region of ORF \textit{jhp0917} (absent in strain J99) and 45 bp in the 3' of \textit{jhp0918} (consist of 37 bp of intergenic region of \textit{jhp0918}-\textit{jhp0919} and 8 bp of 5' region of \textit{jhp0919} in J99) to make 2499 bp of \textit{dupA} in the Japanese population (Figure 1). This variation formed the basis for classification of \textit{dupA} into two types; “long and short types”. The long type of intact 2499 bp (with an additional 615 bp at 5' region of \textit{jhp0917}) has been considered as an actual virulence factor, and the absence of the additional segment should be interpreted with caution\textsuperscript{64}.

None of the \textit{H. pylori} strains from Iraq carried the complete \textit{dupA} cluster containing \textit{virB8, virB9, virB10, virB11, virD4} and \textit{virD2}, but there was a significant association between \textit{dupA1} and DU. Moreover, higher levels of gastric mucosa IL-8 production were documented in \textit{dupA1} than in \textit{dupA2} or \textit{dupA} negative strains\textsuperscript{65}. Further studies with \textit{H. pylori} infected patients showed that \textit{cagA}, complete \textit{dupA} cluster and smoking habit were associated with increased levels of IL-8 production from gastric mucosa\textsuperscript{66}. It was also shown in another study that the high IL-8 level in gastric mucosa was neither significantly associated with \textit{dupA1} positive strains nor with \textit{dupA} negative strains\textsuperscript{67}. A significant association has also been found between \textit{dupA1} and A2147G clarithromycin resistance mutation. However, the result of \textit{dupA1} and IL-8 association in the Iraqi population was not well elucidated. In Brazilian \textit{H. pylori} strains, it was found that \textit{H. pylori} strains had the 45 base at the 3' end of \textit{dupA}, similar to that of \textit{dupA1}\textsuperscript{68}.

\textit{dupA} gene of Indian \textit{H. pylori} strains has been classified into two forms based on the presence of additional 615 bp at the 5' region of \textit{dupA} followed by a stop codon. This includes \textit{dupA1} without any frameshift mutation (either long type or short type) and \textit{dupA2} with the truncated version having frameshift mutation\textsuperscript{69}. Among these, \textit{dupA1} (intact \textit{dupA}) was significantly associated with DU. Phylogenetic analysis of complete \textit{dupA} gene sequencing revealed that Indian \textit{H. pylori} strains intermingled with the East Asian strains, but differed from European strains\textsuperscript{70}. \textit{dupA} is the first known genetic element of Indian \textit{H. pylori} strains, which phylogenetically formed the same cluster with the East Asian strains. In \textit{vitro} study showed that IL-8 production was significantly associated with DU in intact \textit{dupA1} rather than truncated \textit{dupA2} or \textit{dupA} negative strains\textsuperscript{69}. In Chinese strains, the prevalence of long type \textit{dupA} (2499 bp) was significantly higher in patients with GU, GC and DU than in those with gastritis\textsuperscript{71}.

In the Japanese population, prevalence of \textit{dupA} was higher in the group where \textit{H. pylori} cannot be eradicated, indicating that \textit{dupA} may be an associated risk factor in the eradication failure\textsuperscript{72}. A study from Pakistan on the influence of \textit{dupA} in the eradication failure showed that \textit{H. pylori} strains harboring \textit{dupA} and \textit{cagA} were multidrug (metronidazole, clarithromycin and amoxicillin) resistant as compared to strains having other virulence factors. This finding was similar to the observation made in the Japanese population\textsuperscript{73}. In the northern part of Spain, \textit{dupA} was more prevalent in mild diseases (peptic ulcer) than severe diseases (GC)\textsuperscript{74}. In Switzerland and South Africa, \textit{dupA} of \textit{H. pylori} was not associated with severe gastritis or DU\textsuperscript{75,76}. 

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\textbf{References}

\textsuperscript{1} Alam \textit{et al}. Novel virulence factor \textit{dupA} of \textit{Helicobacter pylori}
DUPA CLUSTER: THIRD TYPE IV SECRETION SYSTEM (T4SS) OF H. PYLORI

The T4SS is an important bacterial transport system, and it is involved in the transport of large molecules (e.g., DNA, protein, etc.) across the bacterial cell envelope. Till now, three types of T4SS have been identified in H. pylori, of which much work has been done for the first two categories (cagPAI and ComB) and little is known about the third T4SS termed dupA cluster or tfs3 (Figure 2). The third putative type IV secretion system (tfs3) is a 16 kb gene fragment present in the plasticity zone of H. pylori, whose seven ORFs (virB4, virB8, virB9, virB10, virD4 and virD2) were homologous to virB4/D of Agrobacterium tumefaciens. The function of the tfs3 elements is not yet clear as there is no direct evidence to show its role in transformation, conjugation or mouse colonization. Some researchers divided the tfs3 into tfs3a (all six virB homologues with dupA) and tfs3b (all six virB homologues with virB4), whereas others named all six virB homologues with virB4 as tfs3 and all six virB homologues with dupA as tfs4. In order to avoid confusion, we will use the term tfs3a or dupA cluster (all six virB homologues with dupA). VirB8, VirB9 and VirB10 are expected to form the core complex that bridges cytoplasm and the outer membrane. The VirB4, VirB11, VirD4 may be localized to the inner bacterial membrane and recognize the substrate and energize translocation and assembly of T4SS.

Further, the novel putative T4SS (tfs3a) or dupA cluster has been divided into three groups: Viz, a complete dupA cluster (dupA-positive and all six virB genes-positive), an incomplete dupA cluster (dupA-positive but one/more than one virB genes negative) and dupA-negative group (dupA negative and virB gene negative/positive).

The study of dupA cluster from the United States population showed that the complete dupA cluster (dupA with six virB homologues) was associated with DU rather than dupA gene only. Another report from the northeast part of China showed a significant association of complete dupA cluster with IL-8 production (P < 0.01), but it did not show any correlation between dupA cluster and disease outcome. The studies from United States and China were conducted to check the prevalence of tfs3a or dupA cluster in their population by PCR only. However, the mere presence of the gene does not express functional protein and there is no direct evidence that shows tfs3a or dupA cluster forming a functional T4SS. The earlier studies on tfs3adid not find a direct pathogenic role of tfs3a in H. pylori, but found increased colonization fitness and up-regulation of pro-inflammatory signaling from cultured cells. A novel pathogenicity island (PAI) called tfs3-PAI was identified in China that had 17 ORFs, of which six are functionally homologues of T4SS and coordinate with the well-studied cag-PAI. The complete tfs plasticity cluster was associated with IL-8 induction. The expression of some of the genes of tfs3a/tfs4 (virB2, virB4, virB6, virB8, virB10) in H. pylori is up-regulated in low pH and enhances bacterial adhesion that support the role of tfs3a/tfs4 in the colonization and virulence. It is not known whether the virB genes of dupA cluster work independently or in a coordinated manner by interacting among themselves or complementing each other’s function. We checked the interaction of...
**Figure 2** Organization of three types of type IV secretion system in the Helicobacter pylori compared to Agrobacterium tumefaciens prototype type IV secretion system. Genes are not drawn to scale. H. pylori: Helicobacter pylori; A. tumefaciens: Agrobacterium tumefaciens; T4SS: Type IV secretion system.

dupA with six virB genes of tfs3a to identify the assembly and function of complete tfs3 using in vivo studies (yeast two-hybrid system) and found that dupA gene did not interact directly with any virB gene. It seems that dupA may interact with some intermediates or work independently (unpublished data). This interpretation supports our earlier finding that tfs3 is not significantly associated with DU in Indian population. More studies are required to know the structure, assembly and functions of the VirB proteins in H. pylori.

**THE PROSPECTIVE FUNCTIONS OF DUPA**

The bioinformatics analysis (PDB search tool, UniProt database) showed that the dupA gene is homologous to VirB4 adenosine triphosphate (ATP)ase of virB/virD of A. tumefaciens and is predicted to be involved in DNA/protein transfer. The N-terminal of long type DupA has no homologous motif. Only the middle portion (jhp0917) and C-terminal (part of jhp0918) showed homologous motifs suggesting that the N-terminal region might act as signal sequence. The amino acid sequence (210-406 AA) of jhp0917 gene protein was homologous to CagE_TrbE_VirB family, a component of type IV transporter secretion system. The first middle region of the DupA protein (430-500 AA) is homologous to FtsK/SpoIIIE family, which contains ATP binding P-loop motif. This was found in the Ftsk protein of *Escherichia coli* involved in peptidoglycan synthesis and spoIIIe of *Bacillus subtilis*, facilitating in the intercellular chromosomal DNA transfer.

The second middle region (464-503aa) is homologous to TrwB, which has an ATP binding domain, and a part of T4SS may be responsible for the DNA binding and horizontal DNA transfer. The C-terminal region (668-738aa) is homologous to TraG_C_D, which is involved in the interaction of DNA-processing (Dtr) and mating pair formation (Mpf) system, leading to DNA transfer in bacterial conjugation. Many reports have shown that the growth rate of dupA positive strains is higher in low pH as compared to dupA deleted/negative strains. This phenomenon indicates that DupA protein acts as an interactive protein and hence regulates urease secretion in *H. pylori* ([79]).

The in vitro and in vivo studies showed the role of dupA gene in the activation of transcription factors nuclear factor kappa light chain enhancer of activated B cells and activator protein-1, which leads to IL-8 production. DupA protein act as an ATPase associated efflux pump, which probably confers its virulence. Evidence suggests that DupA is involved in the pathogenesis of *H. pylori* by activating the mitochondria dependent apoptotic pathway of the host’s cell, which ultimately inhibits gastric cell growth.
Studies to understand the apoptotic effect of dupA on human gastric adenocarcinoma epithelial cell line (commonly known as AGS) by propidium iodide staining and fragmentation assay determined that dupA gene can induce apoptosis in AGS cells during an early stage of infection (unpublished data). This finding supports the results of Wang et al (2015) and finds that dupA may act as a pathogenic factor of H. pylori to cause gastroduodenal diseases. Further studies are required to confirm the pathogenic effect of dupA in an in vivo model.

The growth kinetics between wild type dupA positive strains and its isogenic mutant strain showed that exponential phase was retarded in dupA mutant cells as compared to the wild type strain. Our growth curve results, supported by the microarray data, showed that cell division gene in the mutant H. pylori was downregulated (unpublished data). It has also been suggested that motility is an essential feature in the colonization and therefore the pathogenicity of H. pylori. The decrease in motility in dupA mutant strain as compared to wild type inferred the role of dupA gene in the motility. This motility result was further confirmed by the gene expression profile of dupA mutant strain whose flagella proteins (FigE, FliD and FliG) were found to be down-regulated (unpublished data). It might be possible that dupA gene is directly or indirectly involved in negatively affecting the expression of cell division and flagellar genes of H. pylori.

As predicted from the bioinformatics analysis, our experimental data (unpublished data) have shown that natural transformation ability in dupA mutant strains has been totally inhibited in comparison to their wild type counterparts. There is a need for more studies on the heat-shock transformation efficiency, which will confirm the natural transformation assay, if any. Resistance to antimicrobials is of serious concern in H. pylori infection, as this may be the basis for eradication failure. It is important to use therapeutic regimens based on the results of antibiotic susceptibility testing. Metronidazole is considered a key drug in several therapies against H. pylori infection. The results of the metronidazole susceptibility test showed that inactivation of dupA gene transforms the H. pylori strains to resistance phenotype. This phenomenon has not been explained very well. It is possible that the dupA gene might help in the DNA/protein/drug import (unpublished data).

The dupA or dupA cluster may have an intermediate function to link cagPAI and comB system, as dupA gene shows homology with cagE of cagPAI and comB4 of comB system. So, there is a need of an in vivo study to establish the precise function of dupA. It is assumed that the dupA in combination with other six vir genes form a novel third T4SS called tfs3a or dupA cluster that might play a pathogenic role in gastroduodenal diseases.

**CONCLUSION**

H. pylori is one of the most diverse bacterial species. H. pylori demonstrate panmictic population structure. DNA-fingerprint of two strains isolated from two different persons generally displays a non-identical pattern, which suggests genetic exchange along with co-evolution of this gastric pathogen with its host. One study from the Indian population demonstrated that all the tested patients carried multiple H. pylori strains in their gastric mucosa. Analyses of certain genetic loci showed the micro diversity among the colonies from a single patient, which may be due to the recombination events during long-term carriage of the pathogen. From the results of this study, researchers predicted that many patients from the developing world acquired infections of H. pylori due to repeated exposure to this pathogen with different genetic make-up. This may enhance the probability of super infections, which favor genetic exchanges among these unrelated H. pylori strains. As a result, this led to the genesis of certain H. pylori variants with different genetic makeup than the parental strain, which in turn increases the chance of the severe infection. Therefore, the exploration of appropriate biomarker(s) that envisage the clinical condition in H. pylori-infected patient is a challenging area of research.

There is a lack of relevant biomarker(s) capable of predicting important digestive diseases in clinical settings. Even though there is ample information regarding the dupA of H. pylori, many unanswered questions still exist, especially regarding the specificity of the dupA proposed for clinical manifestation. dupA was categorized as long and short types in one study, but in another study, this gene was typed as dupA1 (intact dupA1 may be long type or short type) and dupA2 (truncated version). This gene classification should be resolved for international use to avoid any misperception. We propose the long dupA as dupA1 and short type dupA as dupA2, and the truncated
version of dupA has to be disregarded, as it has no role in pathogenesis. dupA should be screened by PCR, sequencing of the full-length gene (1884 and 2499 nt) and western blotting. Nevertheless, the discrepancy prevails between the association of dupA (short type or long type) or dupA cluster and the disease outcome. Currently, the prevalence of intact dupA in East Asian countries is lower than Western countries. DupA with another six Vir proteins (VirB8, VirB9, VirB10, VirB11, VirD4 and VirD2) predicted to form novel third type-IV secretion system (fts3a), which may be involved in transformation/conjugation or injection of DNA/new effector molecules in gastric epithelial cells. However, the function of specific Vir protein of complete dupA cluster (fts3a) is not well characterized. Recent reports and other unpublished data showed that DupA has multifunctional biological activities, and it can be considered an important biomarker for DU. It is also not clear whether the DupA works alone or in combination with other VirB proteins. There is an urgent need for reliable in vitro and animal models from diverse geographical areas of the world to elucidate further the pathogenic role of dupA and dupA cluster in gastroduodenal diseases, particularly the DU and GC.

REFERENCES

1. Buzás GM. Benign and malignant gastroduodenal diseases associated with Helicobacter pylori: a narrative review and personal remarks in 2018. Minerva Gastroenterol Dietol 2018; 64: 280-296 [PMID: 29458240 DOI: 10.23736/S1121-421X.18.02481-9]

2. Misra V, Pandey R, Misra SP, Dwivedi M. Helicobacter pylori and gastric cancer: Indian enigma. World J Gastroenterol 2014; 20: 1503-1509 [PMID: 24587625 DOI: 10.3748/wjg.v20.i6.1503]

3. Abadi AT, Kusters JG. Management of Helicobacter pylori infections. BMC Gastroenterol 2016; 16: 94 [PMID: 27520775 DOI: 10.1186/s12876-016-0496-2]

4. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacsen PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet 1991; 338: 1175-1176 [PMID: 168259 DOI: 10.1016/0140-6736(91)90235-4]

5. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991; 325: 1127-1131 [PMID: 19910290 DOI: 10.1056/NEJM199110173251603]

6. Suerbaum S, Michetti P. Helicobacter pylori infection. N Engl J Med 2002; 347: 1175-1186 [PMID: 12374879 DOI: 10.1056/NEJMra020542]

7. Pellicano R, Ribuldige DG, Fagounee S, Agostiano M, Saracco GM, Mégraud F. A 2016 panorama of Helicobacter pylori pylori infection: key messages for clinicians. Panminerva Med 2016; 58: 304-317 [PMID: 27716738]

8. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC Monogr Eval Carcinog Risks Hum 1994; 61: 1-241 [PMID: 771568]

9. McGuire S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr 2016; 7: 418-419 [PMID: 26988082 DOI: 10.3945/an.116.012211]

10. Smith S, Fowora M, Pellicano R. Infections with Helicobacter pylori and challenges encountered in Africa. World J Gastroenterol 2019; 25: 3183-3195 [PMID: 31333310 DOI: 10.3748/wjg.v25.i35.3183]

11. Mamishi S, Eshaghi H, Mahmoudi S, Bahador A, Hosseinpour Sadeghi R, Najafi M, Farahmand N, Khodadad A, Pourakbari B. Intrafamilial transmission of Helicobacter pylori: genotyping of facial samples. Br J Biomed Sci 2016; 73: 38-43 [PMID: 27182676 DOI: 10.4269/ajtmh.15-0297]

12. Bui D, Brown HE, Harris RB, Oren E. Serologic Evidence for Fecal-Oral Transmission of Helicobacter pylori. Am J Trop Med Hyg 2016; 94: 82-88 [PMID: 26598653 DOI: 10.4269/ajtmh.15-0297]

13. Ranjarbar H, Khamisaeipour F, Jonaidi-Jafari N, Rahimi E. Helicobacter pylori isolated from Iranian drinking water: vacA, cagA, iceA, oipA and babA2 genotype status and antimicrobial resistance properties. FEBS Open Bio 2016; 6: 433-441 [PMID: 27419049 DOI: 10.1002/2211-5463.12054]

14. Talaei R, Souad N, Montazz H, Dabiri H. Milk of livestock as a possible transmission route of Helicobacter pylori pylori infection. Gastroenterol Hepatol Bed Bench 2015; 8: S30-S36 [PMID: 26171153]

15. Amineva MR, El-Omar EM. Host-bacterial interactions in Helicobacter pylori infection. Gastroenterology 2008; 134: 306-332 [PMID: 18166359 DOI: 10.1053/j.gastro.2007.11.009]

16. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of Helicobacter pylori. Proc Natl Acad Sci USA 2000; 97: 7533-7538 [PMID: 10852959 DOI: 10.1073/pnas.130079797]

17. Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura M. Benign and malignant gastroduodenal diseases associated with Helicobacter pylori pylori: a narrative review and personal remarks in 2018. Minerva Gastroenterol Dietol 2018; 64: 280-296 [PMID: 29458240 DOI: 10.23736/S1121-421X.18.02481-9]

18. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci USA 1996; 93: 14648-14653 [PMID: 8962108 DOI: 10.1073/pnas.93.25.14648]

19. Atherton JC, Cao P, Peek RM Jr, Tummuruk MJ, Blaser MJ, Cover TL. Mosiacism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 1995; 270: 17771-17777 [PMID: 8629377 DOI: 10.1074/jbc.270.17.17717]

20. Glosh P, Sarkar A, Ganguly M, Raghuban, Alam J, De R, Mukhopadhyay AK. Helicobacter pylori strains
Alam J et al. Novel virulence factor dupA of Helicobacter pylori

harboring babA2 from Indian sub population are associated with increased virulence in ex vivo study. Gut Pathog 2016; 8: 1 [PMID: 26759607 DOI: 10.1186/s13099-015-0083-z]

Fischer W, Breithaupt U, Kern B, Smith SI, Spicher C, Haas R. A comprehensive analysis of Helicobacter pylori plasticity zones reveals that they are integrating with intermediate integration specificity. BMC Genomics 2014; 15: 310 [PMID: 24767410 DOI: 10.1186/1471-2164-15-310]

Ganguly M, Sarkar S, Ghosh P, Sarkar A, Alam J, Karmakar BC, De R, Saha DR, Mukhopadhay AK. Helicobacter pylori plasticity region genes are associated with the gastroduodenal diseases manifestation in India. Gut Pathog 2016; 8: 10 [PMID: 27006705 DOI: 10.1186/s13099-016-0093-5]

Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of Helicobacter pylori. Gastroenterology 2005; 128: 833-848 [PMID: 15825067 DOI: 10.1053/j.gastro.2005.01.009]

Arachchi HS, Kalva V, Lal B, Bhutia V, Baba CS, Chakravartty S, Rohatgi S, Sarma PM, Mishra V, Das B, Ahuja V. Prevalence of duodenal ulcer-promoting gene (dupA) of Helicobacter pylori in patients with duodenal ulcer in North Indian population. Helicobacter 2007; 12: 591-597 [PMID: 18001398 DOI: 10.1111/j.1537-3387.2007.00557.x]

Argent RH, Burette A, Miendi Deyi VY, Atherton JC. The presence of dupA in Helicobacter pylori is not significantly associated with duodenal ulceration in Belgium, South Africa, China, or North America. Clin Infect Dis 2007; 45: 1204-1206 [PMID: 17918084 DOI: 10.1086/521277]

Gomes LI, Rocha GA, Rocha AM, Soares TF, Oliveira CA, Bittencourt PF, Queiroz DM. Lack of association between Helicobacter pylori infection with dupA-positive strains and gastroduodenal diseases in Brazilian patients. Int J Med Microbiol 2008; 299: 223-230 [PMID: 17897881 DOI: 10.1016/j.ijmm.2007.05.006]

Pacheco AR, Proença-Médena JL, Sales AI, Fukushima Y, da Silveira WD, Pimenta-Médena JL, de Oliveira RB, Brocchi M. Involvement of the Helicobacter pylori plasticity region and cag pathogenicity island genes in the development of gastroduodenal diseases. Eur J Clin Microbiol Infect Dis 2008; 27: 1053-1059 [PMID: 18569912 DOI: 10.1007/s10096-008-0549-8]

Pereira WN, Ferraz MA, Bazagli LM, de Labro RW, Orcini WA, Bianchi Ximenes JP, Neto AC, Payálo SL, Rassmusen LT. Association among H. pylori virulence markers dupA, cagA and vacA in Brazilian patients. J Venom Anim Toxins Incl Trop Dis 2014; 20: 1 [PMID: 24456629 DOI: 10.1186/1678-9199-20-1]

Hussein NR, Mohammad M, Talebkhani Y, Doraghi M, Letley DP, Muhammad MK, Argent RH, Atherton JC. Differences in virulence markers between Helicobacter pylori strains from Iraq and those from Iran: potential importance of regional differences in H. pylori-associated disease. J Clin Microbiol 2008; 46: 1747-1779 [PMID: 18353934 DOI: 10.1128/JCM.01737-07]

Douraghi M, Mohammad M, Oghaliae A, Abdirad A, Mohaghegh MA, Hosseini ME, Zanati H, Ghasemi A, Esmaeili M, Mohajerani N. dupA, a risk determinant in Helicobacter pylori infection. J Med Microbiol 2008; 57: 554-562 [PMID: 18436537 DOI: 10.1099/jmm.0.47776-6]

Talebi Bezmian Abadi A, Taghvaei T, Wolfram L, Kusters JG. Infection with Helicobacter pylori strains lacking dupA is associated with an increased risk of gastric ulcer and gastric cancer development. J Med Microbiol 2012; 61: 23-30 [PMID: 21903829 DOI: 10.1099/jmm.0.07250-0]

Fatahi G, Talebi Bezmian Abadi A, Peerayeh SN, Forootan M. Carrying a 112 bp-segment in Helicobacter pylori dupA may associate with increased risk of duodenal ulcer. Infect Genet Evol 2019; 73: 21-25 [PMID: 30981881 DOI: 10.1016/j.meegid.2019.04.009]

Farzi N, Yadegar A, Sadeghi A, Asadzadeh Aghdaie H, Marian Smith S, Raymond J, Suzuki H, Zali MR. High Prevalence of Antibiotic Resistance in Iranian Helicobacter pylori isolates: Importance of Functional and Mutational Analysis of Resistance Genes and Virulence Genotyping. J Clin Med 2019; 8: 2004 [PMID: 31744181 DOI: 10.3390/jcm8110200]

Salih AM, Goreal A, Hussein NR, Abdullah SM, Hawrami K, Assafi M. The distribution of cagA and dupA genes in Helicobacter pylori strains in Kurdistan region, northern Iraq. Ann Saudi Med 2013; 33: 290-293 [PMID: 23793434 DOI: 10.1016/j.anscen.2013.02.007]

Haddadi MH, Bazargani A, Khashei R, Fattahi MR, Bagheri Lankarani K, Moini M, Rokni Hosseini SM. Different distribution of Helicobacter pylori cagPA- cagA motifs and dupA genes in the upper gastrointestinal diseases and correlation with clinical outcomes in iranian patients. Gastroenterol Hepatol Bed Bench 2015; 8: S37-S46 [PMID: 26171136 DOI: 10.1016/j.cecphi.2012.02.001]

Soud N, Sarshar M, Dahir H, Montaz H, Kargar M, Mohammadzadeh A, Abdi S. The study of the cagA and dupA genes in Helicobacter pylori strains and their relationship with different gastroduodenal diseases. Gastroenterol Hepatol Bed Bench 2015; 8: S47-S53 [PMID: 26171137]

Tuncel IE, Hussein NR, Bolek IK, Arikam S, Salih BA. Helicobacter pylori virulence factors and their role in peptic ulcer diseases in Turkey. Acta Gastroenterol Belg 2010; 73: 235-238 [PMID: 20690562 DOI: 10.1007/s10262-009-9502-2]

Zhang Z, Zheng Q, Chen X, Xiao S, Liu W, Lu H. The Helicobacter pylori duodenal ulcer promoting gene, dupA in China. BMC Gastroenterol 2008; 8: 49 [PMID: 18950522 DOI: 10.1186/1471-230X-8-49]

Kim JY, Kim N, Nam RH, Suh JH, Chang H, Lee JW, Kim YS, Kim JM, Choi JW, Park JG, Lee YS, Lee DH, Jung HC. Association of polymorphisms in virulence factor of Helicobacter pylori and gastroduodenal diseases in South Korea. J Gastroenterol Hepatol 2014; 29: 984-991 [PMID: 24372834 DOI: 10.1111/jgh.12509]

Yeh YC, Cheng HC, Chang WL, Yang HB, Sheu BS. Matrix metalloproteinase-3 promoter polymorphisms but not dupA-H. pylori correlate to duodenal ulcers in H. pylori-infected females. BMC Microbiol 2010; 10: 218 [PMID: 20707923 DOI: 10.1186/1471-2180-10-218]

Schmidt HM, Andres S, Kaakoush NO, Engstrang L, Eriksson L, Goh KL, Fock KM, Hilmi I, Dhamodaran S, Forman D, Mitchell H. The prevalence of the duodenal ulcer promoting gene (dupA) in Helicobacter pylori isolates varies by ethnic group and is not universally associated with disease development: a case-control study. Gut Pathog 2009; 1: 1 [PMID: 19338650 DOI: 10.1186/1757-4749-1-1]

Osman HA, Hasan H, Surpiatn R, Hassan S, Andee DZ, Abdul Majid N, Zilfalil BA. Prevalence of Helicobacter pylori cagA, babA2, and dupA genotypes and correlation with clinical outcome in Malaysian patients with dyspepsia. Turk J Med Sci 2015; 45: 940-946 [PMID: 26422871 DOI: 10.3906/sag-1409-77]
43 Hussein NR. The association of dupA and Helicobacter pylori-related gastroduodenal diseases. *Eur J Clin Microbiol Infect Dis* 2010; 29: 817-821. [PMID: 20419465 DOI: 10.1007/s10096-010-0933-z]

44 Shiota S, Matsunari O, Watanabe M, Hanada K, Yamaoka Y. Systematic review and meta-analysis: the relationship between Helicobacter pylori dupA gene and clinical outcomes. *Gut Pathog* 2010; 2: 13. [PMID: 20404520 DOI: 10.1186/1757-4749-2-13]

45 Nguyen LT, Uchida T, Tsukamoto Y, Kuroda A, Okimoto T, Kodama M, Murakami K, Fujioka T, Moriyama M. Helicobacter pylori dupA gene is not associated with clinical outcomes in the Japanese population. *Clin Microbiol Infect* 2010; 16: 1264-1269. [PMID: 19832706 DOI: 10.1111/j.1469-0691.2009.03081.x]

46 Imagawa S, Ito M, Yoshihara M, Eguchi H, Tanaka S, Chayama K. Helicobacter pylori dupA and gastric acid secretion are negatively associated with gastric cancer development. *Med Microbiol* 2010; 59: 1484-1489. [PMID: 20829397 DOI: 10.1099/jmm.0.021816-0]

47 Alam J, Maiti S, Ghosh P, De R, Chowdhury A, Das S, Macaden R, Devarbhavi H, Ramamurthy T, Mukhopadhyay AK. Significant association of the dupA gene of Helicobacter pylori with duodenal ulcer development in a South-east Indian population. *J Med Microbiol* 2012; 61: 1295-1302. [PMID: 22653921 DOI: 10.1099/jmm.0.038398-0]

48 Paredes-Osses E, Sáez K, Sanhuaza E, Hebel S, González C, Briceño C, García Cancino A. Association between cagA, vacA, and dupA genes of Helicobacter pylori and gastroduodenal pathologies in Chilean patients. *Folia Microbiol (Prague)* 2017; 62: 437-444. [PMID: 28233946 DOI: 10.1021/s22201-017-0714-y]

49 Molina-Castro S, Garita-Cambronerio J, Malespin-Bendaña W, Une C, Ramírez V. Virulence factor genotyping of Helicobacter pylori isolated from Costa Rican dyspeptic patients. *Microb Pathog* 2019; 128: 276-280. [PMID: 30654009 DOI: 10.1016/j.micpath.2019.01.018]

50 Queiroz DM, Rocha GA, Rocha AM, Moura SB, Saraiva IE, Gomes LI, Soares TF, Melo FF, Cabral MM, Oliveira CA. dupA polymorphisms and risk of Helicobacter pylori-associated diseases. *Int J Med Microbiol* 2011; 301: 225-228. [PMID: 21050811 DOI: 10.1016/j.ijmm.2010.08.019]

51 Moura SB, Costa RF, Anacleto C, Rocha GA, Rocha AM, Queiroz DM. Single nucleotide polymorphisms of Helicobacter pylori dupA gene that lead to premature stop codons. *Helicobacter* 2012; 17: 176-180. [PMID: 22515354 DOI: 10.1111/j.1121-5378.2011.00933.x]

52 Hussein NR, Argent RH, Marx CK, Patel SR, Robinson K, Atherton JC. Helicobacter pylori dupA is polymorphic, and its active form induces proinflammatory cytokine secretion by mononuclear cells. *J Infect* 2010; 202: 261-269. [PMID: 20533870 DOI: 10.1086/653587]

53 Takahashi A, Shiota S, Matsunari O, Watanabe M, Suzuki R, Nakachi S, Kinjo N, Kinjo F, Yamaoka Y. Intact long-type dupA as a marker for gastroduodenal diseases in Okinawan subpopulation, Japan. *Helicobacter* 2013; 18: 66-72. [PMID: 23007336 DOI: 10.1111/j.1323-5787.2012.00994.x]

54 Hussein NR, Abdullah SM, Salih AM, Assafi MA. dupA is associated with duodenal ulcer and high interleukin-8 secretion from the gastric mucosa. *Infect Immun* 2012; 80: 2971-2; author reply 2973. [PMID: 22811495 DOI: 10.1128/IAI.00767-12]

55 Hussein NR, Tuncel IE. Helicobacter pylori dupA and smoking are associated with increased levels of interleukin-8 in gastric mucosa in Iraq. *Hum Pathol* 2015; 46: 929-930. [PMID: 25791584 DOI: 10.1016/j.humpath.2015.01.021]

56 Hussein NR, Tunjel I, Majed HS, Youssif ST, Aswad SI, Assafi MS. Duodenal ulcer promoting gene I (dupA) is associated with A2147G clarithromycin-resistance mutation but not interleukin-8 secretion from gastric mucosa in Iraqi patients. *New Microbes New Infect* 2015; 5: 6-10. [PMID: 26042185 DOI: 10.1016/j.mnni.2015.02.003]

57 Queiroz DM, Moura SB, Rocha AM, Costa RF, Anacleto C, Rocha GA. The genotype of the Brazilian dupA-positive Helicobacter pylori strains is dupA1. *J Infect Dis* 2011; 203: 1033-1034. [PMID: 21402555 DOI: 10.1093/infdis/jiq47]

58 Alam J, Ghosh P, Ganguly M, Sarkar A, De R, Mukhopadhyay AK. Association of intact dupA (dupA1) rather than dupA1 cluster with duodenal ulcer in Indian population. *Gut Pathog* 2015; 7: 9. [PMID: 25829953 DOI: 10.1186/s13099-015-0056-2]

59 Wang MY, Chen C, Gao XZ, Li JY, Yue J, Ling F, Wang XC, Shao SH. Distribution of Helicobacter pylori virulence markers in patients with gastroduodenal diseases in a region at high risk of gastric cancer. *Microb Pathog* 2013; 59-60: 13-18. [PMID: 23583809 DOI: 10.1016/j.micpath.2013.04.001]

60 Shiota S, Nguyen LT, Murakami K, Kuroda A, Mizukami K, Okimoto T, Kodama M, Fujioka T, Yamaoka Y. Association of Helicobacter pylori dupA with the failure of primary eradication. *J Clin Gastroenterol* 2012; 46: 297-301. [PMID: 22290890 DOI: 10.1097/MCG.0b013e31823242310c]

61 Rasheed F, Campbell BJ, Alfazih H, Varro A, Zahra R, Yamaoka Y, Pritchard DM. Analysis of clinical isolates of Helicobacter pylori in Pakistan reveals high degrees of pathogenicity and high frequencies of antibiotic resistance. *Helicobacter* 2014; 19: 387-399. [PMID: 24827414 DOI: 10.1111/hel.12142]

62 Fernández-Reyes M, Tamayo E, Rejas-Rengifo D, Fischer W, Carrasco-García E, Alonso M, Lizasoain J, Bujanda L, Cosme Á, Montes M. Helicobacter pylori pathogenicity and primary antimicrobial resistance in Northern Spain. *Eur J Clin Investig* 2019; 49: e13150. [PMID: 31192451 DOI: 10.1111/eji.13150]

63 Imkamp F, Lauener FN, Poh D, Lehures P, Vale FF, Jehanne Q, Zbinden R, Keller PM, Wagner K. Rapid Characterization of Virulence Determinants in Helicobacter pylori Isolated from Non-Athropic Gastritis Patients by Next-Generation Sequencing. *J Clin Med* 2019; 8: 1030. [PMID: 31330977 DOI: 10.3390/jcm807S030]

64 Idowa A, Muzaka A, Harrison U, Palamides P, Haas R, Mbao A, Mammad R, Bolon J, Jolaya T, Smith S, Ally R, Clarke A, Njong H. Detection of Helicobacter pylori and its virulence genes (cagA, dupA, and vacA) among patients with gastroduodenal diseases in Chris Hani Baragwanath Academic Hospital, South Africa. *BMC Gastroenterol* 2019; 19: 73. [PMID: 31088318 DOI: 10.1186/s12876-019-0986-0]

65 Christie PJ. Type IV secretion: intercellular transfer of macromolecules by systems ancestrally related to conjugation machines. *Mol Microbiol* 2001; 40: 294-305. [PMID: 11309113 DOI: 10.1046/j.1365-2958.2001.02302.x]

66 Fischer W, Püls J, Buhrdorf R, Gebert B, Oderbreit S, Haas R. Systematic mutagenesis of the Helicobacter pylori cag pathogenicity island: essential genes for CagA translocation in host cells and induction of...
\textbf{Alam J et al.} Novel virulence factor \textit{dupA} of \textit{Helicobacter pylori}.

interleukin-8. \textit{Mol Microbiol} 2001; 42: 1337-1348 [PMID: 11886563 DOI: 10.1046/j.1365-2958.2003.03406.x]

67 \textbf{Backert S}, Ziska E, Brinkmann V, \textit{et al}. Translocation of the \textit{Helicobacter pylori} CagA protein in gastric epithelial cells by a type IV secretion apparatus. \textit{Cell Microbiol} 2000; 2: 155-164 [PMID: 11287572 DOI: 10.1046/j.1462-5822.2000.00043.x]

68 \textbf{Karnholz A}, Hoefler C, Odenbreit S, Fischer W, \textit{et al}. Functional and topological characterization of novel components of the \textit{comB} DNA transformation competence system in \textit{Helicobacter pylori}. \textit{J Bacteriol} 2006; 188: 3764-3772 [PMID: 12813069 DOI: 10.1128/JB.188.13.3764-3772.2006]

69 \textbf{Kersulyte D}, \textit{et al}. DNA transfer in the gastric pathogen \textit{Helicobacter pylori}. \textit{J Gastroenterol} 2014; 49: 594-604 [PMID: 24515309 DOI: 10.1007/s00535-014-0938-y]

70 \textbf{Jung SW}, \textit{et al}. Multiple infection and microdiversity among \textit{Helicobacter pylori} isolates in a single host in India. \textit{PLoS One} 2012; 7: e43570 [PMID: 22952670 DOI: 10.1371/journal.pone.0043570]

71 \textbf{Vergunst AC}, \textit{et al}. Site-specific relaxase activity of a VirD2-like protein encoded within the \textit{tfs} genomic island of \textit{Helicobacter pylori}. \textit{J Biol Chem} 2013; 288: 26385-26396 [PMID: 23900838 DOI: 10.1074/jbc.M113.496430]

72 \textbf{Silva B}, \textit{et al}. The expression of \textit{Helicobacter pylori} \textit{tfs} plasticity zone cluster is regulated by pH and adherence, and its composition is associated with differential gastric IL-8 secretion. \textit{Helicobacter} 2017; 22 [PMID: 28436598 DOI: 10.1111/hel.12390]

73 \textbf{Wang GQ}, Xu JT, Xu GS, \textit{et al}. \textit{Helicobacter pylori} with the Intact \textit{dupA} Cluster is more Virulent than the Strains with the Incomplete \textit{dupA} Cluster. \textit{Curr Microbiol} 2015; 71: 16-23 [PMID: 25847580 DOI: 10.1007/s00284-015-0812-z]

74 \textbf{Wang MY}, Chen C, \textit{et al}. Predicting a novel pathogenicity island in \textit{Helicobacter pylori} by genomic barcoding. \textit{World J Gastroenterol} 2013; 19: 5006-5010 [PMID: 23946608 DOI: 10.3748/wjg.v19.i30.5006]

75 \textbf{Wang MY}, Chen C, \textit{et al}. The intact \textit{dupA} Cluster is a more reliable \textit{Helicobacter pylori} virulence marker than \textit{dupA} alone. \textit{Infect Immun} 2012; 80: 381-387 [PMID: 22038914 DOI: 10.1128/IAI.05472-11]

76 \textbf{Wang MY}, Shao C, \textit{et al}. Predicting a novel pathogenicity island in \textit{Helicobacter pylori} by genomic barcoding. \textit{World J Gastroenterol} 2013; 19: 5006-5010 [PMID: 23946608 DOI: 10.3748/wjg.v19.i30.5006]
